AN ANALYSIS OF MICROBIAL CONTAMINATION IN MILITARY AVIATION FUEL SYSTEMS

THESIS

Harold W. Graef, Major, USMC

AFIT/GEE/ENV/03-10

DEPARTMENT OF THE AIR FORCE
AIR UNIVERSITY
AIR FORCE INSTITUTE OF TECHNOLOGY

Wright-Patterson Air Force Base, Ohio

AFIT/GEE/ENV/03-10

AN ANALYSIS OF MICROBIAL CONTAMINATION IN MILITARY AVIATION FUEL SYSTEMS

THESIS

Presented to the Faculty

Department of Systems and Engineering Management

Graduate School of Engineering and Management

Air Force Institute of Technology

Air University

Air Education and Training Command

In Partial Fulfillment of the Requirements for the

Degree of Master of Science in Engineering and Environmental Management

Harold W. Graef, M.S.

Major, USMC

March 2003

APPROVED FOR PUBLIC RELEASE; DISTRIBUTION UNLIMITED.

AFIT/GEE/ENV/03-10

AN ANALYSIS OF MICROBIAL CONTAMINATION IN MILITARY AVIATION FUEL SYSTEMS

Harold W. Graef, M.S.
Major, USMC

Approved:

Charles Bleckmann — 7 Feb 03
Dr. Charles A. Bleckmann, PhD (Chairman) — date

Michelle Rauch-Johnson — 7 Feb 03
Captain Michelle Rauch-Johnson, PhD (Member) — date

Dan E. Reynolds — 10 Feb 03
Professor Daniel E. Reynolds (Member) — date

Acknowledgements

I would like to express my sincere appreciation to my thesis advisor, Dr. Charles Bleckmann. His in-depth knowledge of fuel issues and guidance throughout the process were indispensable in completing my thesis. He provided the vision necessary to keep my thesis efforts focused without confining the learning experience associated with a thesis effort.

Further thanks go to my sponsor, the Air Force Research Laboratory, for support in this endeavor, which went beyond my expectations. Special thanks go to Captain Michelle Rauch-Johnson who was extremely generous with her time and expertise.

I am also very appreciative to Professor Daniel Reynolds. I have never met a man more passionate about his work than Professor Reynolds is about teaching and applying statistics.

I am also very grateful to my late parents who taught me the importance of higher education. It is through their sacrifice that I am able to pursue my dreams.

I'd like to thank the Marine Corps who allowed me the opportunity to attend AFIT. *Semper Fidelis* to God, Country, and the United States Marine Corps.

Finally, and most importantly, a special thank you goes to my family for their support and understanding throughout the thesis process. Without my family's motivation and encouragement, I could not have completed my thesis or the AFIT program. Thanks for listening and helping me to keep things in perspective. This thesis is as much a result of your dedication as it is mine.

Harold W. Graef

Table of Contents

List of Figures

Page

List of Tables

Page

AFIT/GEE/ENV/03-10

Abstract

Military aviation fuel systems can be an ideal environment for microorganisms. Microbial growth in hydrocarbon fuel systems arises because of the impracticality of keeping fuel tanks sterile and the inevitable presence of water from condensation. Microbial contaminants in aviation fuel systems are a concern because of their potential to degrade the fuel, accelerate tank corrosion, and threaten flight safety.

This research addresses the concern of using more environmentally friendly Fuel System Icing Inhibitors (FSII), which are also biocidal. Are significant levels of microorganisms growing in military aviation fuel systems, and if so, are there any common variables?

Forty aviation fuel samples were collected from fuel storage tanks (including flexible expeditionary fuel bladders), refueling trucks, and aircraft from 12 U.S. military bases. Samples were analyzed using peak naming and pattern recognition algorithms of sample extracts processed on a gas chromatograph.

Significant levels of microorganisms were found in military aviation fuel systems. 90% (36 of 40) of fuel samples produced microbial growth. Over 40% of the serial dilutions that produced microbial growth were characterized as moderately or heavily contaminated samples.

The microorganisms isolated were overwhelmingly Gram negative, anaerobic, bacilli with populations varying by orders of magnitude.

AN ANALYSIS OF MICROBIAL CONTAMINATION IN MILITARY AVIATION FUEL SYSTEMS

I. Introduction

Overview

This work expands upon the knowledge of microorganisms present in military aviation fuel systems. Fuel systems can be an ideal environment for microorganisms. Fungi and bacteria need three things to thrive; food, water, and metals – all of which are present in aviation fuel systems.

Significant numbers of microbes are likely to be found wherever water and fuel meet. Microbial growth in hydrocarbon fuel systems arises because of the impracticality of keeping fuel tanks sterile and the inevitable presence of water from condensation.

Microbial contaminants in aviation fuel systems are a concern because of their potential to degrade the fuel, accelerate corrosion within the fuel tank, and threaten flight safety. Flight safety may be compromised due to fuel filter clogging and fuel gauge malfunctioning. Operational and maintenance concerns include fuel tank sealant degradation and metal corrosion. Military aircraft are often affected more than civilian aircraft due to operations in extreme temperature regions.

Many things have changed since the 1950's microbial fuel contamination research including jet fuel and fuel additive compositions. This research asks a fundamental question, yet one that hasn't been asked in nearly half a century. Are significant levels of microorganisms growing in military aviation fuel systems, and if so, are there any common variables linking the contamination?

Purpose of Research

Based on both qualitative and quantitative research, this study identifies the microorganisms isolated from U.S. military aviation fuel systems. From the baseline thus established, further research can be done to improve field-testing and biocide treatment procedures and to reduce the risk of developing in-flight, fuel-related emergencies.

This thesis effort builds upon research conducted nearly a half century ago when the U.S. Air Force recognized that aviation fuels were microbially contaminated. Since the initial recognition of this problem, there have been changes to fuel composition, delivery systems, and housekeeping practices, which have made the original research scientifically inapplicable to today's systems.

Research Objectives

The four primary objectives of this research are:

1. To determine the types and quantities of microorganisms, if any, present in U.S. military aviation fuel storage tanks, aircraft refueling trucks, and aircraft fuel tanks;
2. To determine the characteristic conditions in which microorganisms thrive in U.S. military aviation fuel systems;
3. To determine if current microbial minimization methods are appropriate; and
4. To determine if U.S. military aviation fuel systems are vulnerable to intentional microbial contamination.

Scope of Research

This research was limited to analysis of samples from active duty U.S. Air Force aviation fuel systems consuming and supplying JP-8. Sources of the contamination were not identified. Since military aircraft routinely refuel at other Services' bases, or civilian airports, these multiple sources allow aircraft contamination from outside Air Force fuel systems.

This study emphasized the first three objectives, the current status of the fuel supply system. The fourth objective was addressed indirectly by analyzing the prevalence, treatment, and attitudes encountered while gathering fuel samples from various squadrons and locations. This research, therefore, is limited to analyzing the ease at which microorganisms may be grown and spread physically.

II. Literature Review

Overview

This chapter summarizes the pertinent information regarding microbial contamination in military aviation fuel systems. It reviews the history of the contamination and measures to mitigate them, microbial growth issues and concerns, the gas chromatography microbial identification technique, and the evolution of fuel and additive composition. This literature review also discusses three major aspects of this research:

1. Why microbial growth in military aviation fuel systems is a concern;
2. Why the military's pioneering research of the 1950's and 60's no longer applies to today's fuel systems; and
3. Which methods are most appropriate for this research.

The military first began investigating microbial contamination in fuel systems in the 1950's when flight operations were hampered by the presence of microorganisms. The research was prompted by the discovery of the accumulation of sludge in fuel storage tanks (Bakanauskas, 1958:1). Since then, microbial contamination has been minimized by good housekeeping practices and the addition of anti-icing additives, which also act as biocides.

Definitions

Bacterium

A single cell microorganism characterized by the absence of defined intracellular membranes (prokaryotes). Potential food sources range from single carbon molecules to complex polymers, including plastics (ASTM, 1999:2).

Fungus

Single cell (yeasts) or filamentous (molds) microorganisms that share the property of having the true intracellular membranes (organelles) that characterize all higher life forms (eukaryotes) (ASTM, 1999:3).

Biocide

A poisonous substance that kills living organisms. Biocides are further classified as bactericides (kill bacteria), fungicides (kill fungi), and microbiocides (kill both bacterial and fungi) (ASTM, 1999:3).

Contamination

The process of making inferior or impure by admixture, as well as to making unfit for use by the introduction of unwholesome or undesirable elements (Merriam-Webster Online, 2002). In the case of aviation fuel contamination, the undesirable elements are free phase water, solid particulates, and microorganisms.

Cleanliness

Fuel cleanliness means the absence of solid particulates and free water (Bacha, 2000:9). Particulates such as rust and dirt can obstruct fuel filters and increase fuel pump friction.

Free Phase Water

Visible layer of water separate from the fuel within the same container. Water has three adverse effects in fuel systems. It does not burn in the engine, it freezes at low temperatures encountered during high altitude flights, and it provides an environment in which microorganisms can grow.

Microbially Influenced Corrosion (MIC)

Microbially influenced corrosion occurs directly or indirectly as a result of the metabolic activity of microorganisms. Two different types of MIC are commonly encountered: anaerobic and aerobic (Angeles-Chavez, 2001:292).

Sulfate Reducing Bacteria

Sulfate reducing bacteria live under anaerobic conditions using sulfate as a final electron-acceptor. By the reduction of sulfate the bacteria produce sulfide and by-products, like thio-sulfate and sulfur. Some sulfate reducers contain a hydrogenase enzyme, which enables them to utilize H_2 as an energy source (Keresztes and others, 1998:77).

Evolution of Fuel Additives

Early Discovery and Solution

In 1956, the United States Air Force recognized that its widely-used JP-4 fuels were microbially contaminated when Air Force B-47 and KC-97 flight operations were affected (Finefrock and London, 1966:1). Two years later, a B-52 crash was directly attributed to clogging of fuel system screens and filters by some form of fuel contamination (Finefrock and London, 1966:1). In that same year, the Wright Air Development Center determined that sludge accumulation in tanks used to store kerosene-type fuels was a common occurrence (Bakanauskas, 1958:1).

More instances of contamination and corrosion surfaced in the late 1950's and early 1960's and reached near epidemic proportions in storage tanks and aircraft fuel cells at various locations. At the beginning of 1962, approximately 52 governmental and non-governmental agencies were involved in various phases of research on microbiological contamination of fuels (Finefrock and London, 1966:3).

The practical solution to microbial contamination came from the biocidal action of an anti-icing additive (AIA), now known as a fuel system icing inhibitor (FSII), ethylene glycol monomethyl ether (EGME) (see Figure 1), also known as methoxyethanol, and glycerol, which were added to JP-4 fuels in 1962 (Finefrock and London, 1966:4). The original concentration resulted in 0.1% volume/volume (V/V)

$$CH_3\text{-}O\text{-}CH_2\text{-}CH_2\text{-}OH$$

Figure 1. Ethylene Glycol Monomethyl Ether (EGME)
(J. T. Baker Company, 2000:1).

(Johnston and others, 1964:1), which consisted of 90% EGME and 10% glycerine (by volume). The composition of the mixture was changed to 99.6% EGME and 0.4% glycerine on the basis of solubility data. The solubility of glycerine in JP-4 fuel, when in the presence of 0.1% EGME, was determined to be approximately 4 parts per million (Finefrock and London, 1966:4). This concentration was designated in military specification, MIL-I-27686C. In experimental studies, effective inhibition of microbial growth was observed in water bottoms containing 20% EGME, and some inhibition with 10% EGME. Lower concentrations allowed microbial growth. It was also found that the addition of 20% EGME effectively sterilized the contents of a tank in which growth was already established (Johnston and others, 1964:1). Concentrations of 20% or more in the water-phase are considered biocidal (Finefrock and London, 1966:4).

Two years later, in 1964, the microbial contamination of Air Force JP-4 fuels appeared to be under control. The reduction in the number of microorganisms found in the operational JP-4 fuel systems was attributed to "good housekeeping" procedures (Finefrock and London, 1966:4,5,68). Although variations of EGME are still used as an anti-icing agent, fuel handling procedures and the elimination of the use of floating-roof tanks reduced the amount of water and contaminants in the fuel systems, and therefore, the requirement for the use of EGME as an anti-microbial agent.

Health Concerns Caused By Anti-Icing Agents

Although good housekeeping now prevents and controls much of the microbial contamination in jet fuel, fuel system icing inhibitors are currently used to aid in combating microbial activity. Fuel system icing inhibitors, however, do pose a potential risk to personnel who work with or come in contact with the chemicals. EGME is delivered from the manufacturer with a warning that exposure to the chemical may adversely affect the central nervous system, blood and blood forming organs, liver, testes, and kidneys. The warning also states that exposure may cause birth defects and has caused birth defects in animal testing (J. T. Baker Co., 2000:1-2). Exposure to di-EGME (the military's current anti-icing agent), under most circumstances, has fewer severe effects such as discomfort and signs of central nervous system disturbances. However, taken in large quantities, di-EGME could cause severe kidney and liver damage or even death (Del Rey Chemical Co., 1992:2).

Environmental Concerns of Anti-Icing Agents

Aside from the potential health risks of direct exposure to these chemicals, an ecological concern is present as well. When released into the soil, EGME may leach into nearby groundwater. If this were to happen, EGME may biodegrade, but only to a moderate extent (J. T. Baker Co., 2000:6). Di-EGME causes slightly less environmental concern when released into the soil. The manufacturer lists no neutralizing agent and water dilution is the recommended method of spill response procedures (Del Rey Chemical Co., 1992:2). Of course, the jet fuel spilled with it has ecological concerns of its own. Jet fuel is expected to be toxic to aquatic organisms and is considered ultimately (but not readily) biodegradable (Chevron Products Co., 2000:1).

Controlling microorganisms with biocides is not a feasible solution. Biocides, by definition, are toxic (ASTM, 1999:3). Biocides may endanger the fuel handlers, personnel working in areas where fuel volatilization may occur, and organisms in the environment receiving wastes from the system (or spills) (Gaylarde, 1999:8).

In a pollution prevention effort, the U.S. Air Force and Navy formed a joint initiative to find safer, more environmentally acceptable jet fuel system icing inhibitors for military aircraft (Meshako and others, 1999:383; Geiss and Frazier, 2001:210; Mattie, 1995:295). EGME was replaced with di-EGME because of concern over potential environmental toxicity. Di-EGME, however, has been shown to be more toxic during simple microbial toxicity tests than some potential alternatives such as dipropylene glycol and glycerol formal (Meshako and others, 1999:383). The search for even more environmentally friendly fuel system icing inhibitors is ongoing. Due to different fuel properties and FSII compositions, it is doubtful that di-EGME or any future replacement FSII, will display the exact same biocidal characteristics as EGME that was tested so many years ago. Because of these significant changes, fuel system microbial research is warranted.

Types of Military Aviation Fuels

Two types of Jet Propulsion (JP) fuel are currently widely used by the U.S. military (Bacha, 2000:13). The Navy and Marine Corps use JP-5 during carrier operations. The Air Force, Navy and Marine Corps use JP-8 primarily during land-based operations. Both are kerosene-type fuels. The primary difference between JP-5 and JP-8 is the flash point. JP-5 has a higher minimum flash point, which provides an additional level of safety in handling jet fuel in the unforgiving environment of carrier aviation. A brief summary of military jet fuels is provided in Table 1. In this study, only JP-8 will be examined.

Table 1. Summary of Military Jet Fuels

Fuel	Year Introduced	Type	Freeze Point ºC max	Flash Point ºC min	Comments
JP-1	1944	kerosene	– 60	43	obsolete
JP-2	1945	wide-cut	– 60		obsolete
JP-3	1947	wide-cut	– 60		obsolete
JP-4	1951	wide-cut	– 72		obsolete
JP-5	1952	kerosene	– 46	60	U.S. Navy / Marine Corps fuel
JP-6	1956	kerosene	– 54		XB-70 program, obsolete
JPTS	1956	kerosene	– 53	43	Higher thermal stability, lower freeze point
JP-7	1960	kerosene	– 43	60	Lower volatility, higher thermal stability
JP-8	1979	kerosene	– 47	38	U.S. Department of Defense fuel
JP-8+100	1998	kerosene	– 47	38	U.S. Air Force fuel containing an additive that provides improved thermal stability

(Derived from Bacha, 2000:13)

JP-8

Combat experience in Vietnam demonstrated that jet aircraft damage (and losses) due to the use of JP-4 was clearly higher than damage encountered by the Navy using JP-5 which has a higher minimum flash point. This difference in aircraft damage and losses was the motivation behind the development of JP-8 (Maurice and others, 2001:752). JP-8 is essentially a common civilian jet fuel, Jet A, with a military additive package. This package contains three components: FSII to prevent water in the fuel from freezing, corrosion inhibitors (CI) to prevent fuel pump failures, and Static Dissipater Additive (SDA) to prevent mishaps due to static discharge while refueling. The desire to move toward a single fuel, coupled with the JP-4 safety hazards, led the Air Force to begin the conversion of all its aircraft and fuel systems to JP-8 in 1993. Conversion was completed in 1995.

Kerosene-based fuels like JP-8 are mixtures of thousands of hydrocarbons. For JP-8, these hydrocarbons can be divided into three broad classes: aromatics (approx. 20%), *n*-paraffins and isoparaffins (approx. 60%), and cycloparaffins (naphthenes, approx. 20%) (Maurice and others, 2001:752).

Jet fuel has two roles. First is to provide enough propulsive energy to the aircraft so that it will leave the ground. Second is to provide a coolant for airframe and engine subsystems (Maurice and others, 2001:752). Unfortunately, the heavier JP-8 led to increased maintenance costs at Air Force bases worldwide. Fuel degradation was found to have caused fouling/coking in engine fuel nozzles, fuel controls, and fuel manifolds costing millions of dollars per year. This led to a joint government/industry/academia program to develop an additive package for JP-8.

The additive agreed upon contained a detergent/dispersant (fuel injector cleaner), in addition to the standard additives. JP-8 with the additive package, added at approximately 250 ppm (1 quart of additive to 1000 gallons of fuel), is referred to as JP-8 + 100 (Maurice and others, 2001:752). The "plus 100" additive allows the bulk fuel temperature to increase by 38^{o} C (from 163 to 218^{o} C) without generating harmful fuel system deposits, thereby increasing the thermal stability of the fuel. The Air Force is converting all fighters, bombers, trainers, and many cargo aircraft to JP-8 + 100.

Microbial Growth in Aviation Fuel Systems

Microbial growth in hydrocarbon fuel systems results from the impossibility of keeping storage facilities sterile and the always-present water from condensation or poor housekeeping procedures (Bailey and May, 1979:871; Chesneau, 2000:8).

Aviation fuel is sterile when it is first produced due to the high refinery processing temperatures (Bacha, 2000:9; ASTM, 1999:4). However, shortly thereafter, it is contaminated with microorganisms present in the environment. Microorganisms found in aviation fuel include bacteria, fungi, mold and yeast (Bacha, 2000:9; Finefrock and London, 1966:1).

Since microorganisms need water to reproduce, microbial growth is usually concentrated at the fuel-water interface. Some organisms are aerobic and thus require oxygen. Others are anaerobic and grow in the absence of oxygen.

The best approach to control microbial contamination is prevention through good housekeeping procedures. Keeping the amount of water in the fuel tank as low as possible is the most critical step (Bacha, 2000:9; Finefrock and London, 1966:5).

Common Types of Microorganisms Found in Fuel Systems

There is consistency among studies from the 1950's to the late 1990's which show that, although many types of microorganisms have been discovered in fuel systems, only a few have been found to have the ability to survive and multiply in tank bottoms and other water associated with aviation fuel (Bakanauskas, 1958:15; Ferrari and others, 1998; 106; Gaylarde, 1999:5; de Schiapparelli and de Meybaum, 1980:47). Table 2 provides a listing of common microorganisms shown to thrive in a jet fuel environment.

Table 2. Common Microorganisms That Thrive in a Jet Fuel Environment

Bacteria	Fungi	Yeast
Bacillus	*Hormoconis resinae*	*Candida*
Micrococcus	*Aspergillus*	
Pseudomonas	*Fusarium*	
Arthrobacter	*Penicillum*	

(Derived from Bakanauskas, 1958:15; Ferrari and others, 1998:106; Gaylarde, 1999:5; de Schiapparelli and de Meybaum, 1980:47)

Consequences of Microbial Growth in Fuel Systems

A host of problems will likely surface when uncontrolled microbial growth is allowed to develop (Table 3). Microbial activity has been shown to cause degradation of fuel hydrocarbons (Pardede and Batts, 1996:1132). Flight safety also will likely be compromised, as well as increased maintenance and cost. Not all microorganisms, however, cause the same problems.

Table 3. Consequences of Microorganisms in Military Aviation Fuel Systems

Problem	Principal Types of Microorganisms
Blockage of pipes, valves, filters	Fungi; polymer-producing bacteria
Increased water content	All
Sludge formation	All
Surfactant production	Fungi and aerobic bacteria
Corrosion of storage tanks and lines	Fungi and anaerobic bacteria
Production of suspended solids in the fuel	All
Breakdown of hydrocarbons	Fungi and aerobic bacteria
Shortened filter life	All
Fouling of injectors	Aerobic bacteria and fungi
Increased sulfur content of fuel	Sulfur-reducing bacteria (SRB)
Shortened life of engine parts	Undetermined
Penetration of protective tank linings	Fungi
Health problems	Endotoxin-producing bacteria, SRB

(Derived from Gaylarde, 1999:3)

Fuel Distribution System

Aviation fuel produced at a refinery is tested to ensure that it meets or exceeds all applicable specifications. From the refinery, it must be moved to the base or airport and then, ultimately, into the aircraft fuel cells. These transportation mechanisms may include tankers, pipelines, rail cars, or trucks (Bacha, 2000:72). Usually, the transportation process includes one or more intermediate storage facilities (terminals). When the fuel is transported, regardless of method, it will typically become contaminated with particulate matter and water (Bacha, 2000:73).

The most common type of particulate matter contamination is a solid corrosion product, commonly referred to as rust or scale. Because steel is extremely common in fuel transportation and storage equipment and because some water is always present, almost any distribution process will result in some rust contamination.

Water is introduced to the fuel mostly from condensation. Because most pipelines are buried, batch shipments tend to be cooled during transmission (Bacha, 2000:75). This cooling will cause droplets of water to form in the fuel if the fuel was close to being saturated with water when it was placed into the pipe. Even if the fuel was not close to saturation when placed into the pipe, it may pick up water deposited in low spots in the pipeline by the batch shipments of other fuel products.

Since that decade of discovery, the 1950's, the focus of both research and field application has been on controlling the contamination rather than eliminating it. In a perfect world, fuel would arrive at the aircraft from the refinery with zero microbes, water, or contamination, and then remain "clean" while being stored and consumed in the aircraft. However, such a distribution and storage system is impractical (Bacha, 2000:9).

Microbially Influenced Corrosion (MIC)

Microbially influenced corrosion was first discovered and reported around the turn of the last century. However, it was not studied seriously until the 1960's and 70's when the scientific community debated the theory of anaerobic corrosion of iron by sulfate reducing bacteria (Videla, 2001:176). Microbially influenced corrosion is a process that occurs directly or indirectly as a result of the metabolic activity of microorganisms. A connection between the presence of microorganisms and corrosion is somewhat obvious, but not totally understood.

Although the scientific community still does not fully comprehend the mechanisms of microbially influenced corrosion, significant improvements have been made in the technology, allowing scientists to move closer to an understanding of MIC. The advancement of numerous microelectrodes that allow the detailed mapping at fairly high resolution (tens of microns) is providing significant insights into biofilms and MIC (Angell, 1999:271).

General Characteristics of Inorganic Corrosion

Generally, a metal surface remains stable for an indefinite period of time, provided that it does not contact moisture. Corrosion occurs during the natural tendency of the elemental metals (except noble metals) to revert to a combined form. Metals become unstable when contacting moisture, this instability results from charge-transfer reactions at the interface between the metal and the aqueous environment (Videla, 2001:177).

Corrosion is an electrochemical reaction caused by a flow of electrons from one metal to another (or any other electron sink). For corrosion to occur, the reaction needs an aqueous environment (electrolyte) to conduct the electrical flow. Essentially, inorganic corrosion is accomplished at the interface between the metal and the electrolyte.

General Characteristics of Microbially Influenced Corrosion

The electrochemical characteristics of corrosion remain valid for MIC. However, instead of analyzing only metal and electrolyte reactions, we must analyze reactions of the metal, the electrolyte, and microorganisms. Because of the synergistic effect of microorganisms, such as high growth rate and high surface to volume ratio, microbial action can be a major contributor to the corrosion process (Videla, 2001:177). Figure 2 depicts a simplified scheme of microbially influenced corrosion beneath a bacterial colony. The electrochemical process is similar to that of simple inorganic corrosion.

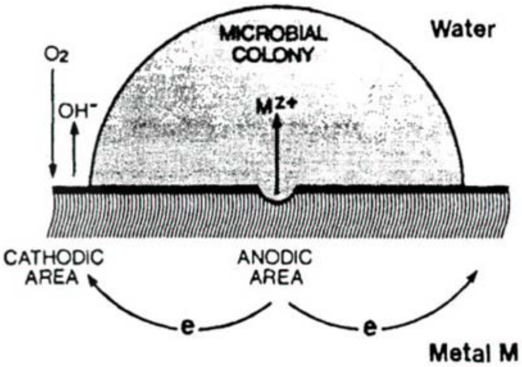


Figure 2. Simplified Scheme of MIC Beneath a Bacterial Colony
(Videla, 2001:178)

A single microorganism is rarely responsible for the microbial effects of corrosion (Videla, 2001:179). Normally, several types of microorganisms influence the corrosion process by operating together or consecutively.

Gas Chromatography and Microbial Identification

Historical methods for the identification (and classification) of microorganisms have been based on their biological, morphological, serological, and toxigenic characteristics (Gharaibeh and Voorhees, 1996:2805). These historical methods usually required whole workable organisms and several tests requiring the incubation of the microorganisms. The limitations of these methods have led to the development of "analytical microbiology" (Fox and others, 1990:63). Analytical microbiology refers to analytical methods using instruments to be applied to the identification of microorganisms. With this logic, microbial identification is based on determining the chemical make-up of fractions of the microorganism, such as the profiling of lipids using gas chromatography (GC) techniques.

Gas chromatography separates chemical components by a combination of three processes: partition chromatography, adsorption chromatography, and volatility of components in the gaseous state. The sample is passed through a coiled column of glass fused silica oxide located inside an oven. The sample in the column is heated until the components vaporize. Because the molecules possess characteristic size, similarity with the stationary phase, and boiling point, chemical compounds are carried through the column at different times. The period between a compound's injection and their detection by the sensor is called retention time (RT). Retention time is a unique characteristic for each compound. Hence, comparing sample RT with standard RT, the compounds are identified.

The most common approach in the classification of bacteria through lipid profiling is the analysis of their fatty acid methyl esters (FAME) (Gharaibeh and

Voorhees, 1996:2805). Commercial instruments, like the one used in this study, have been brought to market, which correlate the fatty acid composition to bacterial type (MIS, 2001:1). The Microbial Identification, Inc. (MIDI) techniques use conventional saponification of the bacterial cells and derivatization (methylation) of the fatty acids, followed by gas chromatography analysis of the fatty acid methyl esters.

The Microbial Identification System provides standard GC FAME distributions in five database types for over 2000 bacteria and has achieved classification to the subspecies level (MIS, 2001:3; Gharaibeh and Voorhees, 1996:2806). The MIDI Research and Development Laboratory has also found more than 300 fatty acids and related compounds that can be used for microbial identification (MIDI, Inc. Technical Note, 2001:1).

Gas chromatography techniques have been successfully applied to identification of jet fuel and its contaminates. Johnson and Synovec used the GC methodology coupled with pattern recognition software to determine the classification of fuel type (Johnson and Synovec, 2002:225). The authors demonstrated that gas chromatography was a useful tool for distinguishing between JP-5, JP-7, JP-8, and JP-TS. Not only did the GC assist in identifying neat samples of each, but also in various combinations of mixtures. Gas chromatography has also been helpful in identifying jet fuel contaminated with hydraulic fluid in a Boeing 707 in-flight refueler (Spila and others, 1999:331-337).

Unlike the antiquated and error-prone techniques used nearly 50 years ago, new scientific methods and equipment have allowed for advances in the field of microbial contamination identification. For example, GC/MS techniques have proven to be very beneficial in identifying microbial contamination in jet fuel (Pardede and Batts, 1996:1134; Spila and others, 1999:331; Jung and others, 2002:128).

Changing Properties

Properties of jet fuel have changed since the 1960's. No longer is the U.S. military using JP-4, the fuel much research was based upon. Modern fuels such as JP-5, JP-8, and JP-8 + 100 all have properties different from those of JP-4.

The military's initial solution to microbial contamination was to continue the use of the FSII, ethylene glycol monomethyl ether (EGME), and not pursue additional anti-microbial additives. That has also changed over the years. The only fuel system icing inhibitor (FSII) currently approved and required for Jet A, Jet A-1, and U.S. military fuels is di-ethylene glycol monomethyl ether (di-EGME) (Department of Defense, 1992:10). However, concerns over di-EGME's potential environmental impact have driven the search for a less toxic, environmentally friendly substitute (Meshako and others, 1999:383-384; Geiss and Frazier, 2001:210; Mattie, 1995:295).

Because the fuel and fuel additives have changed, research is needed to update the baseline from which specifications are made. It is theorized that a change in fuel and fuel additives would cause a difference in microorganism types and quantities. Different constituents, different hydrocarbons, and different additive molecules are now available for microorganisms to metabolize.

Summary

The first purpose of this literature review was to establish the reasons for concern over microbial contamination of fuel. No first-rate military can tolerate unacceptable losses due to clogged fuel filters, erroneous fuel quantity indications, or accelerated corrosion within fuel systems. Although not every microbial contamination event ends in the catastrophic loss of the aircraft and crew, microbial contamination is a serious hazard to military aviation.

The second topic of concern was an evaluation of the findings of the military's pioneering research of the 1950's and 60's for use with today's fuels and additives. The evolution of jet fuel, from JP-4 to JP-8, and the ongoing search for more environmentally friendly anti-icing agents, has made the original research of nearly a half-century ago no longer applicable to today's fuel and fuel systems.

The third and final topic of this literature review concerns the methods of microbial identification. Identification of jet fuel contaminants has progressed markedly over the last several decades. The literature suggests that traditional laboratory techniques, such as selective growth media, are limited. Traditional techniques are slow and imprecise compared to today's new technology (Fox and others, 1990:63). My research seeks to employ the latest technology and up-to-date techniques to properly identify microorganisms. Gas chromatography has been and remains a viable methodology for identifying the microorganisms found in aviation fuel. It has proven itself to be a reliable and practical approach (Pardede and Batts, 1996:1134; Spila and others, 1999:331; Jung and others, 2002:128).

III. Methodology

Experimental Overview

The identification techniques used in this study were based upon the recommendations from the Microbial Identification, Inc (MIDI) training manual entitled *MIS Whole Cell Fatty Acid Analysis by Gas Chromatography* (MIDI, Inc. Training Manual, 2002). The Microbial Identification System (MIS) consists of an Agilent Technologies, Inc., (formerly Hewlett-Packard) 5890 Gas Chromatograph unit coupled to a desktop computer using Agilent ChemStation® software as the operating system for the GC (MIS Operating Manual, 2001:2). The Microbial Identification, Inc. software, Sherlock®, sets the operating parameters of the GC each time a sample is processed (MIDI, Inc. Training Manual, 2002:A-1). The operator enters sample information using the Sherlock® software, which, in turn, interfaces with the ChemStation® software, which sets the GC parameters and controls the injection by the automatic liquid sampler.

The Sherlock® system is a fully automated gas chromatographic system, which identifies bacteria based on their unique fatty acid profiles. The naming is highly objective and reproducible because no subjective tests are required (MIDI, Inc. Training Manual, 2002:F-3).

The MIS uses peak naming and pattern recognition algorithms to identify sample extracts and to provide a "Similarity Index" to known organisms in a database. Currently there are about 1,135 organisms in the three libraries used in this study.

Sample Collection

Number of samples in the study

The number of samples used in this study was determined by the availability of relevant samples. Samples were collected from bases that reported to routinely collect water from their tank sumps and therefore were good candidates for microbial contamination. A maximum of six samples were collected from each of 12 bases, for a total of 40 samples. Three samples were usually collected from each base. Samples were collected from fuel storage tanks, refueling trucks, aircraft, and in the case of an undisclosed overseas air base, a flexible expeditionary fuel bladder.

Geographical location

The geographical locations to collect samples were chosen based on the worst-case scenario; that being the bases with the most suspected microbial problems. Consideration was given to colder-climate bases to include them in the study due to a suspicion that microbial contamination is more prevalent in a cold environment due to more condensation and water being present in fuel tanks.

Samples were taken from Air Force bases located in the Northern, Southern, Eastern, and Western regions of the continental United States, as well as from a Middle Eastern air base. Locations of continental United States bases are depicted in Figure 3.

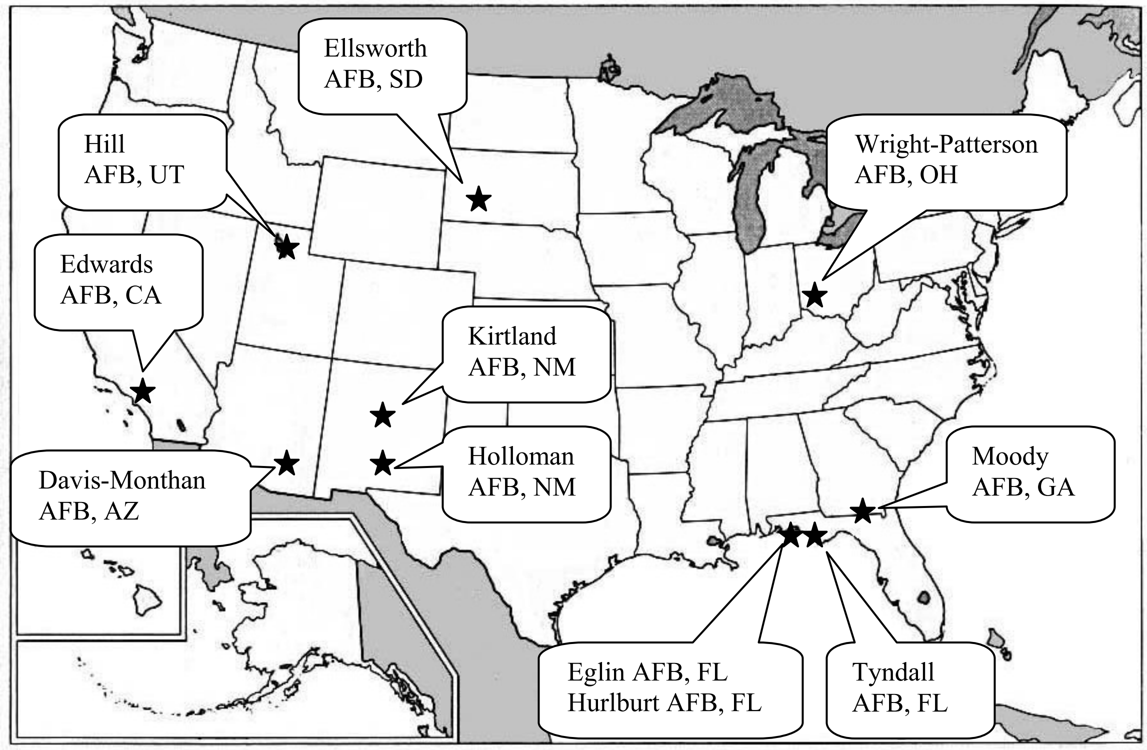


Figure 3. Locations of Samples Collected Within the Continental U.S.

Sampling Quality Assurance

To minimize the variances in sampling techniques, only two individuals collected the samples within the continental United States. Both individuals were intimately familiar with the study and collection standards.

Collection kits

The fuel samples were collected in sterilized one-liter clear glass bottles.

Shipping and handling methods

The sampling container fuel kit, NSN 8115-00-719-4111, was used to transport the samples. The kit is a small steel drum, similar in size to a “pony keg” meeting United

Nations (UN) specification UN1863 for transporting aviation fuel, a flammable hazardous cargo. The one-liter glass bottles were cushioned by foam-type packing material, NSN 8115-00-719-4825.

The sampling dates listed in the worksheets (Appendix A-L) are the dates the samples were drawn from the field. All samples were sent by overnight express to arrive in the lab within 24 hours of shipment (with the exception of Wright-Patterson AFB where samples were transported locally). All samples began laboratory analysis within three days of the field sampling (with the exception of Wright-Patterson AFB storage tank samples that were drawn on different dates when significant amounts of water were present in the daily samples).

Sample Preparation

Sterilization

All equipment and media sterilization was done in the Tuttnaur Brinkmann 3870 autoclave. The standard autoclaving procedure used in this work was 121° C and 15 psi for 15 minutes. No deviations were made to this sterilization protocol during the thesis work.

For steam sterilization and sterility assurance, the B/T Check (distributed by Barnstead/Thermolyne) sterilization chemical monitoring strips were used (Part number AY759X2). The multi-parameter indicator strips gave a visible indication that sterilizing conditions were met.

Prevention of contamination was a priority for this study. All microbial transfer work was accomplished using the NAPCO NapFLOW™ 1200 Microbiological Safety Cabinet. The NapFLOW™ 1200 is a Class II safety cabinet certified to NSF standard 49.

Sample Testing

The samples were tested using the drained water samples and not merely the fuel itself. This is important due to the fact that the majority of the microbial contaminants were expected to be present in this aqueous phase (or close to the fuel/water interface). It has been shown that fuel may contain less than 50 organisms per liter, while at the same time, the associated water may carry greater than 1,000 (Gaylarde, 1999:6).

Dilutions

Fuel/aqueous samples were diluted with sterilized water. A total of four concentrations were used for each type of growth medium: neat, 1:10, 1:100, and 1:1000. The concentrations were obtained by preparing serial dilutions. For example, the 1:10 sample contained 1 mL of jet fuel (aqueous phase) and 9 mL of sterilized water. To prepare the 1:100 sample, 1 mL of the 1:10 sample was diluted in 9 mL of sterilized water. Each mixture was vortexed thoroughly before further dilutions using the Daigger Vortex Genie 2® to homogenize the concentration of microorganisms.

Cultures

From the dilutions described above, 0.1 mL was pipetted onto each of three growth medium types including those favorable for the growth of aerobes, anaerobes, and yeast. For each Standard Library, a standard medium was chosen based on the following reasons (MIS Operating Manual, 2001:8):

1. It will support growth for most of the organisms in the library.
2. It does not contain a significant amount of fatty acids that, if extracted from the medium, would interfere with the analysis.
3. It is commercially available.
4. Most laboratories are familiar with it.

Types and Amounts of Agar Used

Approximately 25 mL of agar was used for each 100 x 15 mm sterile, polystyrene Petri dish (Fisher, Ct # 08-757-12). Agar was between 2.5 and 3.2 mm deep.

Trypticase Soy Broth Agar (TSBA) plates were chosen in this study because it is a standard media for aerobes. The agar was prepared in accordance with MIS Operating Manual recommendations (MIS Operating Manual, 2001:8) by dissolving 30 grams dehydrated BBL® Trypticase Soy Broth (TSB) and 15 grams dehydrated BBL® Granulated Agar (both manufactured by Becton Dickinson Microbiology Systems) to 1 L distilled water. The agar was then sterilized prior to dispensing aseptically into sterile Petri dishes. The agar was allowed to solidify at room temperature.

All plate-grown anaerobes were grown on supplemented brain heart infusion with blood (BHIBLA) plates. Brain Heart Infusion agar is used for cultivating fastidious microorganisms. BHIBLA plates were prepared by combining REMEL Brain Heart Infusion Agar (REMEL part number 452452) with distilled water at a concentration of 52 grams of medium per 1L water. The agar was then sterilized prior to dispensing aseptically into sterile Petri dishes. The agar was allowed to solidify at room temperature. Some BHIBLA plates were also purchased pre-poured from REMEL (part number 01-158).

Yeast cultures were grown on Sabouraud Dextrose Agar (SDA). SDA was prepared by dissolving 65 grams BBL® Sabouraud Dextrose Agar (manufactured by Becton Dickinson Microbiology Systems) in 1 L of distilled water. The agar was then sterilized prior to dispensing aseptically into sterile Petri dishes. The agar was allowed to solidify at room temperature.

Microbial Growth and Incubation

Cultures from samples were grown on Petri dishes using the different types of agar. All fuel/aqueous samples were grown initially for a minimum of 48 hours and a maximum of five days before streaking for purity. Aerobic and fungal microorganisms were grown at 28 +/- 1^{o} C in an Imperial III® incubator (manufactured by Lab-Line Instruments, Inc., model number 305). Anaerobic microorganisms were grown at 35 +/- 1^{o} C in a Quincy Lab, Inc. incubator (model number 10-140) using the BBL® GasPak Pouch Anaerobic System (part number 260651) to create the oxygen-free environment needed.

Following the streaking for isolation of pure cultures, aerobic and fungal microorganisms were grown for an additional 24 +/- 2 hours at 28 +/- 1^{o} C prior to extraction. Anaerobic microorganisms were grown for an additional 48 +/- 2 hours at 35 +/- 1^{o} C also using the GasPak Pouch Anaerobic System. Care was taken to eliminate the possibility of cross contamination. No two samples were handled together at any one time. The work area was cleaned with chlorine bleach solution before and after all work with microbial cultures.

Cells alter the fatty acid composition of their lipids to maintain membrane fluidity as environmental conditions vary (MIDI Inc. Training Manual, 2002:D-1). Hence, it was imperative to control the selection of a culture medium and the time and temperature of incubation prior to comparing fatty acid compositions with the MIS libraries.

Trustworthy identifications require that the same conditions be used for processing unknowns that were used to generate the library. Deviations from the recommended procedures were avoided, as this could alter the fatty acid profiles, resulting in failure to identify organisms or give rise to poor Similarity Indexes.

Gram Staining

The Gram staining technique is one of the most important stain techniques in bacteriology. This technique divided bacteria into two general classes: Gram-positive bacteria (those retaining the primary stain) and Gram-negative bacteria (those losing the primary stain). The Gram stain procedure used here consisted of staining a fixed smear with the primary dye crystal violet. An iodine solution was applied as a mordant. The primary stain was next decolorized with acetone/alcohol and the smear was counterstained with safranin. The difference between the cell wall compositions of two large groups of bacteria provided a basis for this differential stain. One group of bacteria retained the crystal violet-iodine complex and stained blue-purple and classified as Gram-positive. The other group was decolorized and counterstained with safranin and stained red-pink. This group is classified as Gram-negative (Hucker and Conn, 1927:1-37).

Gram staining for this study was conducted in accordance with Fisher Diagnostics Gram Stain Set (Catalog number SG 100D).

Gram-negative bacteria usually contain a combination of straight-chain, unsaturated, hydroxy, and cyclo fatty acids. Gram-positive bacteria usually contain a combination of straight-chain, unsaturated, iso and anteiso fatty acids only. They do not contain meaningful amounts of hydroxy fatty acids (MIDI Inc. Training Manual, 2002:B-5).

Transfer of Microorganisms

A sterilized wire loop was used to transfer the microorganisms during checks for purity, as well as to spread the microorganisms across the agar.

Streaking Plates

The Quadrant Streak pattern was used for culturing cells on plates for identification by Sherlock Microbial Identification System (see Fig 4). The goal of this pattern was to create four distinct densities of cells and to verify culture purity. Quadrant 3 should have cells in the late log phase of growth (MIS Operating Manual, 2001:14).

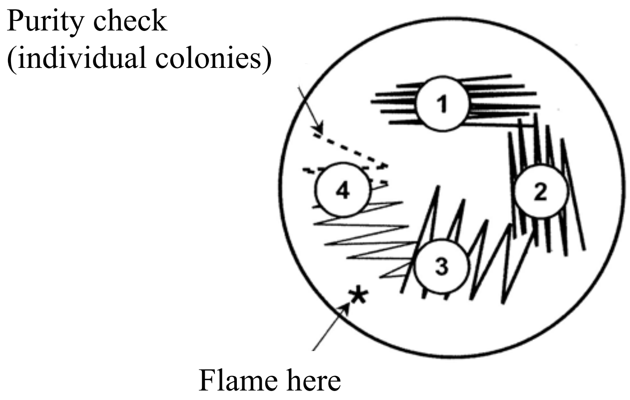


Figure 4. Quadrant Streak Pattern
(MIS Operating Manual, 2001:14).

Colony Counting

The Leica Quebec Darkfield Colony Counter® was used to quantify the number of colonies. The intent of the colony counting was to provide an order of magnitude estimation of the number of microbial colonies present. Results are given in colonies/mL.

Reagent Preparation

Four reagents were needed to saponify the cells, esterify, extract, and wash the fatty acids. Recipes for the four reagents were prepared in accordance with the MIDI Training Manual. A summary of reagent chemicals, quantities, and sources are provided in Table 4.

Table 4. Summary of Reagent Chemicals, Quantities, and Sources

Reagent	Chemical	Amount	Source	Part Number
1	Sodium Hydroxide, ACS grade Methanol, HPLC grade Deionized distilled water	45 grams 150 mL 150 mL	Fisher Fisher	S318-500 A451-4
2	6.00N Hydrochloric acid Methanol, HPLC grade	325 mL 275 mL	Fisher Fisher	LC15370-2 A451-4
3	Hexane, HPLC grade Methyl-tert Butyl Ether (MTBE), HPLC grade	200 mL 200 mL	Fisher Aldrich	H302-1 29321-0
4	Sodium Hydroxide, ACS grade Deionized distilled water	10.8 grams 900 mL	Fisher	S318-500

(Derived from MIDI Inc. Training Manual, 2002:D-7 and Appendix TM-1)

Preparing GC-Ready Extracts

There are five basic steps in the preparation of GC-ready extracts from cell cultures for fatty acid composition analysis. Figure 5 summarizes the extract preparation activities.

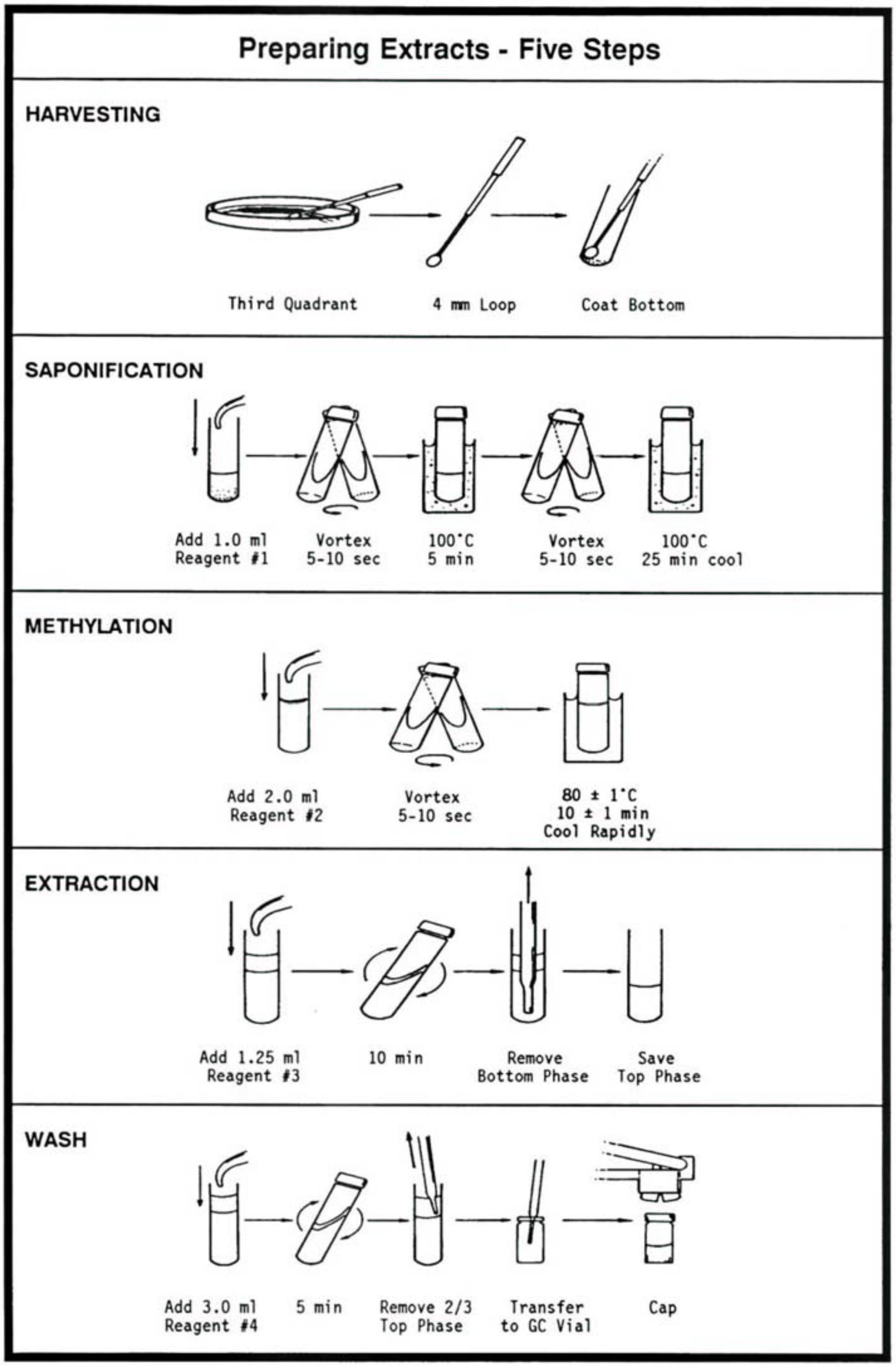


Figure 5. Extract Preparation Activities
(MIDI Inc. Training Manual, 2002:D-13)

Harvesting

Each quadrant in the streak dilutes the microorganisms so that quadrant 4 should contain well-isolated colonies (check for purity). The colonies were harvested from the most dilute quadrant displaying confluent growth (late log phase). This area usually yields the most stable fatty acid compositions. The optimum area for harvesting usually occurs in quadrant 3 (MIDI Inc. Training Manual, 2002:D-14).

Saponification

A strong methanolic base, combined with heat, killed and destroyed the membranes of the cells. Fatty acids were cleaved from the cell lipids and were converted to their sodium salts.

Methylation

Methylation converted the fatty acids (as sodium salts) to fatty acid methyl esters (FAME), which increased the volatility of the fatty acids for the GC analysis.

Extraction

Fatty acid methyl esters were removed from the acidic aqueous phase and transferred to an organic phase with a liquid-liquid extraction procedure. Care was taken working with Reagent #3 due to the flammability of hexane and MTBE (as well as all the fuel samples in the laboratory).

Base Wash

A mild base solution was added to the sample preparation tubes to remove free fatty acids and residual reagents from the organic extract. Residual reagents will damage the chromatographic system, resulting in loss of the hydroxy fatty acid methyl esters.

Quality Control

A reagent (negative) control tube was processed with each batch of samples. Each reagent was added to the tube that was processed with the samples, but no cells were added.

A procedure (positive) control tube was processed with each batch of samples. A known strain, either *Hormoconis resinae* (fungi) or *Stenotrophomonas maltophilia* (bacteria), with a valid library entry in the MIDI database was processed to verify the complete procedure.

Microscope

The instrument used for this work was a Zeiss Axioskop transmitted light microscope. A Zeiss AxioCam microscope digital camera was affixed to the microscope to document images.

Gas Chromatography

The MIS software uses peak naming and pattern recognition algorithms to identify sample extracts processed on the Agilent Technologies, Inc. 5890 gas chromatograph (MIDI, Inc. Training Manual, 2002:A-3) coupled to a 7683 automatic liquid sampler, injector, controller, and 100-vial tray. Care was taken to ensure that the samples were prepared using the same procedure and chromatographic method that was used to construct the identification library (database).

Well-characterized strains of reference cultures from microbiologists specializing in many areas contributed to the development of each library (MIDI Inc. Training Manual, 2002:D-1). Strains were obtained from around the globe to avoid potential geographical bias (MIDI, Inc. Technical Note, 2001:4). Each library entry is a computer-generated composite of the reference strains of each species or subspecies group of organisms. Strain-to-strain and experimental variability has been taken into consideration (MIDI Inc. Training Manual, 2002:D-1). The MIS can identify only those microorganisms for which fatty acid composition profiles of a correctly named reference strain entered into a standard library. Microbial Identification System standard libraries used in this study include TSBA40 Version 4.10 (aerobe), BHIBLA Version 3.8 (anaerobe), and YST28 Version 3.8 (yeast).

Following a sample run of the GC, the software generates a vector of retention times/area percentage pairs. The vector is compared to an internal table called the Peak Naming Table. Each peak is named based on relative retention times.

Following peak naming, the named peak is compared to the feature vectors in the identification library. Statistical pattern recognition techniques are used to identify the most likely matches.

Calibration Standard

Calibration analyses were automatically run in accordance with the operating manual. When a calibration analysis was due, the software checked the results against the Peak Naming Table for a specific number of peaks and a pattern of retention times and area percent amounts.

The standard aerobe, anaerobe, and yeast packages used Calibration Standard 1, which was used for the first two injections of every sequence and was automatically reanalyzed every 11th sample injection (MIDI, Inc. Training Manual, 2002:E-2). The straight-chain C9:0 to C20:0 (9 to 20 carbons in length) fatty acid methyl esters (FAME) were used by the system to calibrate and compensate for peak area discrimination between the low and high boiling point fatty acids. Five hydroxy acids were added to the mixture to detect injection port liner or column degradation, which can result in poor peak shape or a loss of hydroxy acid peak area. The Peak Naming Table for each method contains the expected retention time and the amount for each peak in the calibration analysis (MIDI, Inc. Training Manual, 2002:F-3).

A second function of Calibration Standard 1 was to provide accurate retention times for the straight-chain saturated fatty acid methyl esters C9:0 to C20:0. These retention times are used to calculate the Equivalent Chain Length (ECL) values by which peaks in subsequent analysis are named (see Equation 1). The software calculates how much the calibration analysis has deviated from the expected retention times and reports the Root Mean Square (RMS) fit error. The ECL value for each fatty acid was derived as a function of its retention time in relation to the retention times of a known series of straight-chain fatty acids (MIDI, Inc. Technical Note, 2001:4).

$$ECL_x = \frac{R_{tx} - R_{tn}}{R_{t(n+1)} - R_{tn}} + n \qquad (1)$$

where

R_{tx} is the retention time of x

R_{tn} is the retention time of the saturated FAME preceding x

$R_{t(n+1)}$ is the retention time of the saturated FAME eluding after x

GC Conditions

The fatty acids were analyzed with an Agilent Technologies, Inc. 5890 gas chromatograph equipped with a flame ionization detector (FID). The FID allowed for a large dynamic range and provided good sensitivity (MIDI, Inc. Technical Note, 2001:3). Hydrogen was used as the carrier gas, helium as the "makeup" gas, and air was used to support the flame. The GC conditions used were as follows: injector 285^o C; temperature program at 60^o C for 1.5 min, 60-180^o C at 4^o C per minute, 180-275^o C at 25^o C per minute, 275^o C held for 10 minutes.

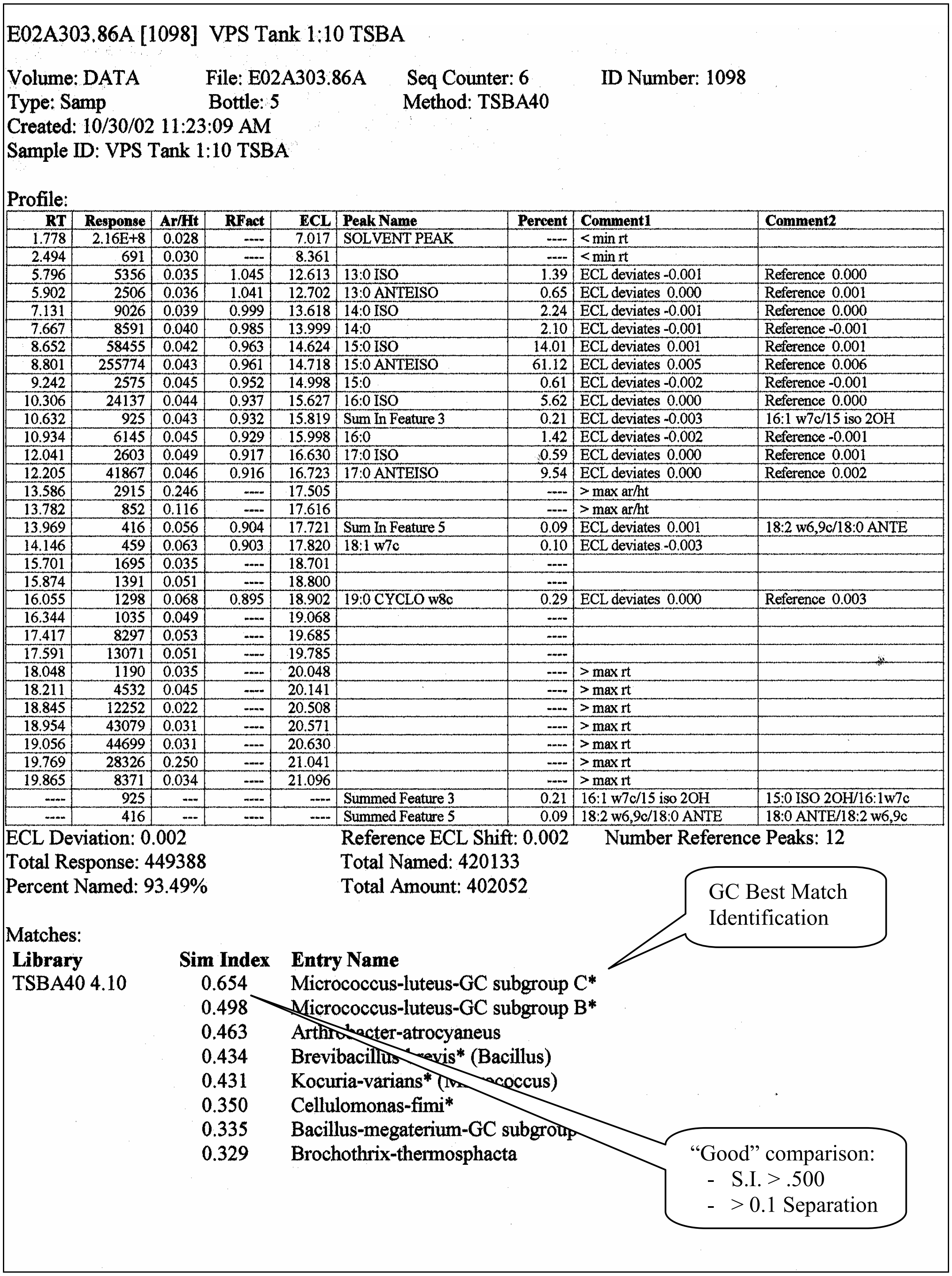
GC Composition Report

At the end of each GC run, the peak retention time, width, and area data are transmitted to the computer software. The data are processed, peaks are named, libraries are searched, and composition reports are printed. Both calibration and sample analyses can have a printed composition report which names the peaks separated by the GC (MIDI, Inc. Training Manual, 2002:F-8).

Each peak from the analysis is listed by retention time (RT), area, and area/height ratio (AR/HT). The composition report also includes the equivalent chain length (ECL), a linear interpolation of each peak's retention between two saturated straight-chain fatty acid methyl ester reference peaks. The MIS software compares the ECL of each peak with the expected ECL of the fatty acids in the Peak Naming Table (MIDI, Inc. Training Manual, 2002:F-8). The fatty acid name (Peak Name) is then determined and printed on the report. A typical GC report is shown in Figure 6.

The percentage of named peaks is listed in the report. After correcting each peak's area by the response factor and summing, the total amount (Total Amount) is listed (MIDI, Inc. Training Manual, 2002:F-8).

A number of reference peaks are used as qualitative internal standards to further adjust the ECL values for more reliable peak naming. The error between the actual ECLs and the expected ECLs (ECL Deviation) is a measure of the system accuracy in naming peaks. The drift from the last calibration (Reference ECL Shift) is a measure of the chromatographic stability. Several of the above performance measures are checked by the system during operation, and warning messages are printed if limits are exceeded (MIDI, Inc. Training Manual, 2002:F-8).



E02A303.86A [1098] VPS Tank 1:10 TSBA

Volume: DATA File: E02A303.86A Seq Counter: 6 ID Number: 1098
Type: Samp Bottle: 5 Method: TSBA40
Created: 10/30/02 11:23:09 AM
Sample ID: VPS Tank 1:10 TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.778	2.16E+8	0.028	----	7.017	SOLVENT PEAK	----	< min rt	
2.494	691	0.030	----	8.361		----	< min rt	
5.796	5356	0.035	1.045	12.613	13:0 ISO	1.39	ECL deviates -0.001	Reference 0.000
5.902	2506	0.036	1.041	12.702	13:0 ANTEISO	0.65	ECL deviates 0.000	Reference 0.001
7.131	9026	0.039	0.999	13.618	14:0 ISO	2.24	ECL deviates -0.001	Reference 0.000
7.667	8591	0.040	0.985	13.999	14:0	2.10	ECL deviates -0.001	Reference -0.001
8.652	58455	0.042	0.963	14.624	15:0 ISO	14.01	ECL deviates 0.001	Reference 0.001
8.801	255774	0.043	0.961	14.718	15:0 ANTEISO	61.12	ECL deviates 0.005	Reference 0.006
9.242	2575	0.045	0.952	14.998	15:0	0.61	ECL deviates -0.002	Reference -0.001
10.306	24137	0.044	0.937	15.627	16:0 ISO	5.62	ECL deviates 0.000	Reference 0.000
10.632	925	0.043	0.932	15.819	Sum In Feature 3	0.21	ECL deviates -0.003	16:1 w7c/15 iso 2OH
10.934	6145	0.045	0.929	15.998	16:0	1.42	ECL deviates -0.002	Reference -0.001
12.041	2603	0.049	0.917	16.630	17:0 ISO	0.59	ECL deviates 0.000	Reference 0.001
12.205	41867	0.046	0.916	16.723	17:0 ANTEISO	9.54	ECL deviates 0.000	Reference 0.002
13.586	2915	0.246	----	17.505		----	> max ar/ht	
13.782	852	0.116	----	17.616		----	> max ar/ht	
13.969	416	0.056	0.904	17.721	Sum In Feature 5	0.09	ECL deviates 0.001	18:2 w6,9c/18:0 ANTE
14.146	459	0.063	0.903	17.820	18:1 w7c	0.10	ECL deviates -0.003	
15.701	1695	0.035	----	18.701		----		
15.874	1391	0.051	----	18.800		----		
16.055	1298	0.068	0.895	18.902	19:0 CYCLO w8c	0.29	ECL deviates 0.000	Reference 0.003
16.344	1035	0.049	----	19.068		----		
17.417	8297	0.053	----	19.685		----		
17.591	13071	0.051	----	19.785		----		
18.048	1190	0.035	----	20.048		----	> max rt	
18.211	4532	0.045	----	20.141		----	> max rt	
18.845	12252	0.022	----	20.508		----	> max rt	
18.954	43079	0.031	----	20.571		----	> max rt	
19.056	44699	0.031	----	20.630		----	> max rt	
19.769	28326	0.250	----	21.041		----	> max rt	
19.865	8371	0.034	----	21.096		----	> max rt	
----	925	---	----	----	Summed Feature 3	0.21	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c
----	416	---	----	----	Summed Feature 5	0.09	18:2 w6,9c/18:0 ANTE	18:0 ANTE/18:2 w6,9c

ECL Deviation: 0.002 Reference ECL Shift: 0.002 Number Reference Peaks: 12
Total Response: 449388 Total Named: 420133
Percent Named: 93.49% Total Amount: 402052

Matches:

Library	Sim Index	Entry Name
TSBA40 4.10	0.654	Micrococcus-luteus-GC subgroup C*
	0.498	Micrococcus-luteus-GC subgroup B*
	0.463	Arthrobacter-atrocyaneus
	0.434	Brevibacillus-brevis* (Bacillus)
	0.431	Kocuria-varians* (Micrococcus)
	0.350	Cellulomonas-fimi*
	0.335	Bacillus-megaterium-GC subgroup
	0.329	Brochothrix-thermosphacta

GC Best Match Identification

“Good” comparison:
- S.I. > .500
- > 0.1 Separation

Figure 6. Typical GC Composition Report

Similarity Index

Similarity Indexes are displayed to the operator to provide the extent to which the sample fatty acid composition compares to the mean fatty acid composition of the strains used to create the library entry listed as its match. The database search presents the best matches and associated similarity indices. The Similarity Index is a software-generated calculation of the distance (in multi-dimensional space) between the profile of the unknown and the mean profile of the closest library entry (MIDI Inc. Training Manual, 2002:F-11). Therefore, the Similarity Index is not a probability or percentage, but a manifestation of the distance from the population mean. An exact match of the unknown fatty acid composition and the mean of the library entry would result in a Similarity Index of 1.000. As each fatty acid varies from the mean percentage, the index will decrease in proportion to the cumulative variance between the composition of the unknown and the library entry (MIDI Inc. Training Manual, 2002:F-11).

The software that computes the Similarity Index assumes that characteristics of species of microorganisms have a Gaussian distribution (classic “bell-shaped curve”). It also assumes that the mean of the population in any series of traits (fatty acid compositions) characterizes the group. According to the Empirical Rule, 68% of the measurements will fall within one standard deviation of the mean, 95% within two standard deviations of the mean, and 99.7% within three standard deviations of the mean (McClave and others, 2001:73). Therefore, nearly all of the correctly identified samples will fall within three standard deviations of the mean.

The Similarity Index can be visualized by examining the Gaussian distribution of the fatty acid composition. As seen in Figure 7, the ideal mean percentage for all fatty acids in a single sample (no variance of any of the fatty acid measurements) is the vertical line at the center. This would equate to a perfect Similarity Index of 1.000. As variance increases, the Similarity Index drops. As seen in Figure 7, a strain with a Similarity Index of 0.600 (or higher) falls three standard deviations from the mean.

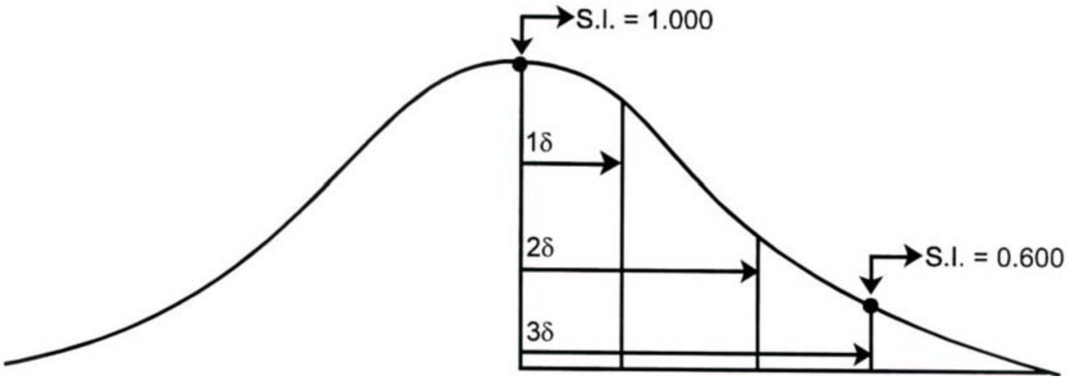


Figure 7. δ Similarity Index
(MIDI Inc. Training Manual, 2002:F-13)

Similarity Index Interpretation

Interpretation of the Similarity Index is in accordance with Microbial Identification System guidelines. Good library comparisons are those strains with a Similarity Index of 0.500 or higher with a separation of at least 0.100 between the first and second choice (Figure 6). Strains may be a good match (but an atypical strain), if the Similarity Index is between 0.300 and 0.500 (more than three standard deviations from the mean) and has a separation of at least 0.100 between the first and second choice. A

Similarity Index of less than 0.300 suggests that the species is not in the database (although the most closely related species will be indicated).

Presentation of Findings

Results of laboratory testing and associated data will be consolidated and translated into a usable form to analyze the extent and identification of microbial contamination (see Appendices). Gas chromatograph identifications will be considered along with partial 16S rRNA gene sequencing and more traditional taxonomy methods to confirm the accuracy of the microbial identification.

Appendices Organization

Appendices will be organized by bases in chronological order in which samples were tested. For example, all relevant sample information for Kirtland Air Force Base will be found in Appendix A. Information for Holloman Air Force Base will be found in Appendix B. Information includes laboratory worksheets containing site information, sample description, light microscopy data, colony description, identification data, gas chromatographic reports (visual plot of the electronic signal generated by the flame ionization detector (FID)), and MIS reports (files containing all peak retention times, widths, and areas).

The focus of the presentation will center on determining whether the study achieved the thesis purpose of determining if significant levels of microorganisms are growing in military aviation fuel systems, and if so, offering insight into any common variables.

IV. Results and Discussion

Overall

Results primarily consist of quantitative and qualitative analysis of the organisms isolated from 40 samples of military aviation fuel from 12 military bases. Results will be presented initially by base, and then compared among all bases in the research. The emphasis of the results and discussion will be placed on information relevant to the first two research objectives presented in Chapter One:

1. To determine the types and quantities of microorganisms, if any, present in U.S. military aviation fuel storage tanks, aircraft refueling trucks, and aircraft fuel tanks; and
2. To determine the characteristic conditions in which microorganisms thrive in U.S. military aviation fuel systems.

In general, microorganisms were found to be present in the fuel systems of all bases sampled. Visible cultures were isolated from at least one sample at each base (Table 5). In all, 123 of 480 serial dilutions led to visible cultures.

Table 5. Summary of Microbially Contaminated Samples

Base	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Total No. Contaminated
Kirtland	—	√	—	N/A	N/A	N/A	1 of 3
Holloman	√	√	√	N/A	N/A	N/A	3 of 3
Eglin	√	√	√	N/A	N/A	N/A	3 of 3
Hurlburt	√	√	√	N/A	N/A	N/A	3 of 3
Tyndall	√	√	√	N/A	N/A	N/A	3 of 3
Ellsworth	√	√	√	N/A	N/A	N/A	3 of 3
Edwards	√	√	√	N/A	N/A	N/A	3 of 3
Middle Eastern	√	√	√	√	N/A	N/A	4 of 4
Davis-Monthan	√	√	√	N/A	N/A	N/A	3 of 3
Hill	√	√	—	N/A	N/A	N/A	2 of 3
Wright-Patterson	√	√	√	√	√	√	6 of 6
Moody	√	—	√	N/A	N/A	N/A	2 of 3

— indicates no visible colonies isolated from sample

Identification of each microorganism was accomplished through the use of gas chromatograph techniques, taxonomy verification, and DNA sequencing, when available. Microorganisms identified with Similarity Indexes of less than .5 should be treated as possible matches (see Chapter 3).

Random samples of GC-identified microorganisms were DNA sequenced by MIDI LABS, Inc. These bacterial identifications were based on 16S rRNA gene sequence similarity. Sequence was performed using Applied Biosystems MicroSeq™ microbial analysis software and database (MIDI, Inc. Report Interpretation Guide, 2002:1). Results are presented with the closest GenBank® match, along with the percent identification, which is a percent identity (essentially the percent similarity) (MIDI, Inc. Alignment Report, 2002:1). GC-identified and DNA sequenced microorganisms used independent databases.

Some of the samples processed by the gas chromatograph had no identification. This “No match found” result is explained by the likelihood that the microorganism isolated is not in the library. Only a few environmental organisms are in current databases.

Appearance of Samples

Appearance of samples varied greatly from base to base. Some samples were visibly clean, clear, and with only trace amounts of water (see Figure 8). Other samples were dark in color (yellow, gray, or brown) with obvious solids (including insects) and significant amounts of water (see Figure 9).

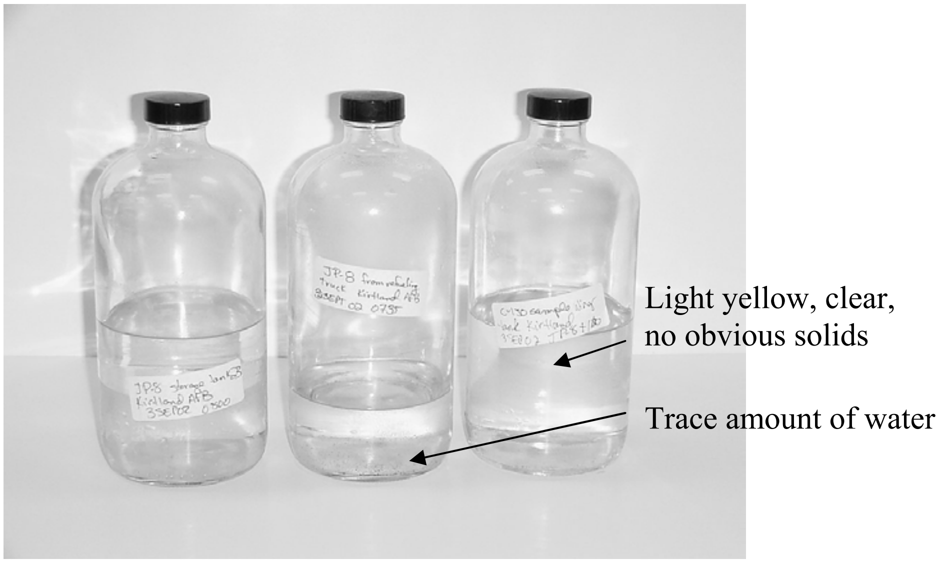


Figure 8. Fuel Samples Taken From Kirtland Air Force Base

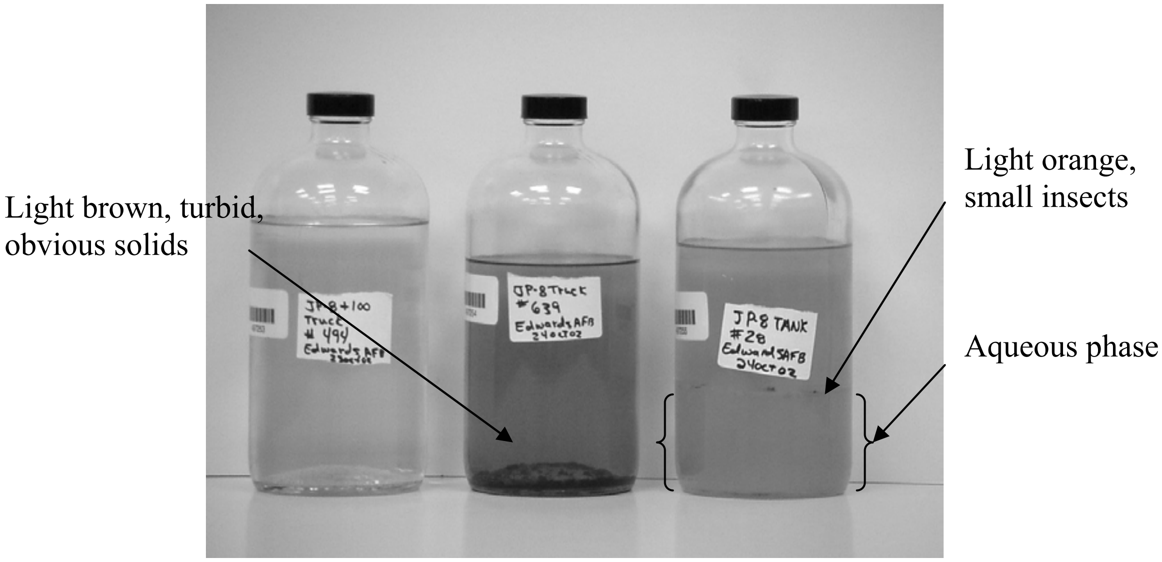


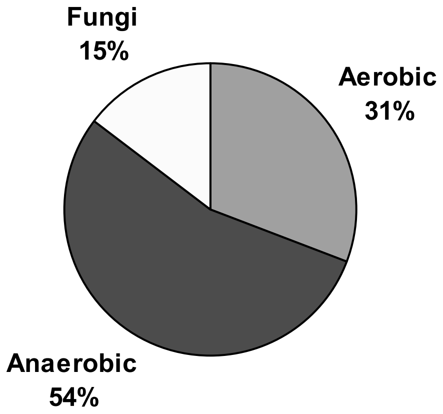
Figure 9. Fuel Samples Taken From Edwards Air Force Base

Sample Degradation

Fuel samples were expeditiously analyzed due to a concern that time may alter the environment (temperature, oxygen content, etc) that the fuel was stored in. There is no reason to suspect sample degradation in this study. Fuel samples stored over three months showed no signs of obvious change (color, clarity, etc).

Types of Organisms

The growth medium used was selected to promote the growth of a wide variety of microorganisms. Since, at a pH near 7, bacteria generally grow faster than fungi, we would expect mostly bacteria on the TSBA plates, although some fungi may also grow. The SDA plates are somewhat lower in pH, giving an advantage to fungi, although some bacteria may also grow. The BHIBLA plates are grown under reduced oxygen, near or at anaerobic conditions, so that only organisms that can grow with little or no oxygen will be found. The results in this chapter are displayed by grouping the organisms into three categories: aerobic bacteria, fungi, and anaerobic bacteria. As shown in Figure 10, most of the microorganisms were anaerobic.



Frequencies

Type	Number
Aerobic	38
Anaerobic	67
Fungi	18
Total	**123**

Figure 10. Summary of Types of Organisms for All Bases

Quantities of Microorganisms

Quantitative results are presented in numerical form in the tables that follow, and by displaying the highest number of colonies per mL for a given sample in the histograms. For example, if a tank sample has 400 colonies per mL of a neat dilution grown on TSBA and 230 colonies per mL of a 1:10 dilution, then 400 colonies per mL will be displayed on that base's histogram as aerobic bacteria.

Serial Dilutions

Several bases provided more than one serial dilution forming visible colonies (Figure 11).

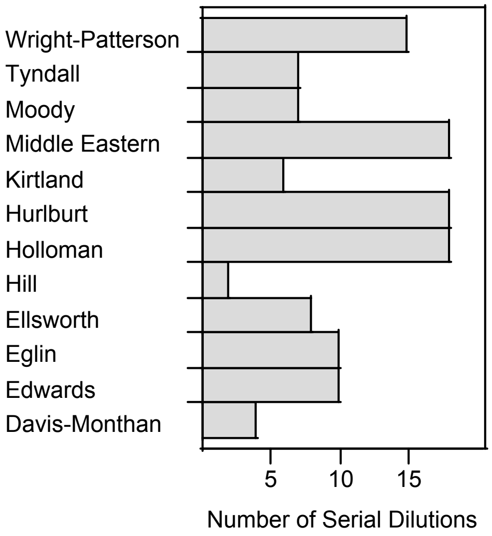
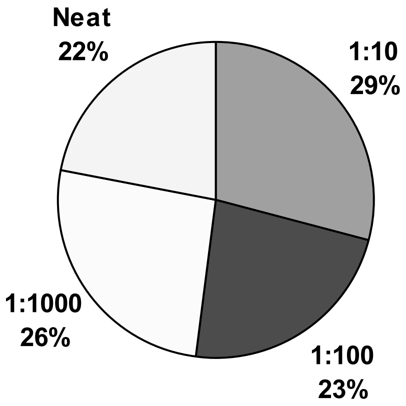


Figure 11. Number of Serial Dilutions per Base Which Produced Visible Colonies

Not all samples resulted in the neat solution supplying the most isolated colonies. In some samples, the most dilute solution provided the most organisms isolated from a given sample. It is theorized that the presence of a toxin may prohibit luxurious microbial growth in the neat and less diluted solutions, yet provide an environment favorable to rapid growth and reproduction in the more diluted solutions. The more dilute solutions also provide the microorganisms with an environment that has less competition for nutrients and oxygen (except anaerobic bacteria). Figure 12 displays a summary of dilution frequencies.



Frequencies

Dilution	Number with Growth
1:10	36
1:100	28
1:1000	32
Neat	27
Total	**123**

Figure 12. Summary of Serial Dilution Frequencies among All Sample Bases

Definition of Significant Contamination

The quantitative results are listed later in this chapter, however, the data need to be put into perspective. Although the presence of any microorganisms in military aviation fuel systems may potentially pose a problem, it is impractical to completely eliminate all presence or growth, or to characterize any amount of microorganisms as significant. Therefore, guidelines need to be established.

There are no agreed or regulatory microbiological standards for fuel supplied to aircraft (Echa Microbiology Ltd., 2002: 3). Although commercially available on-site microbiological test kits are available such as MicrobMonitor[2] and FUELSTAT™ (Echa Microbiology Ltd., 2002: 1-5; Conidia Bioscience, 2002: 1) there is no reason to believe their methods, analysis, and conclusions are applicable to the serial dilution and gas chromatograph methods presented in this research.

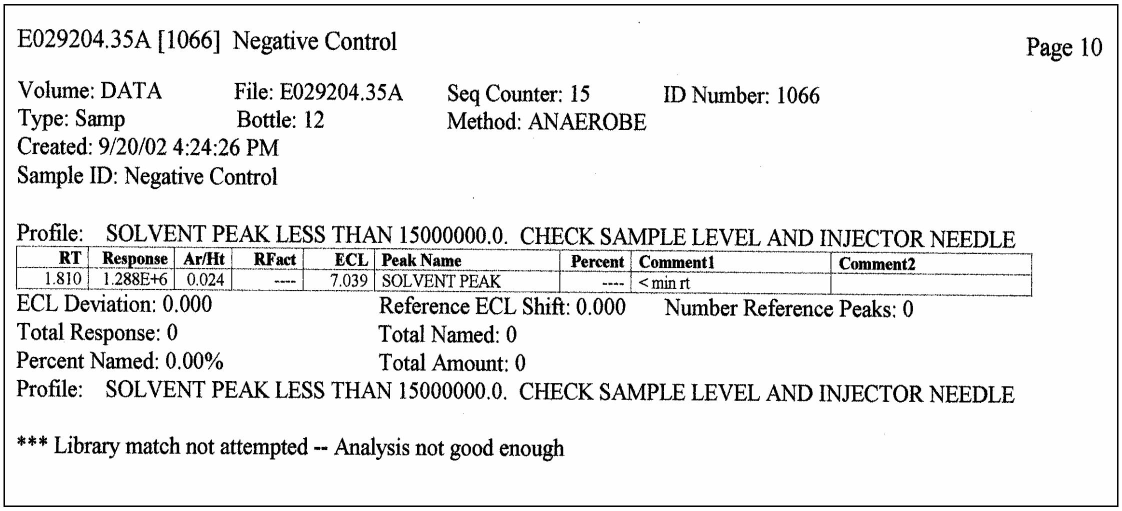
In this research, the levels of microbial contamination are classified into three categories: negligible, moderate, and heavy, based on empirical data. These classifications are shown in Table 6.

Table 6. Classifications of Microbial Contamination

Col/mL	Level of Contamination	Suggested Course of Action
Below 100	Negligible	Continue to monitor program.
100 – 100,000	Moderate	Retest. If confirmed, consider biocide treatment and/or aircraft maintenance.
Above 100,000	Heavy	Retest. If confirmed, consider cleaning and inspecting tank and then biocide treatment and aircraft maintenance.

Observations on Quality Control

The growth, harvesting, and sample preparation proceeded as outlined in the previous chapter. None of the reagent (negative) control samples used for quality control purposes registered any identification of organisms. A typical reagent control GC Composition Report is shown in Figure 13. Note that there are no reference peaks to analyze and that the library match was not attempted.



E029204.35A [1066] Negative Control

Page 10

Volume: DATA File: E029204.35A Seq Counter: 15 ID Number: 1066
Type: Samp Bottle: 12 Method: ANAEROBE
Created: 9/20/02 4:24:26 PM
Sample ID: Negative Control

Profile: SOLVENT PEAK LESS THAN 15000000.0. CHECK SAMPLE LEVEL AND INJECTOR NEEDLE

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.810	1.288E+6	0.024	----	7.039	SOLVENT PEAK	----	< min rt	

ECL Deviation: 0.000 Reference ECL Shift: 0.000 Number Reference Peaks: 0
Total Response: 0 Total Named: 0
Percent Named: 0.00% Total Amount: 0
Profile: SOLVENT PEAK LESS THAN 15000000.0. CHECK SAMPLE LEVEL AND INJECTOR NEEDLE

*** Library match not attempted -- Analysis not good enough

Figure 13. Typical Reagent Control Composition Report

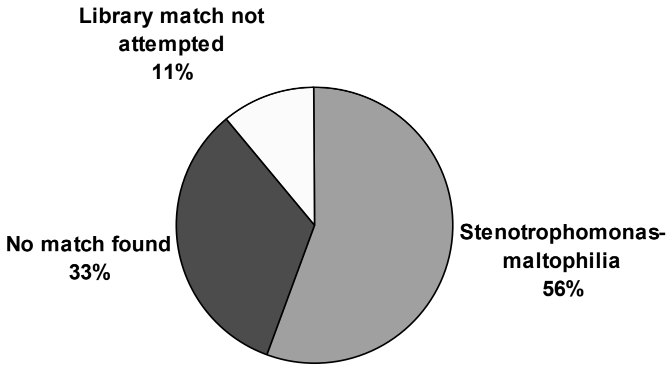
Nine procedure (positive) control samples were used. Although none registered a misidentification of a known strain of *Stenotrophomonas maltophilia*, not all were identified. Five of nine were properly identified as *Stenotrophomonas maltophilia* with Similarity Indexes ranging from 0.271 to 0.808 (Table 7).

Table 7. Summary of Procedure Control Samples

Air Force Base	Similarity Index	> 0 .1 S.I. Separation	GC Best Match
Kirtland	0.769	yes	*Stenotrophomonas maltophilia*
Holloman	0.425	yes	*Stenotrophomonas maltophilia*
Eglin, Hurlburt, Tyndall	0.808	yes	*Stenotrophomonas maltophilia*
Ellsworth			No match found *
Edwards	0.271	yes	*Stenotrophomonas maltophilia*
Undisclosed Overseas			No match found
Davis-Monthan, Hill			Library match not attempted
Wright-Patterson	0.491	yes	*Stenotrophomonas maltophilia*
Moody			No match found

* GC vial cap separated, contents evaporated

The results of the procedure control samples emphasize that GC methods are not entirely predictable. Even under near identical circumstances, over a third of the samples failed to register a proper identification (Figure 14).

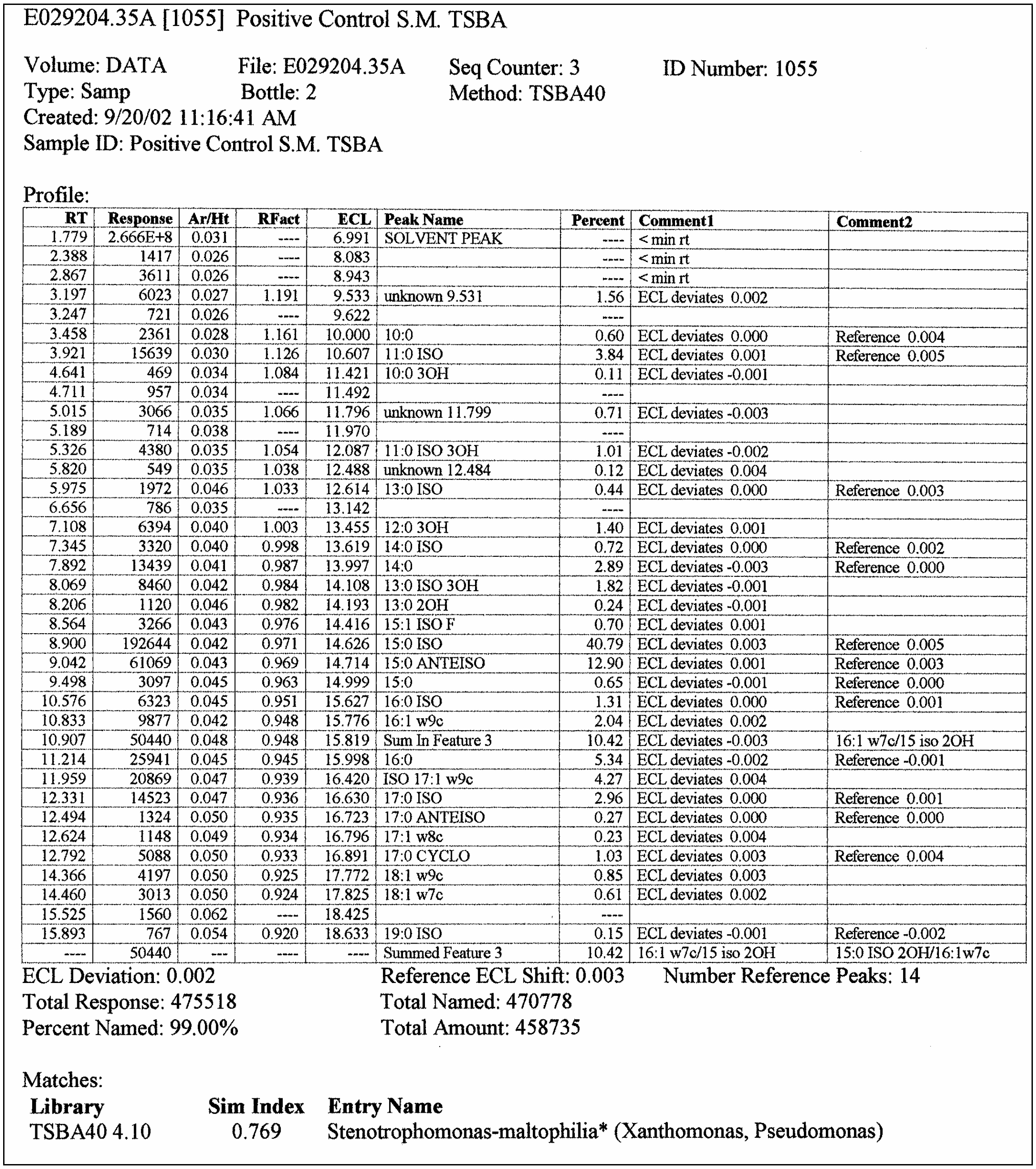


Frequencies

Procedure Control	Number
Stenotrophomonas maltophilia	5
No match found	3
Library match not attempted	1
Total	**9**

Figure 14. Summary of Procedure Control Samples

A typical procedure control GC Composition Report is shown in Figure 15. Note the presence of 14 reference peaks.



E029204.35A [1055] Positive Control S.M. TSBA

Volume: DATA File: E029204.35A Seq Counter: 3 ID Number: 1055
Type: Samp Bottle: 2 Method: TSBA40
Created: 9/20/02 11:16:41 AM
Sample ID: Positive Control S.M. TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.779	2.666E+8	0.031	----	6.991	SOLVENT PEAK	----	< min rt	
2.388	1417	0.026	----	8.083		----	< min rt	
2.867	3611	0.026	----	8.943		----	< min rt	
3.197	6023	0.027	1.191	9.533	unknown 9.531	1.56	ECL deviates 0.002	
3.247	721	0.026	----	9.622		----		
3.458	2361	0.028	1.161	10.000	10:0	0.60	ECL deviates 0.000	Reference 0.004
3.921	15639	0.030	1.126	10.607	11:0 ISO	3.84	ECL deviates 0.001	Reference 0.005
4.641	469	0.034	1.084	11.421	10:0 3OH	0.11	ECL deviates -0.001	
4.711	957	0.034	----	11.492		----		
5.015	3066	0.035	1.066	11.796	unknown 11.799	0.71	ECL deviates -0.003	
5.189	714	0.038	----	11.970		----		
5.326	4380	0.035	1.054	12.087	11:0 ISO 3OH	1.01	ECL deviates -0.002	
5.820	549	0.035	1.038	12.488	unknown 12.484	0.12	ECL deviates 0.004	
5.975	1972	0.046	1.033	12.614	13:0 ISO	0.44	ECL deviates 0.000	Reference 0.003
6.656	786	0.035	----	13.142		----		
7.108	6394	0.040	1.003	13.455	12:0 3OH	1.40	ECL deviates 0.001	
7.345	3320	0.040	0.998	13.619	14:0 ISO	0.72	ECL deviates 0.000	Reference 0.002
7.892	13439	0.041	0.987	13.997	14:0	2.89	ECL deviates -0.003	Reference 0.000
8.069	8460	0.042	0.984	14.108	13:0 ISO 3OH	1.82	ECL deviates -0.001	
8.206	1120	0.046	0.982	14.193	13:0 2OH	0.24	ECL deviates -0.001	
8.564	3266	0.043	0.976	14.416	15:1 ISO F	0.70	ECL deviates 0.001	
8.900	192644	0.042	0.971	14.626	15:0 ISO	40.79	ECL deviates 0.003	Reference 0.005
9.042	61069	0.043	0.969	14.714	15:0 ANTEISO	12.90	ECL deviates 0.001	Reference 0.003
9.498	3097	0.045	0.963	14.999	15:0	0.65	ECL deviates -0.001	Reference 0.000
10.576	6323	0.045	0.951	15.627	16:0 ISO	1.31	ECL deviates 0.000	Reference 0.001
10.833	9877	0.042	0.948	15.776	16:1 w9c	2.04	ECL deviates 0.002	
10.907	50440	0.048	0.948	15.819	Sum In Feature 3	10.42	ECL deviates -0.003	16:1 w7c/15 iso 2OH
11.214	25941	0.045	0.945	15.998	16:0	5.34	ECL deviates -0.002	Reference -0.001
11.959	20869	0.047	0.939	16.420	ISO 17:1 w9c	4.27	ECL deviates 0.004	
12.331	14523	0.047	0.936	16.630	17:0 ISO	2.96	ECL deviates 0.000	Reference 0.001
12.494	1324	0.050	0.935	16.723	17:0 ANTEISO	0.27	ECL deviates 0.000	Reference 0.000
12.624	1148	0.049	0.934	16.796	17:1 w8c	0.23	ECL deviates 0.004	
12.792	5088	0.050	0.933	16.891	17:0 CYCLO	1.03	ECL deviates 0.003	Reference 0.004
14.366	4197	0.050	0.925	17.772	18:1 w9c	0.85	ECL deviates 0.003	
14.460	3013	0.050	0.924	17.825	18:1 w7c	0.61	ECL deviates 0.002	
15.525	1560	0.062	----	18.425		----		
15.893	767	0.054	0.920	18.633	19:0 ISO	0.15	ECL deviates -0.001	Reference -0.002
----	50440	---	----	----	Summed Feature 3	10.42	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c

ECL Deviation: 0.002 Reference ECL Shift: 0.003 Number Reference Peaks: 14
Total Response: 475518 Total Named: 470778
Percent Named: 99.00% Total Amount: 458735

Matches:

Library	Sim Index	Entry Name
TSBA40 4.10	0.769	Stenotrophomonas-maltophilia* (Xanthomonas, Pseudomonas)

Figure 15. Typical Procedure Control GC Composition Report

Kirtland Air Force Base, New Mexico

Three samples were obtained from Kirtland Air Force Base: tank, truck, and aircraft. Microbial colonies were isolated from one of the three samples. The truck sample showed significant growth of microorganisms on all three growth medium (Tables 8 and 9).

The following tables were derived from data presented in Appendix A.

Table 8. Summary of Microbial Identification of Kirtland AFB Samples

Sample	Dilution	Date Sampled	Medium	Fuel Type	Bacteria vs. Fungi	Similarity Index	GC Best Match	DNA % ID	DNA Best Match
Truck	Neat	9/3/2002	TSBA	JP-8	bacteria	0.541	*Paenibacillus apiarius*	99.81	*Bacillus mojavensis*
Truck	1:10	9/3/2002	TSBA	JP-8	bacteria	0.678	*Paenibacillus apiarius*	99	*Bacillus endophyticus*
Truck	Neat	9/3/2002	SDA	JP-8	bacteria		No match found		
Truck	1:10	9/3/2002	SDA	JP-8	bacteria		No match found		
Truck	Neat	9/3/2002	BHIBLA	JP-8	bacteria		No match found		
Truck	1:10	9/3/2002	BHIBLA	JP-8	bacteria		No match found		

Table 9. Quantity of Microorganisms Isolated From Kirtland AFB Samples

Sample	Dilution	Medium	pH	Gram	Organism Shape	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours	Level of Contamination
Truck	Neat	TSBA	6.3	neg	bacillus	23	32	230	Moderate
Truck	1:10	TSBA	6.3	neg	bacillus	4	5	400	Moderate
Truck	Neat	SDA	6.3	neg	bacillus	10	18	100	Moderate
Truck	1:10	SDA	6.3	neg	bacillus	1	2	100	Moderate
Truck	Neat	BHIBLA	6.3	neg	bacillus	23	39	230	Moderate
Truck	1:10	BHIBLA	6.3	neg	bacillus	3	6	300	Moderate

Although no matches were found for the microorganisms isolated on SDA and BHIBLA that does not indicate that no organisms were present. The unidentified organisms were fast growing and plentiful.

As seen in Figure 16, the truck contained the majority of the microorganisms isolated. Aerobic and anaerobic bacteria comprised most of the microbial growth, yet growth of all three types of microorganisms were on the same order of magnitude.

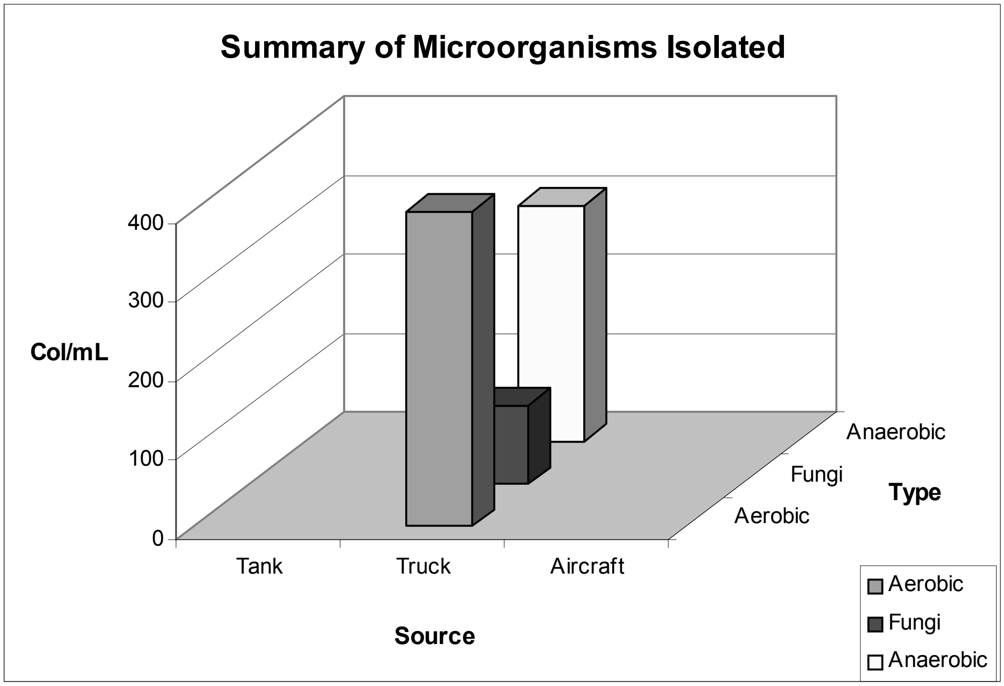


Figure 16. Summary of Microorganism Types Isolated from Kirtland AFB

Holloman Air Force Base, New Mexico

Three samples were obtained from Holloman Air Force Base: tank, truck, and aircraft. Microbial colonies were isolated from all three samples. Each sample showed growth of microorganisms on all three growth medium (see Table 10).

The following tables were derived from data presented in Appendix B.

Table 10. Summary of Microbial Identification of Holloman AFB Samples

Sample	Type / Number	Date Sampled	Dilution	Medium	Fuel Type	Bacteria vs. Fungi	Similarity Index	GC Best Match	DNA % ID	DNA Best Match
Tank	recovery	9/9/2002	Neat	TSBA	JP-8	bacteria	0.016	*Brevundimonas vesicularis*	99	*Sphingomonas sanguinis*
Tank	recovery	9/9/2002	1:1000	TSBA	JP-8	bacteria		No match found		
Tank	recovery	9/9/2002	1:1000	SDA	JP-8	bacteria		No match found		
Tank	recovery	9/9/2002	1:100	BHIBLA	JP-8	bacteria		No match found		
Truck	#375	9/9/2002	1:10	TSBA	JP-8	bacteria	0.144	*Brevundimonas vesicularis*	99.91	*Bacillus licheniformis*
Truck	#375	9/9/2002	1:100	TSBA	JP-8	bacteria		No match found		
Truck	#375	9/9/2002	1:1000	TSBA	JP-8	bacteria		No match found		
Truck	#375	9/9/2002	1:10	SDA	JP-8	bacteria		No match found		
Truck	#375	9/9/2002	1:100	SDA	JP-8	bacteria		No match found		
Truck	#375	9/9/2002	1:1000	SDA	JP-8	bacteria		Library match not attempted		
Truck	#375	9/9/2002	1:10	BHIBLA	JP-8	bacteria		No match found		
Aircraft	F-117	9/9/2002	Neat	TSBA	JP-8	bacteria	0.027	*Brevundimonas vesicularis*	99.91	*Bacillus licheniformis*
Aircraft	F-117	9/9/2002	1:10	TSBA	JP-8	bacteria	0.011	*Brevundimonas vesicularis*	99	*Sphingomonas sanguinis*
Aircraft	F-117	9/9/2002	1:100	TSBA	JP-8	bacteria	0.368	*Cellulomonas flavigena*	99.81	*Bacillus pumilus*
Aircraft	F-117	9/9/2002	1:1000	TSBA	JP-8	bacteria		No match found		
Aircraft	F-117	9/9/2002	1:100	SDA	JP-8	bacteria		No match found		
Aircraft	F-117	9/9/2002	1:1000	SDA	JP-8	bacteria		No match found		
Aircraft	F-117	9/9/2002	1:10	BHIBLA	JP-8	bacteria		No match found		

As seen in Table 11, the microorganisms isolated were slow growing and took five days to grow before any visible colonies were seen. After streaking for purity, the organisms were harvested at 24 (aerobes and fungi) and 48 hour (anaerobes) intervals, as per the methodology described in Chapter 3.

Table 11. Quantity of Microorganisms Isolated From Holloman AFB Samples

Sample	Dilution	Medium	pH	Gram	Organism Shape	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Colonies/mL 24 hours	Level of Contamination
Tank	Neat	TSBA		neg	bacillus	< 1	< 1	5 days to grow	Negligible
Tank	1:1000	TSBA		neg	coccus	< 1	< 1	5 days to grow	Negligible
Tank	1:1000	SDA		neg	coccus	< 1	< 1	5 days to grow	Negligible
Tank	1:100	BHIBLA		neg	coccus	< 1	< 1	5 days to grow	Negligible
Truck	1:10	TSBA	6.3	neg	bacillus	< 1	< 1	5 days to grow	Negligible
Truck	1:100	TSBA	6.3	neg	bacillus	< 1	< 1	5 days to grow	Negligible
Truck	1:1000	TSBA	6.3	neg	coccus	< 1	< 1	5 days to grow	Negligible
Truck	1:10	SDA	6.3	neg	bacillus	< 1	< 1	5 days to grow	Negligible
Truck	1:100	SDA	6.3	neg	bacillus	< 1	< 1	5 days to grow	Negligible
Truck	1:1000	SDA	6.3	neg	coccus	< 1	< 1	5 days to grow	Negligible
Truck	1:10	BHIBLA	6.3	neg	coccus	< 1	< 1	5 days to grow	Negligible
Aircraft	Neat	TSBA		neg	bacillus	< 1	< 1	5 days to grow	Negligible
Aircraft	1:10	TSBA		neg	bacillus	< 1	< 1	5 days to grow	Negligible
Aircraft	1:100	TSBA		neg	bacillus	< 1	< 1	5 days to grow	Negligible
Aircraft	1:1000	TSBA		neg	coccus	< 1	< 1	5 days to grow	Negligible
Aircraft	1:100	SDA		neg	coccus	< 1	< 1	5 days to grow	Negligible
Aircraft	1:1000	SDA		neg	coccus	< 1	< 1	5 days to grow	Negligible
Aircraft	1:10	BHIBLA		neg	coccus	< 1	< 1	5 days to grow	Negligible

As seen in Figure 17, microbial growth was consistent among the three types of medium. All microorganisms were slow to grow initially with less than 1 visible colony at the end of the 24-hour period.

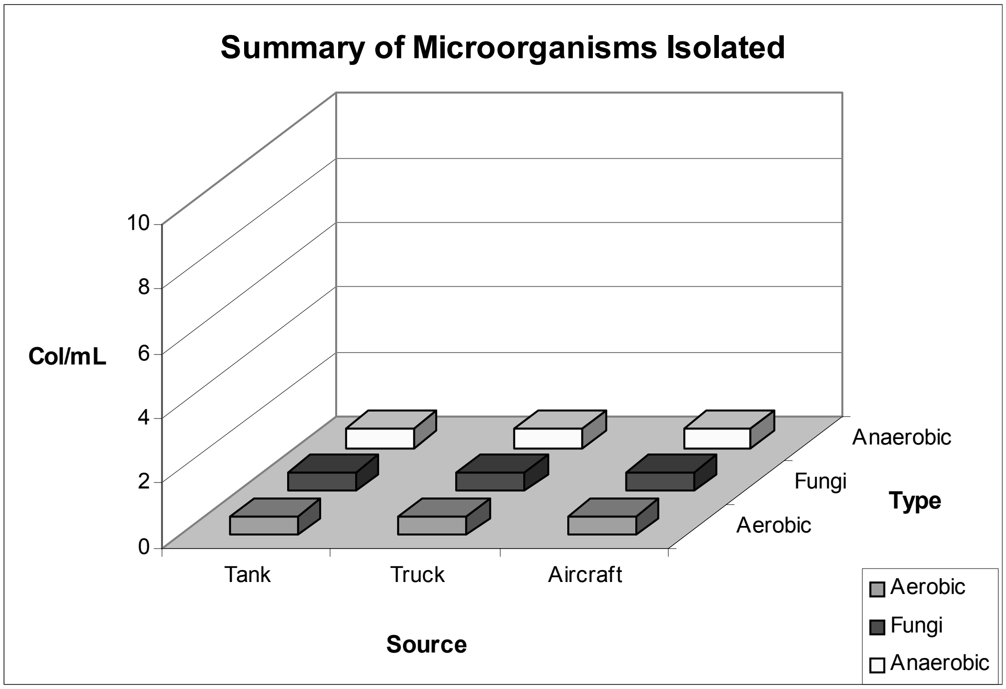


Figure 17. Summary of Microorganism Types Isolated from Holloman AFB

Eglin Air Force Base, Florida

Three samples were obtained from Eglin Air Force Base: tank, truck, and aircraft. Microbial colonies were isolated from all three samples (Table 12), with the most microbial growth in the form of anaerobic bacteria (Table 13).

Unusual circumstances were present for the Eglin fuel sample collection. Samples were collected within two days of the nearby passing of Hurricane Isidore. Base weather officials noted that with the hurricane, monthly rainfall measurements were approximately 2-3 times their normal values.

The following tables were derived from data presented in Appendix C.

Table 12. Summary of Microbial Identification of Eglin AFB Samples

Sample	Type / Number	Date Sampled	Dilution	Medium	Fuel Type	Bacteria vs. Fungi	Similarity Index	GC Best Match
Tank	29	9/30/2002	1:10	TSBA	JP-8	bacteria	0.654	*Micrococcus luteus*
Tank	29	9/30/2002	Neat	BHIBLA	JP-8	fungi		No match found
Tank	29	9/30/2002	1:10	BHIBLA	JP-8	bacteria		No match found
Tank	29	9/30/2002	1:100	BHIBLA	JP-8	bacteria		Library match not attempted
Truck	89L545	9/30/2002	1:10	TSBA	JP-8	fungi	0.018	*Actinomadura yumaensis*
Truck	89L545	9/30/2002	1:10	SDA	JP-8	fungi		No match found
Truck	89L545	9/30/2002	1:100	BHIBLA	JP-8	bacteria		No match found
Truck	89L545	9/30/2002	1:1000	BHIBLA	JP-8	bacteria		No match found
Aircraft	F-15	9/30/2002	Neat	BHIBLA	JP-8 +100	bacteria		No match found
Aircraft	F-15	9/30/2002	1:100	BHIBLA	JP-8 +100	bacteria		No match found

Table 13. Quantity of Microorganisms Isolated From Eglin AFB Samples

Sample	Dilution	Medium	pH	Gram	Organism Shape	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours	Level of Contamination
Tank	1:10	TSBA		neg	coccus	24	150	2,400	Moderate
Tank	Neat	BHIBLA			varied	12	30	120	Moderate
Tank	1:10	BHIBLA		neg	bacillus	50	190	5,000	Moderate
Tank	1:100	BHIBLA		neg	bacillus	910	3,000	910,000	Heavy
Truck	1:10	TSBA	7.2		varied	< 1	< 1	5 days to grow	Negligible
Truck	1:10	SDA	7.2		varied	29	185	2,900	Moderate
Truck	1:100	BHIBLA	7.2	neg	bacillus	< 1	< 1	5 days to grow	Negligible
Truck	1:1000	BHIBLA	7.2	neg	bacillus	< 1	< 1	5 days to grow	Negligible
Aircraft	Neat	BHIBLA		neg	bacillus	< 1	< 1	5 days to grow	Negligible
Aircraft	1:100	BHIBLA		neg	bacillus	< 1	< 1	5 days to grow	Negligible

As seen in Figure 18, the overwhelming majority of the microbial growth occurred in the form of anaerobic bacteria isolated from the tank. Although growth was present in all three fuel storage types, they differed by several orders of magnitude.

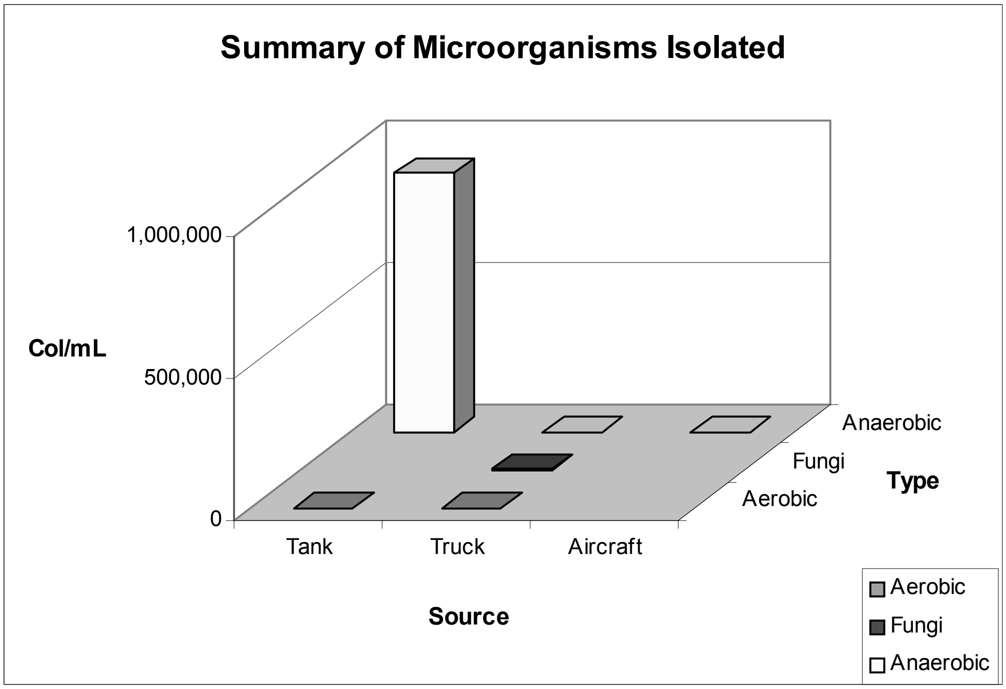


Figure 18. Summary of Microorganism Types Isolated from Eglin AFB

Hurlburt Air Force Base, Florida

Three samples were obtained from Hurlburt Air Force Base: tank, truck, and aircraft. Microbial colonies were isolated from all three samples (Table 14), with the most microbial growth in the form of aerobic bacteria from the tank and aircraft (Table 15 and Figure 19).

As with the Eglin AFB fuel collection, the same unusual circumstances were present for the Hurlburt fuel sample collection. Samples were collected within three days of the nearby passing of Hurricane Isidore. Base weather officials noted that with the hurricane, monthly rainfall measurements were approximately 2-3 times their normal values.

The following tables were derived from data presented in Appendix D.

Table 14. Summary of Microbial Identification of Hurlburt AFB Samples

Sample	Type / Number	Date Sampled	Dilution	Medium	Fuel Type	Bacteria vs. Fungi	Similarity Index	GC Best Match
Tank	TK-2	10/1/2002	1:10	TSBA	JP-8	bacteria	0.238	*Staphylococcus warneri*
Tank	TK-2	10/1/2002	1:100	TSBA	JP-8	bacteria	0.299	*Staphylococcus cohnii cohnii*
Tank	TK-2	10/1/2002	Neat	SDA	JP-8	bacteria		Library match not attempted
Tank	TK-2	10/1/2002	1:100	SDA	JP-8	bacteria		No match found
Tank	TK-2	10/1/2002	1:100	BHIBLA	JP-8	bacteria		No match found
Tank	TK-2	10/1/2002	1:1000	BHIBLA	JP-8	bacteria		No match found
Truck	90L-510	10/1/2002	Neat	TSBA	JP-8	bacteria	0.780	*Bacillus pasteurii*
Truck	90L-510	10/1/2002	1:100	TSBA	JP-8	bacteria	0.292	*Staphylococcus warneri*
Truck	90L-510	10/1/2002	Neat	BHIBLA	JP-8	bacteria		Library match not attempted
Truck	90L-510	10/1/2002	1:10	BHIBLA	JP-8	bacteria		No match found
Truck	90L-510	10/1/2002	1:100	BHIBLA	JP-8	bacteria		Library match not attempted
Truck	90L-510	10/1/2002	1:1000	BHIBLA	JP-8	bacteria		No match found
Aircraft	AC-130	10/1/2002	Neat	TSBA	JP-8	bacteria	0.248	*Staphylococcus warneri*
Aircraft	AC-130	10/1/2002	1:10	TSBA	JP-8	bacteria	0.325	*Staphylococcus cohnii cohnii*
Aircraft	AC-130	10/1/2002	Neat	BHIBLA	JP-8	bacteria		Library match not attempted
Aircraft	AC-130	10/1/2002	1:10	BHIBLA	JP-8	bacteria		No match found
Aircraft	AC-130	10/1/2002	1:100	BHIBLA	JP-8	bacteria		No match found
Aircraft	AC-130	10/1/2002	1:1000	BHIBLA	JP-8	bacteria		No match found

Table 15. Quantity of Microorganisms Isolated From Hurlburt AFB Samples

Sample	Dilution	Medium	pH	Gram	Organism Shape	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours	Level of Contamination
Tank	1:10	TSBA		neg	coccus	7	12	700	Moderate
Tank	1:100	TSBA		neg	coccus	< 1	1	< 1	Negligible
Tank	Neat	SDA		pos	coccus	< 1	1	< 1	Negligible
Tank	1:100	SDA		neg	coccus	< 1	< 1	4 days to grow	Negligible
Tank	1:100	BHIBLA		pos	coccus	< 1	< 1	4 days to grow	Negligible
Tank	1:1000	BHIBLA		neg	coccus	< 1	< 1	4 days to grow	Negligible
Truck	Neat	TSBA	6.3	pos	coccus	1	2	10	Negligible
Truck	1:100	TSBA	6.3	pos	coccus	< 1	< 1	4 days to grow	Negligible
Truck	Neat	BHIBLA	6.3	neg	bacillus	< 1	< 1	4 days to grow	Negligible
Truck	1:10	BHIBLA	6.3	neg	bacillus	< 1	< 1	4 days to grow	Negligible
Truck	1:100	BHIBLA	6.3	neg	bacillus	< 1	< 1	4 days to grow	Negligible
Truck	1:1000	BHIBLA	6.3	neg	bacillus	< 1	< 1	4 days to grow	Negligible
Aircraft	Neat	TSBA	6.9	neg	coccus	< 1	1	4 days to grow	Negligible
Aircraft	1:10	TSBA	6.9	pos	coccus	1	2	100	Moderate
Aircraft	Neat	BHIBLA	6.9	neg	bacillus	< 1	< 1	4 days to grow	Negligible
Aircraft	1:10	BHIBLA	6.9	pos	coccus	< 1	< 1	4 days to grow	Negligible
Aircraft	1:100	BHIBLA	6.9	neg	bacillus	< 1	< 1	4 days to grow	Negligible
Aircraft	1:1000	BHIBLA	6.9	pos	coccus	< 1	< 1	4 days to grow	Negligible

As seen in Figure 19, the overwhelming majority of the microbial growth occurred in the form of aerobic bacteria in the tank and aircraft. Although growth was present in all three fuel storage types, they differed by orders of magnitude.

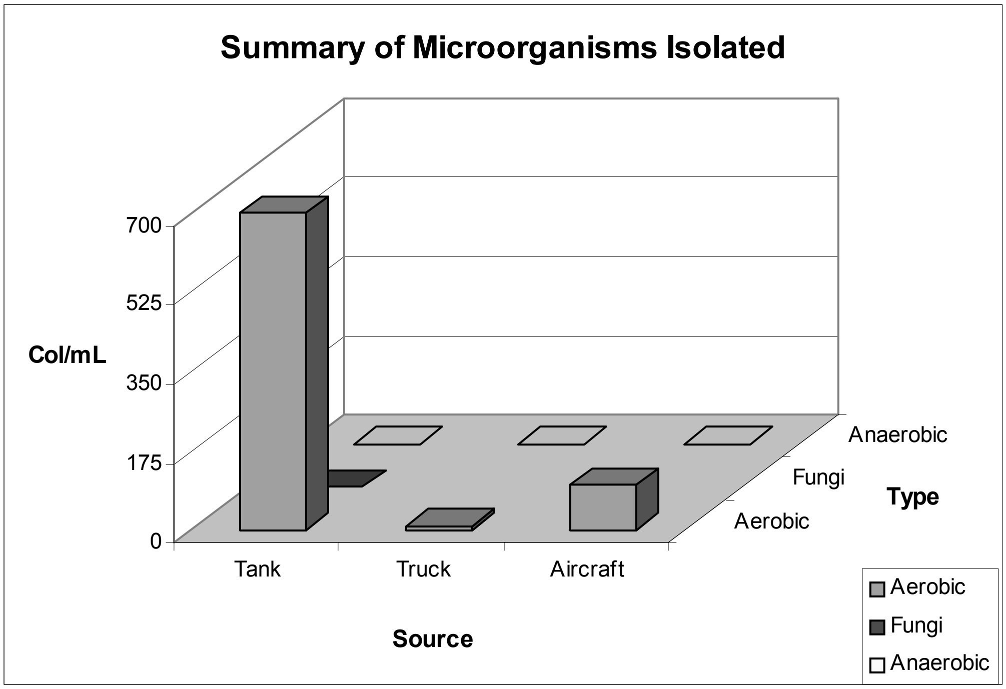


Figure 19. Summary of Microorganism Types Isolated from Hurlburt AFB

Tyndall Air Force Base, Florida

Three samples were obtained from Tyndall Air Force Base: tank, truck, and aircraft. Microbial colonies were isolated from all three samples (Table 16), with the most microbial growth in the form of fungi (Table 17).

Unusual weather circumstances were also present for the Tyndall fuel sample collection. Samples were collected within four days of the nearby passing of Hurricane Isidore. Base weather officials noted that with the hurricane, monthly rainfall measurements were approximately twice their normal values.

The following tables were derived from data presented in Appendix E.

Table 16. Summary of Microbial Identification of Tyndall AFB Samples

Sample	Type / Number	Date Sampled	Dilution	Medium	Fuel Type	Bacteria vs. Fungi	Similarity Index	GC Best Match
Tank	407	10/2/2002	1:1000	BHIBLA	JP-8	bacteria	0.010	*Bacteroides melaninogenicus*
Truck	124	10/2/2002	1:100	TSBA	JP-8 +100	bacteria	0.170	*Staphylococcus aureus*
Truck	124	10/2/2002	1:100	BHIBLA	JP-8 +100	bacteria		No match found
Truck	124	10/2/2002	1:1000	BHIBLA	JP-8 +100	bacteria		No match found
Aircraft	F-15	10/2/2002	1:1000	SDA	JP-8 +100	fungi		No match found
Aircraft	F-15	10/2/2002	1:100	BHIBLA	JP-8 +100	bacteria		Library match not attempted
Aircraft	F-15	10/2/2002	1:1000	BHIBLA	JP-8 +100	bacteria	0.027	*Propionibacterium propionicus (Arachnia propionica)*

Table 17. Quantity of Microorganisms Isolated From Tyndall AFB Samples

Sample	Dilution	Medium	pH	Gram	Organism Shape	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours	Level of Contamination
Tank	1:1000	BHIBLA		neg	coccus	<1	<1	4 days to grow	Negligible
Truck	1:100	TSBA	6.3	neg	coccus	1	2	1,000	Moderate
Truck	1:100	BHIBLA	6.3	neg	coccus	<1	<1	4 days to grow	Negligible
Truck	1:1000	BHIBLA	6.3	neg	coccus	<1	<1	4 days to grow	Negligible
Aircraft	1:1000	SDA			varied	2	7	20,000	Moderate
Aircraft	1:100	BHIBLA		neg	bacillus	<1	<1	4 days to grow	Negligible
Aircraft	1:1000	BHIBLA		neg	coccus	<1	<1	4 days to grow	Negligible

As seen in Figure 20, the overwhelming majority of the microbial growth occurred in the form of fungi in the aircraft fuel tank. Although growth was present in all three fuel storage types, they differed by orders of magnitude.

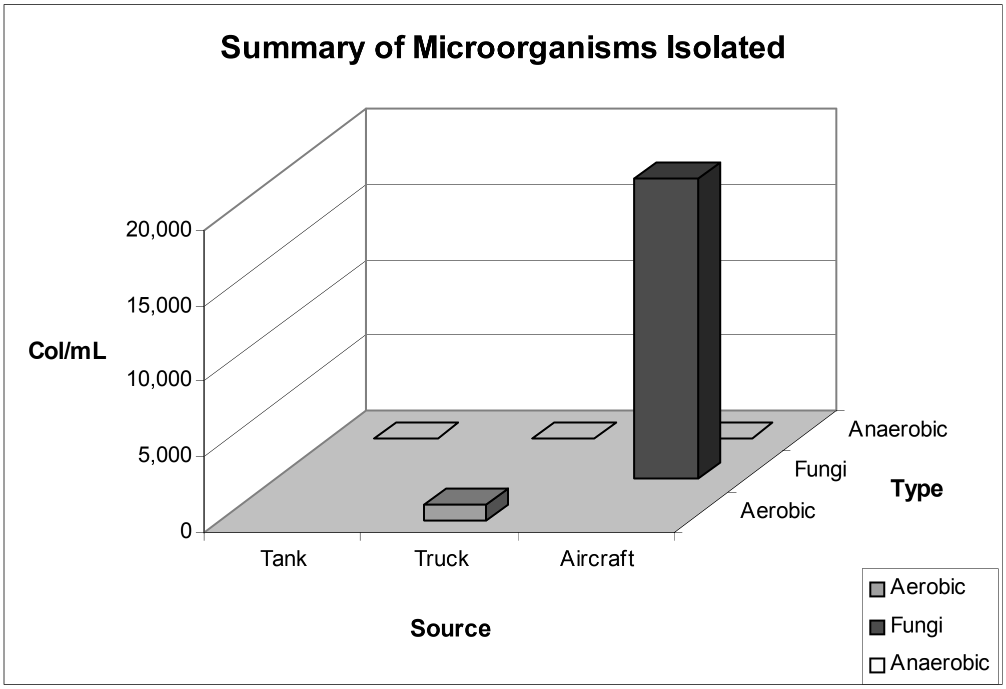


Figure 20. Summary of Microorganism Types Isolated from Tyndall AFB

Ellsworth Air Force Base, South Dakota

Three samples were obtained from Ellsworth Air Force Base: tank, truck, and aircraft. Microbial colonies were isolated from all of the three samples. The tank sample showed growth of microorganisms on more than one medium (Tables 18 and 19).

The following tables were derived from data presented in Appendix F.

Table 18. Summary of Microbial Identification of Ellsworth AFB Samples

Sample	Type / Number	Date Sampled	Dilution	Medium	Fuel Type	Bacteria vs. Fungi	Similarity Index	GC Best Match
Tank	15	10/10/2002	Neat	TSBA	JP-8	bacteria	0.530	*Bacillus megaterium*
Tank	15	10/10/2002	1:100	TSBA	JP-8	bacteria	0.612	*Bacillus licheniformis*
Tank	15	10/10/2002	1:1000	TSBA	JP-8	bacteria	0.426	*Micrococcus luteus*
Tank	15	10/10/2002	1:100	BHIBLA	JP-8	bacteria		No match found
Truck	97L359	10/10/2002	1:10	BHIBLA	JP-8	bacteria		No match found
Aircraft	B-1 #83	10/10/2002	1:10	BHIBLA	JP-8	bacteria		No match found
Aircraft	B-1 #83	10/10/2002	1:100	BHIBLA	JP-8	bacteria		Library match not attempted
Aircraft	B-1 #83	10/10/2002	1:1000	BHIBLA	JP-8	bacteria		No match found

Table 19. Quantity of Microorganisms Isolated From Ellsworth AFB Samples

Sample	Dilution	Medium	pH	Gram	Organism Shape	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours	Level of Contamination
Tank	Neat	TSBA	6.4	neg	bacillus	<1	1	<1	Negligible
Tank	1:100	TSBA	6.4	neg	bacillus	<1	<1	4 days to grow	Negligible
Tank	1:1000	TSBA	6.4	neg	coccus	<1	1	<1	Negligible
Tank	1:100	BHIBLA	6.4	neg	coccus	<1	2,980	<1	Negligible
Truck	1:10	BHIBLA		neg	bacillus	<1	1,750	<1	Negligible
Aircraft	1:10	BHIBLA		neg	bacillus	<1	<1	4 days to grow	Negligible
Aircraft	1:100	BHIBLA		neg	bacillus	<1	1	<1	Negligible
Aircraft	1:1000	BHIBLA		neg	bacillus	<1	<1	4 days to grow	Negligible

As seen in Figure 21, the microorganisms isolated were aerobic and anaerobic bacteria in all the types of storage tanks. All growth was slow and small in number of visible colonies.

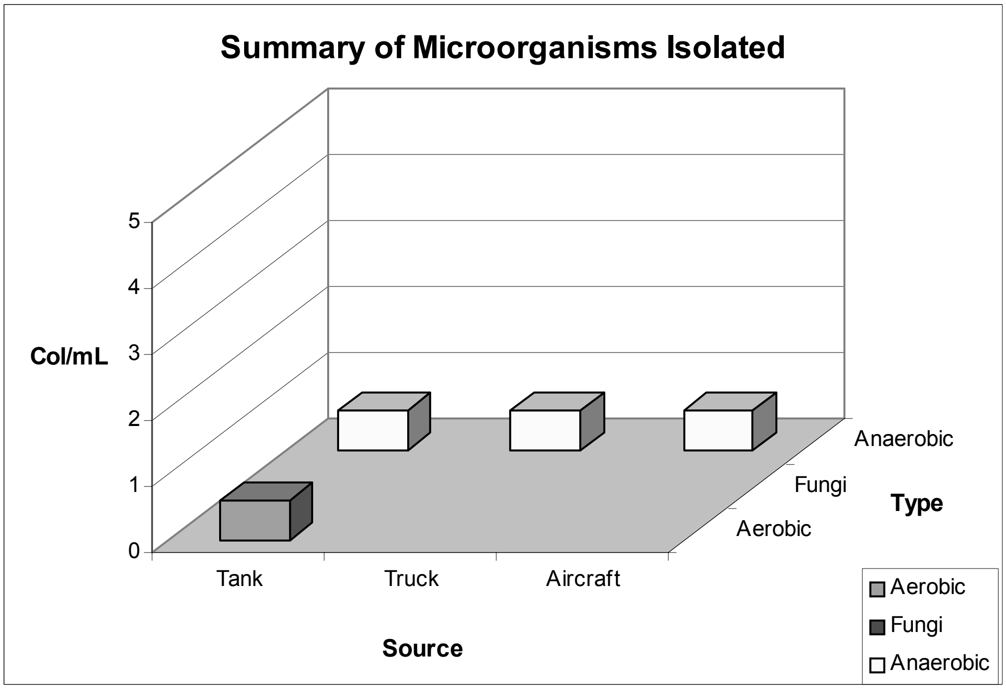


Figure 21. Summary of Microorganism Types Isolated from Ellsworth AFB

Edwards Air Force Base, California

Three samples were obtained from Edwards Air Force Base: tank, truck #1, and truck #2. Microbial colonies were isolated from all three samples (Table 20), with the most microbial growth in the form of aerobic bacteria (Table 21).

The following tables were derived from data presented in Appendix G.

Table 20. Summary of Microbial Identification of Edwards AFB Samples

Sample	Type / Number	Date Sampled	Dilution	Medium	Fuel Type	Bacteria vs. Fungi	Similarity Index	GC Best Match
Tank	28	10/24/2002	1:100	TSBA	JP-8	bacteria	0.588	*Xenorhabdus nematophilus*
Tank	28	10/24/2002	1:1000	TSBA	JP-8	fungi		No match found
Tank	28	10/24/2002	1:10	SDA	JP-8	fungi		No match found
Tank	28	10/24/2002	1:100	SDA	JP-8	fungi		No match found
Tank	28	10/24/2002	1:1000	SDA	JP-8	fungi		No match found
Tank	28	10/24/2002	1:100	BHIBLA	JP-8	fungi		No match found
Truck 1	494	10/23/2002	Neat	TSBA	JP-8 +100	bacteria	0.583	*Xenorhabdus nematophilus*
Truck 1	494	10/23/2002	1:10	BHIBLA	JP-8 +100	fungi		No match found
Truck 2	639	10/24/2002	1:1000	TSBA	JP-8	bacteria	0.675	*Xenorhabdus nematophilus*
Truck 2	639	10/24/2002	1:1000	SDA	JP-8	bacteria		No match found

Table 21. Quantity of Microorganisms Isolated From Edwards AFB Samples

Sample	Dilution	Medium	pH	Gram	Organism Shape	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours	Level of Contamination
Tank	1:100	TSBA	7.8	neg	coccus	112	422	112,000	Heavy
Tank	1:1000	TSBA	7.8		varied	5	11	50,000	Moderate
Tank	1:10	SDA	7.8		varied	1	1	100	Moderate
Tank	1:100	SDA	7.8		varied	149	518	149,000	Heavy
Tank	1:1000	SDA	7.8		varied	1	3	10,000	Moderate
Tank	1:100	BHIBLA	7.8		varied	398	574	398,000	Heavy
Truck 1	Neat	TSBA			varied	1	1	10	Negligible
Truck 1	1:10	BHIBLA			varied	520	880	52,000	Moderate
Truck 2	1:1000	TSBA			varied	89	210	890,000	Heavy
Truck 2	1:1000	SDA		neg	coccus	21	51	210,000	Heavy

As seen in Figure 22, the majority of the microbial growth occurred in the form of aerobic activity in truck #2's fuel tank. Although growth was present in all three fuel storage systems, they differed by orders of magnitude.

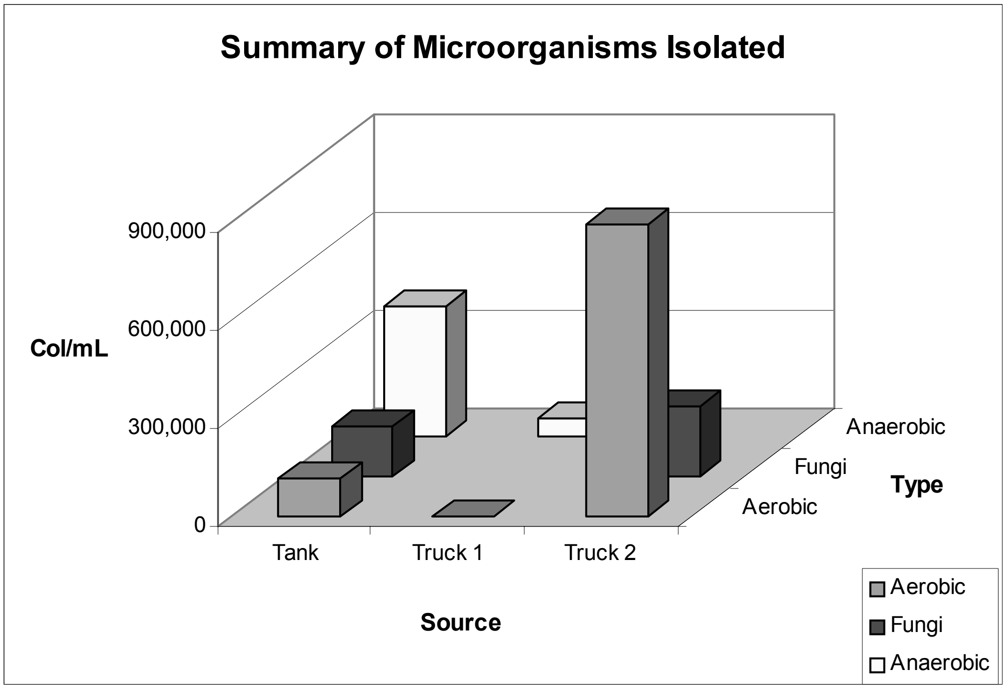


Figure 22. Summary of Microorganism Types Isolated from Edwards AFB

Undisclosed Overseas Base

Four samples were obtained from an undisclosed Middle Eastern air base: bladder, liner, drain, and vent. All samples are from the same expeditionary fuel storage system known as a "bladder".

An explanation of the sampling process is in order. The sample labeled bladder was taken as the bag was dissected. The sample labeled liner was taken once the bladder was removed. A blackened discoloration was observed underneath the lining. The discoloration was determined to be a stain coming from underneath. The underside of the liner was reported to be black and the black stain penetrated to approximately 8” into the sand underneath the expeditionary fuel storage system and had an odor of H_2S. Base Bio-environmental personnel checked for the presence of mercaptan sulfur, and found none. The same personnel then verified the presence of H_2S. Over 50 ppm H_2S was verified from readings taken from the center of the bladder where the fuel was pooled.

General observations were made during the sampling process. The inside of the bladder was in near perfect condition. There was no visible water in the lowest part of the slope. There were no depressions containing small pools of water. There was no evidence that there had been any biological activity. There were no areas of the bladder showing any discoloration (Mudry, 2002:1).

Microbial colonies were isolated from all four samples (Table 22), with the most microbial growth in the form of anaerobic bacteria (Table 23).

The following tables were derived from data presented in Appendix H.

Table 22. Summary of Microbial Identification of Undisclosed Overseas Air Base Samples

Sample	Date Sampled	Dilution	Medium	Fuel Type	Bacteria vs. Fungi	Similarity Index	GC Best Match	DNA % ID	DNA Best Match
Bladder	10/25/2002	1:1000	TSBA	JPTS	fungi	0.746	*Kocuria kristinea (Micrococcus)*		
Bladder	10/25/2002	Neat	BHIBLA	JPTS	bacteria		No match found	99.91	*Bacillus licheniformis*
Bladder	10/25/2002	1:10	BHIBLA	JPTS	fungi		No match found		
Bladder	10/25/2002	1:100	BHIBLA	JPTS	bacteria		No match found		
Bladder	10/25/2002	1:1000	BHIBLA	JPTS	fungi		Library match not attempted		
Liner	10/25/2002	1:1000	TSBA	JPTS	bacteria	0.314	*Staphylococcus xylosus*		
Liner	10/25/2002	Neat	BHIBLA	JPTS	bacteria	0.019	*Prevotella loescheii (Bacteroides loescheii)*		
Liner	10/25/2002	1:10	BHIBLA	JPTS	bacteria		Library match not attempted		
Liner	10/25/2002	1:1000	BHIBLA	JPTS	bacteria		No match found		
Drain	10/25/2002	Neat	TSBA	JPTS	bacteria	0.378	*Bacillus megaterium GC subgroup B*	99.91	*Micrococcus luteus*
Drain	10/25/2002	1:10	TSBA	JPTS	fungi		No match found		
Drain	10/25/2002	Neat	BHIBLA	JPTS	bacteria		No match found	99.91	*Bacillus pumilus*
Drain	10/25/2002	1:10	BHIBLA	JPTS	bacteria		No match found		
Drain	10/25/2002	1:1000	BHIBLA	JPTS	bacteria		No match found		
Vent	10/25/2002	1:10	TSBA	JPTS	bacteria	0.341	*Actinomadura yumaensis (72h)*		
Vent	10/25/2002	Neat	BHIBLA	JPTS	bacteria		No match found	99.91	*Bacillus licheniformis*
Vent	10/25/2002	1:100	BHIBLA	JPTS	fungi		Library match not attempted		
Vent	10/25/2002	1:1000	BHIBLA	JPTS	fungi		No match found		

Table 23. Quantity of Microorganisms Isolated From Undisclosed Overseas Air Base Samples

Sample	Dilution	Medium	pH	Gram	Organism Shape	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours	Level of Contamination
Bladder	1:1000	TSBA			varied	<1	<1	5 days to grow	Negligible
Bladder	Neat	BHIBLA		neg	coccus	<1	<1	5 days to grow	Negligible
Bladder	1:10	BHIBLA			varied	<1	<1	5 days to grow	Negligible
Bladder	1:100	BHIBLA		neg	bacillus	640	2,520	640,000	Heavy
Bladder	1:1000	BHIBLA			varied	710	3,450	7,100,000	Heavy
Liner	1:1000	TSBA		neg	coccus	<1	<1	5 days to grow	Negligible
Liner	Neat	BHIBLA		neg	coccus	<1	<1	5 days to grow	Negligible
Liner	1:10	BHIBLA		neg	coccus	605	1,200	60,500	Moderate
Liner	1:1000	BHIBLA		neg	coccus	<1	<1	5 days to grow	Negligible
Drain	Neat	TSBA		neg	coccus	<1	<1	5 days to grow	Negligible
Drain	1:10	TSBA			varied	<1	<1	5 days to grow	Negligible
Drain	Neat	BHIBLA		neg	bacillus	610	1,200	61,000	Moderate
Drain	1:10	BHIBLA		neg	bacillus	<1	<1	5 days to grow	Negligible
Drain	1:1000	BHIBLA		neg	bacillus	690	3,450	6,900,000	Heavy
Vent	1:10	TSBA		neg	bacillus	<1	<1	5 days to grow	Negligible
Vent	Neat	BHIBLA			varied	210	345	2,100	Moderate
Vent	1:100	BHIBLA			varied	<1	<1	5 days to grow	Negligible
Vent	1:1000	BHIBLA			varied	490	890	4,900,000	Heavy

As seen in Figure 23, the majority of the microbial growth occurred in the form of anaerobic activity in the bladder and vent fuel tanks. Although growth was present in all four fuel storage compartments, they differed by orders of magnitude.

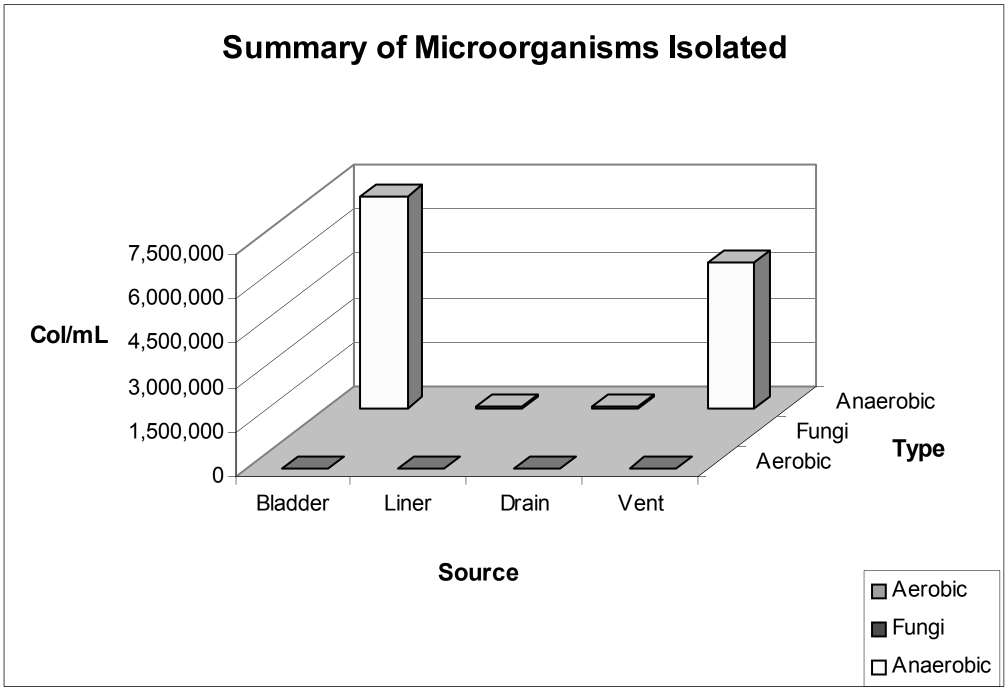


Figure 23. Summary of Microorganism Types Isolated from Undisclosed Overseas Air Base

Davis-Monthan Air Force Base, Arizona

Three samples were obtained from Davis-Monthan Air Force Base: tank, truck, and aircraft. Microbial colonies were isolated from all three samples (Table 24), with the most microbial growth in the form of anaerobic bacteria (Table 25).

The following tables were derived from data presented in Appendix I.

Table 24. Summary of Microbial Identification of Davis-Monthan AFB Samples

Sample	Type / Number	Date Sampled	Dilution	Medium	Fuel Type	Bacteria vs. Fungi	Similarity Index	GC Best Match
Tank	25	11/8/2002	Neat	BHIBLA	JP-8	bacteria		No match found
Truck	570	11/8/2002	1:10	TSBA	JP-8	bacteria		Library match not attempted
Truck	570	11/8/2002	1:1000	BHIBLA	JP-8	bacteria		No match found
Aircraft	A-10	11/8/2002	1:1000	BHIBLA	JP-8	bacteria		No match found

Table 25. Quantity of Microorganisms Isolated From Davis-Monthan AFB Samples

Sample	Dilution	Medium	pH	Gram	Organism Shape	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours	Level of Contamination
Tank	Neat	BHIBLA			varied	<1	<1	5 days to grow	Negligible
Truck	1:10	TSBA	6.6		varied	<1	1	<1	Negligible
Truck	1:1000	BHIBLA	6.6		varied	<1	<1	5 days to grow	Negligible
Aircraft	1:1000	BHIBLA		pos	coccus	1	3	10,000	Moderate

As seen in Figure 24, the majority of the microbial growth occurred in the form of anaerobic activity in the aircraft fuel tank. Although growth was present in all three fuel storage tanks, they differed by orders of magnitude.

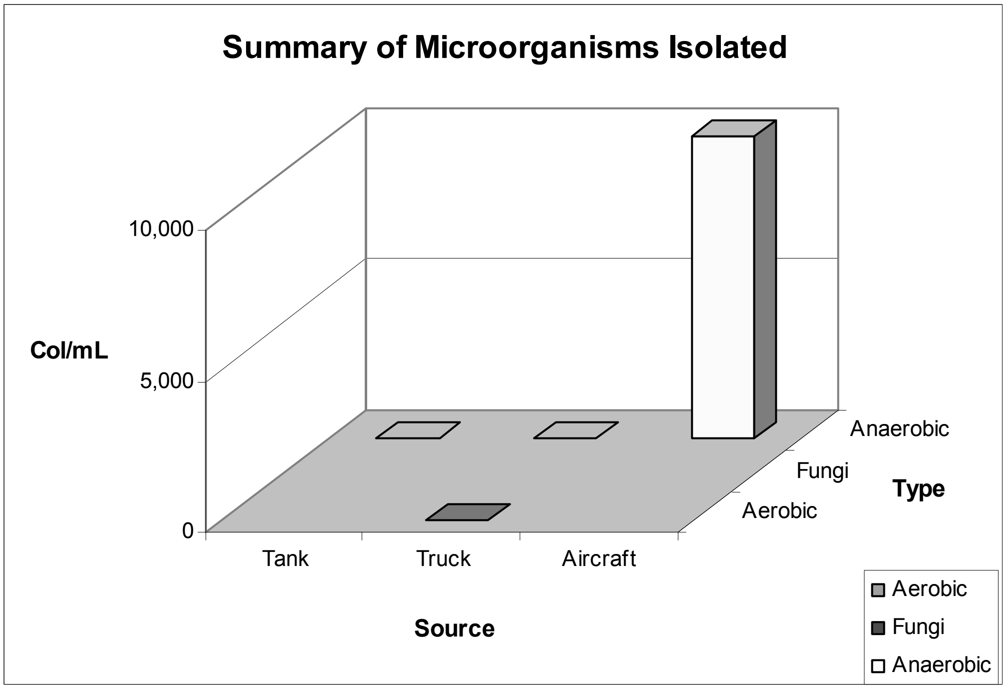


Figure 24. Summary of Microorganism Types Isolated from Davis-Monthan AFB

Hill Air Force Base, Utah

Three samples were obtained from Hill Air Force Base: tank, truck, and aircraft. Microbial colonies were isolated from two samples (Table 26), with only minor microbial growth (Table 27).

The following tables were derived from data presented in Appendix J.

Table 26. Summary of Microbial Identification of Hill AFB Samples

Sample	Type / Number	Date Sampled	Dilution	Medium	Fuel Type	Bacteria vs. Fungi	Similarity Index	GC Best Match
Tank	40	11/12/2002	1:10	BHIBLA	JP-8	bacteria		No match found
Truck	34	11/12/2002	1:1000	BHIBLA	JP-8	bacteria		Library match not attempted

Table 27. Quantity of Microorganisms Isolated From Hill AFB Samples

Sample	Dilution	Medium	pH	Gram	Organism Shape	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours	Level of Contamination
Tank	1:10	BHIBLA			varied	<1	185	<1	Negligible
Truck	1:1000	BHIBLA	6.3		varied	<1	98	<1	Negligible

As seen in Figure 25, the majority of the microbial growth occurred in the form of anaerobic activity in the tank and truck’s fuel tanks.

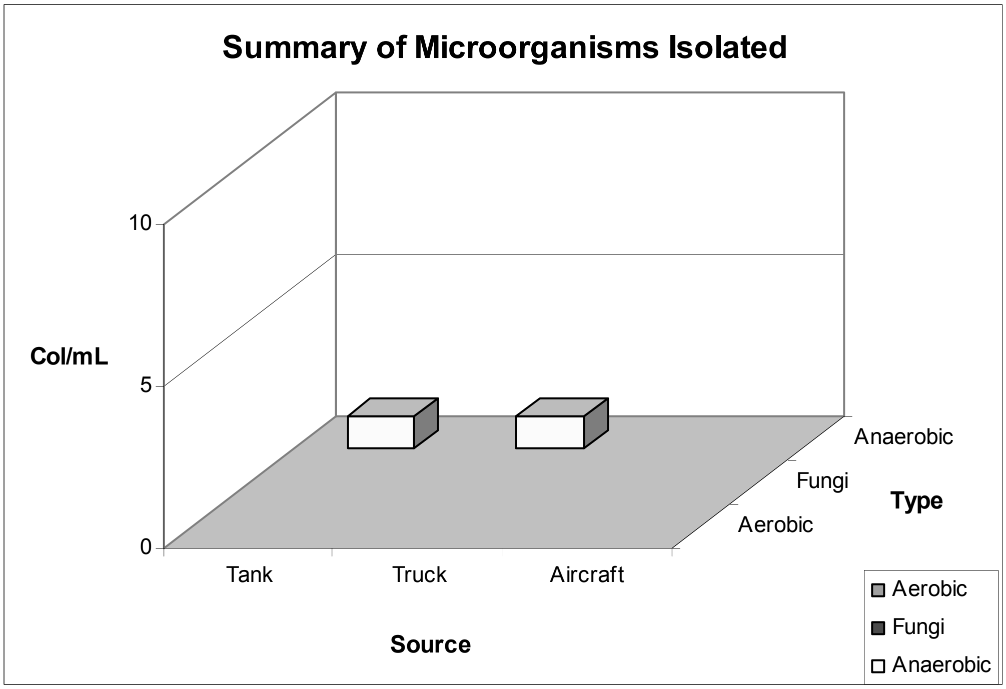


Figure 25. Summary of Microorganism Types Isolated from Hill AFB

Wright-Patterson Air Force Base, Ohio

Six samples were obtained from Wright-Patterson Air Force Base: tank #1, tank #2, tank #3, tank #4, truck, and aircraft. Multiple samples were taken of the various tanks due to the recent drop in average ambient temperatures (normal winter season), which led to an increase in condensation within the tanks. One-liter fuel samples from tanks #1-4 contained approximately half a liter of aqueous phase each.

Microbial colonies were isolated from all six samples (Table 28), with the most microbial growth in the form of anaerobic bacteria (Table 29).

The following tables were derived from data presented in Appendix K.

Table 28. Summary of Microbial Identification of Wright-Patterson AFB Samples

Sample	Type / Number	Date Sampled	Dilution	Medium	Fuel Type	Bacteria vs. Fungi	Similarity Index	GC Best Match
Tank 1	F/S 1	11/9/2002	1:10	BHIBLA	JP-8	bacteria		Library match not attempted
Tank 1	F/S 1	11/9/2002	1:100	BHIBLA	JP-8	bacteria		No match found
Tank 1	F/S 1	11/9/2002	1:1000	BHIBLA	JP-8	fungi		No match found
Tank 2	F/S 2	11/9/2002	1:10	BHIBLA	JP-8	bacteria		No match found
Tank 2	F/S 2	11/9/2002	1:100	BHIBLA	JP-8	bacteria		No match found
Tank 2	F/S 2	11/9/2002	1:1000	BHIBLA	JP-8	bacteria		Library match not attempted
Tank 3	Unloading	11/9/2002	Neat	TSBA	JP-8	bacteria	0.435	*Bacillus cereus GC subroup A*
Tank 3	Unloading	11/9/2002	1:10	TSBA	JP-8	bacteria	0.897	*Bacillus licheniformis*
Tank 3	Unloading	11/9/2002	1:10	BHIBLA	JP-8	bacteria		No match found
Tank 3	Unloading	11/9/2002	1:1000	BHIBLA	JP-8	bacteria		No match found
Tank 4	Bldg 159	9/1/2002	1:10	TSBA	JP-8	bacteria	0.253	*Bacillus cereus GC subroup A*
Tank 4	Bldg 159	9/1/2002	1:100	BHIBLA	JP-8	fungi		No match found
Truck	821	11/22/2002	1:10	BHIBLA	JP-8	bacteria		No match found
Aircraft	134	11/22/2002	Neat	BHIBLA	JP-8	fungi		No match found
Aircraft	134	11/22/2002	1:10	BHIBLA	JP-8	fungi		Library match not attempted

Table 29. Quantity of Microorganisms Isolated From Wright-Patterson Samples

Sample	Dilution	Medium	pH	Gram	Organism Shape	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours	Level of Contamination
Tank 1	1:10	BHIBLA	6.0	neg	bacillus	4	6	400	Moderate
Tank 1	1:100	BHIBLA	6.0	neg	bacillus	102	180	102,000	Heavy
Tank 1	1:1000	BHIBLA	6.0		varied	49	61	490,000	Heavy
Tank 2	1:10	BHIBLA	6.2	pos	bacillus	5	7	500	Moderate
Tank 2	1:100	BHIBLA	6.2	pos	bacillus	19	26	19,000	Moderate
Tank 2	1:1000	BHIBLA	6.2	pos	bacillus	35	42	350,000	Heavy
Tank 3	Neat	TSBA	5.6	neg	bacillus	1	2	10	Negligible
Tank 3	1:10	TSBA	5.6	neg	bacillus	1	2	100	Moderate
Tank 3	1:10	BHIBLA	5.6	neg	bacillus	107	245	10,700	Moderate
Tank 3	1:1000	BHIBLA	5.6	neg	bacillus	190	295	1,900,000	Heavy
Tank 4	1:10	TSBA	5.4		varied	1	2	100	Moderate
Tank 4	1:100	BHIBLA	5.4		varied	95	148	950,000	Heavy
Truck	1:10	BHIBLA	6.6	pos	bacillus	110	204	11,000	Moderate
Aircraft	Neat	BHIBLA			varied	32	51	320	Moderate
Aircraft	1:10	BHIBLA			varied	45	62	4,500	Moderate

As seen in Figure 26, the majority of the microbial growth occurred in the form of anaerobic activity in tank #3's storage tank. Although growth was present in all six fuel storage systems, they differed by orders of magnitude.

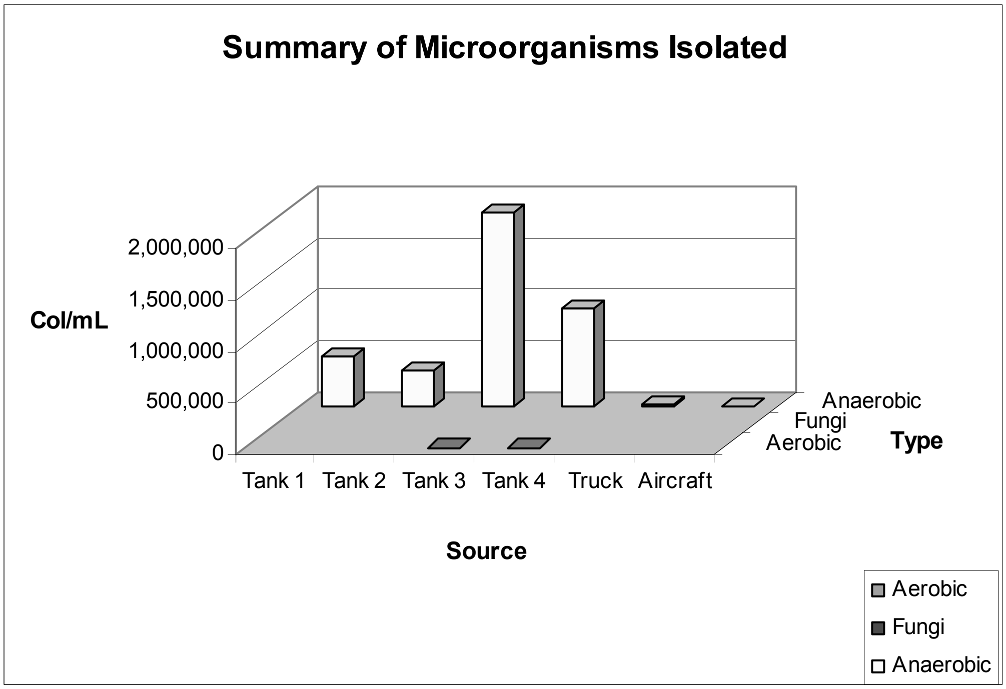


Figure 26. Summary of Microorganism Types Isolated from Wright-Patterson AFB

Moody Air Force Base, Georgia

Three samples were obtained from Moody Air Force Base: tank, truck, and aircraft. Microbial colonies were isolated from two of the three samples, tank and aircraft (Table 30), with the most microbial growth in the form of anaerobic bacteria (Table 31).

The following tables were derived from data presented in Appendix L.

Table 30. Summary of Microbial Identification of Moody AFB Samples

Sample	Type / Number	Date Sampled	Dilution	Medium	Fuel Type	Bacteria vs. Fungi	Sim Index	GC Best Match	DNA % ID	DNA Best Match
Tank	Tank 1	12/5/2002	Neat	TSBA	JP-8	bacteria	0.012	*Flavobacterium johnsoniae*		
Tank	Tank 1	12/5/2002	1:10	SDA	JP-8	bacteria		No match found		
Tank	Tank 1	12/5/2002	Neat	BHIBLA	JP-8	bacteria		No match found		
Tank	Tank 1	12/5/2002	1:10	BHIBLA	JP-8	bacteria		No match found		
Aircraft	C-130	12/5/2002	Neat	TSBA	JP-8	bacteria	0.443	*Bacillus megaterium GC subroup A*		
Aircraft	C-130	12/5/2002	Neat	SDA	JP-8	fungi		Library match not attempted		
Aircraft	C-130	12/5/2002	Neat	BHIBLA	JP-8	bacteria		No match found		

Table 31. Quantity of Microorganisms Isolated From Moody AFB Samples

Sample	Dilution	Medium	pH	Gram	Organism Shape	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours	Level of Contamination
Tank	Neat	TSBA	6.6	neg	bacillus	13	32	130	Moderate
Tank	1:10	SDA	6.6	neg	bacillus	1	1	100	Moderate
Tank	Neat	BHIBLA	6.6	neg	bacillus	78	920	780	Moderate
Tank	1:10	BHIBLA	6.6	neg	bacillus	2	34	200	Moderate
Aircraft	Neat	TSBA			varied	1	2	10	Negligible
Aircraft	Neat	SDA			varied	1	1	10	Negligible
Aircraft	Neat	BHIBLA		neg	bacillus	3	3	30	Negligible

As seen in Figure 27, the majority of the microbial growth occurred in the form of anaerobic activity in the storage tank. Although growth was present in two fuel storage systems, they differed by orders of magnitude.

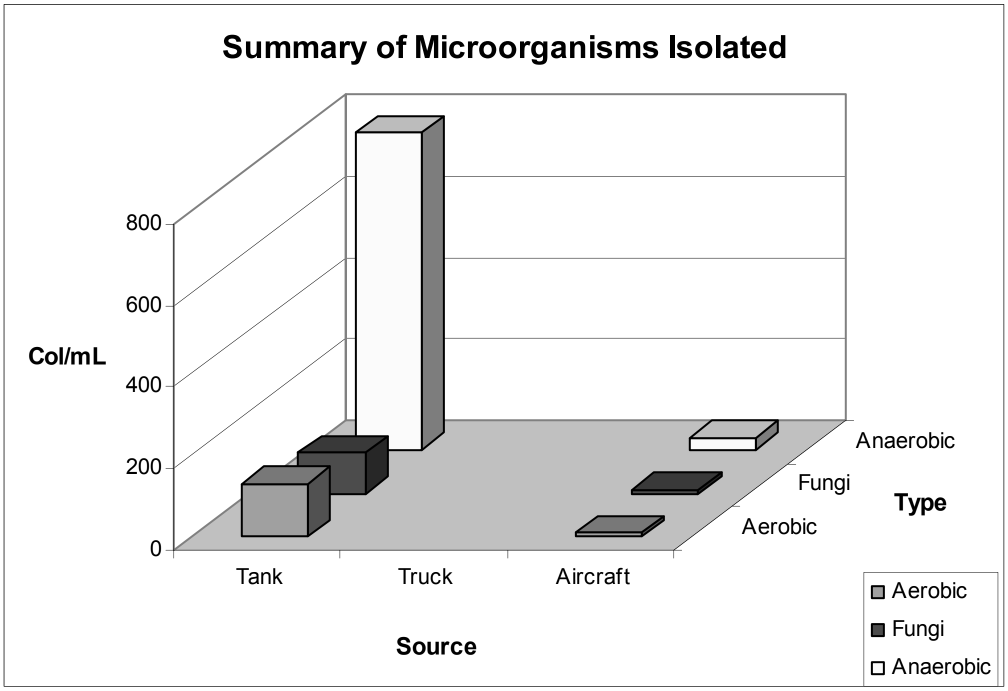


Figure 27. Summary of Microorganism Types Isolated from Moody AFB

Identification of All Samples

Totaling the samples from all 12 bases, 123 of 480 serial dilutions registered visible colonies of microorganisms. Figure 28 contains a summary of the gas chromatograph best matches. Slightly more than half (72 of 123) of the colonies were reported as No Match Found by the GC. This highlights the fact that current GC libraries are not focused on identifying environmental microorganisms.

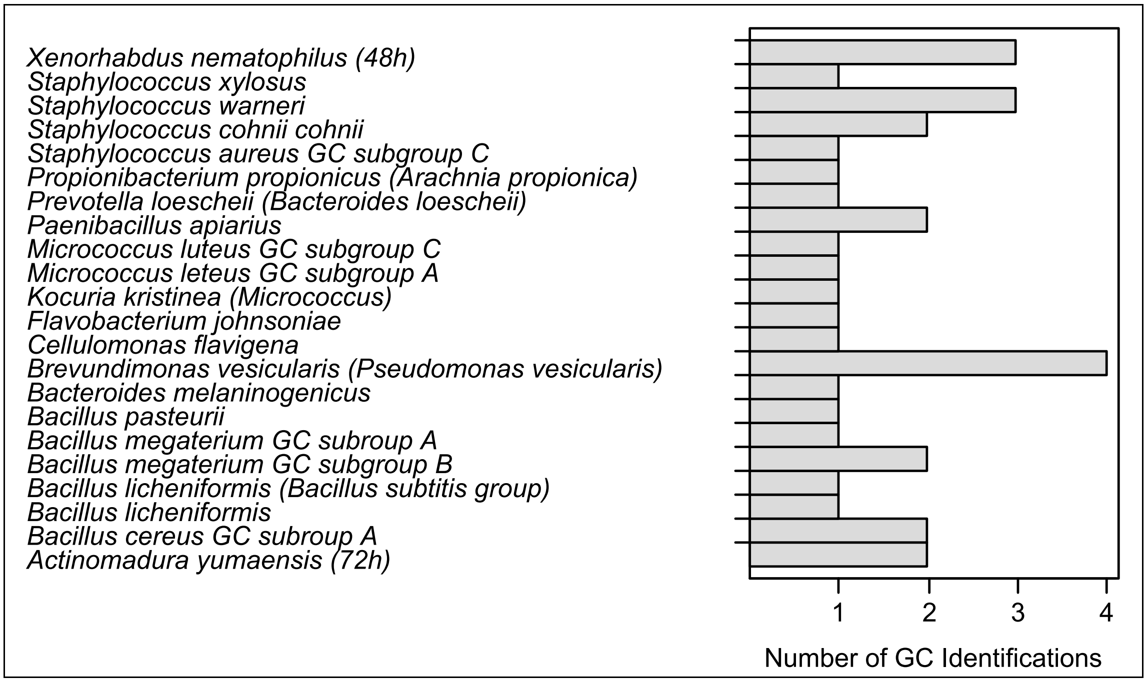


Figure 28. GC Identification of Microorganisms among Entire Study

Figure 29 displays the DNA sequencing identifications of the random samples that were analyzed by 16S rRNA gene sequence similarity.

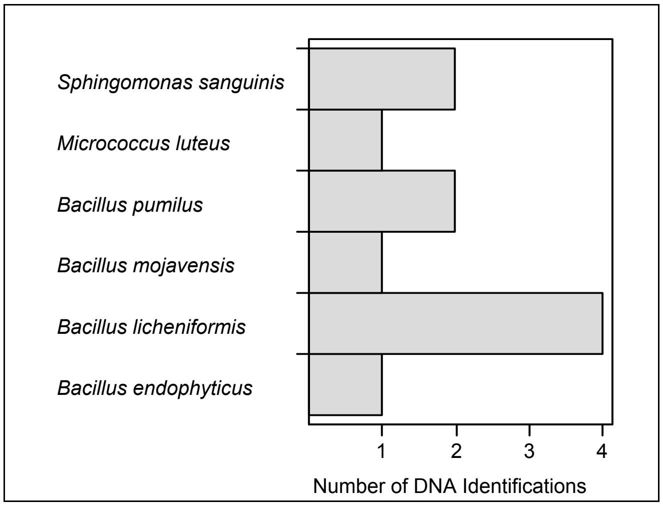


Figure 29. DNA Gene Sequencing Identifications

Quantities of All Fuel Samples

Tank Samples

As seen in Figure 30, microorganisms were isolated from tanks of all 12 bases. Anaerobic bacteria were most common.

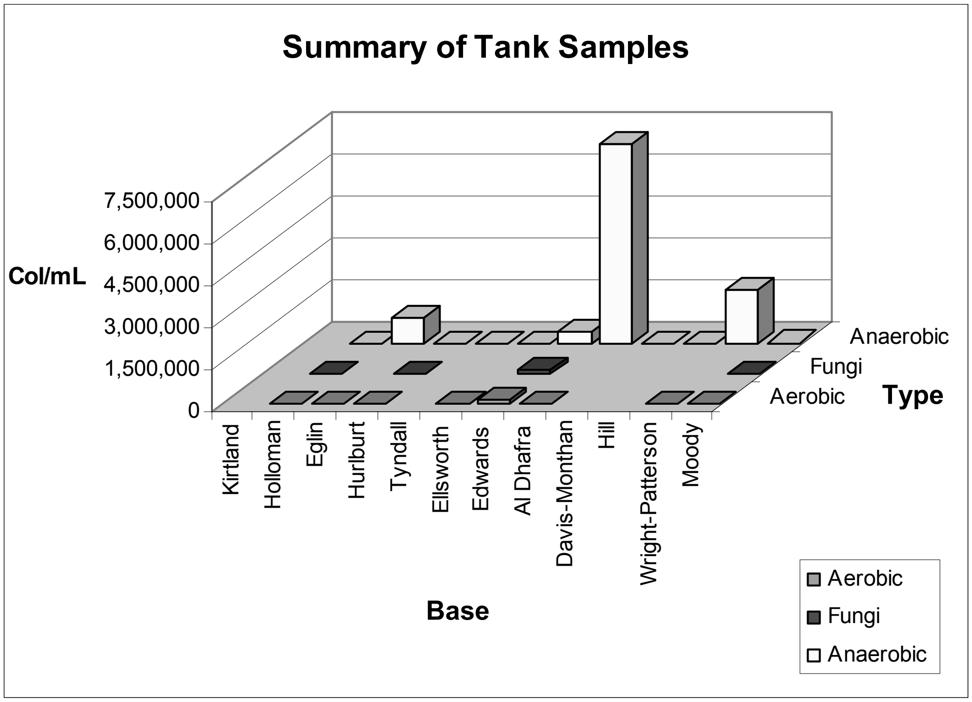


Figure 30. Summary of Microorganism Types Isolated From All Tank Samples

Truck Samples

As seen in Figure 31, microorganisms were isolated from truck samples of nearly every base.

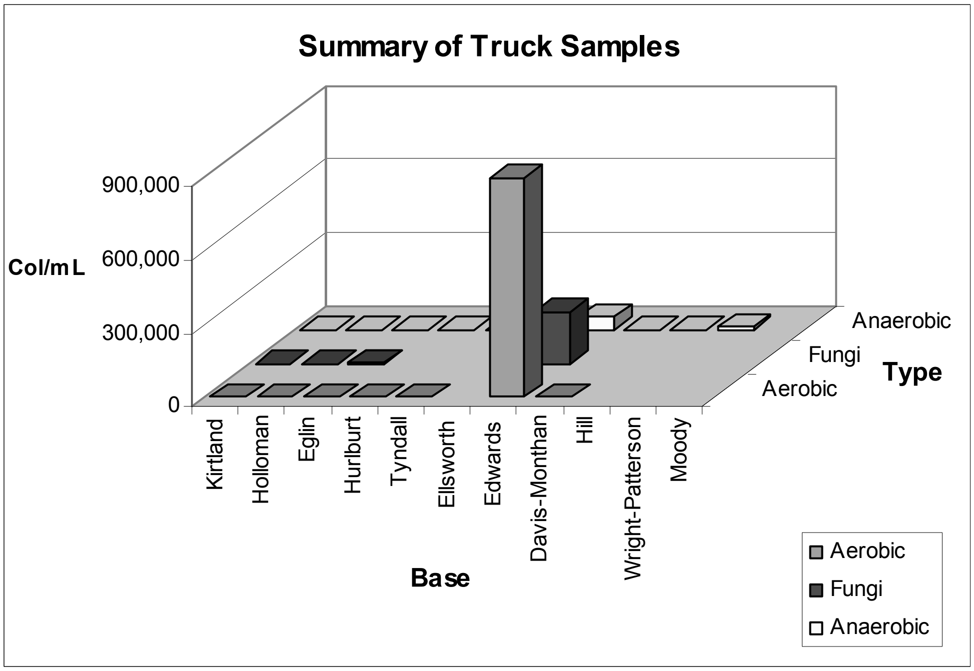


Figure 31. Summary of Microorganism Types Isolated From All Truck Samples

Aircraft Samples

As seen in Figure 32, microorganisms were isolated from aircraft samples of nearly every base. Aside from the single fungi outlier, anaerobic bacteria were most common.

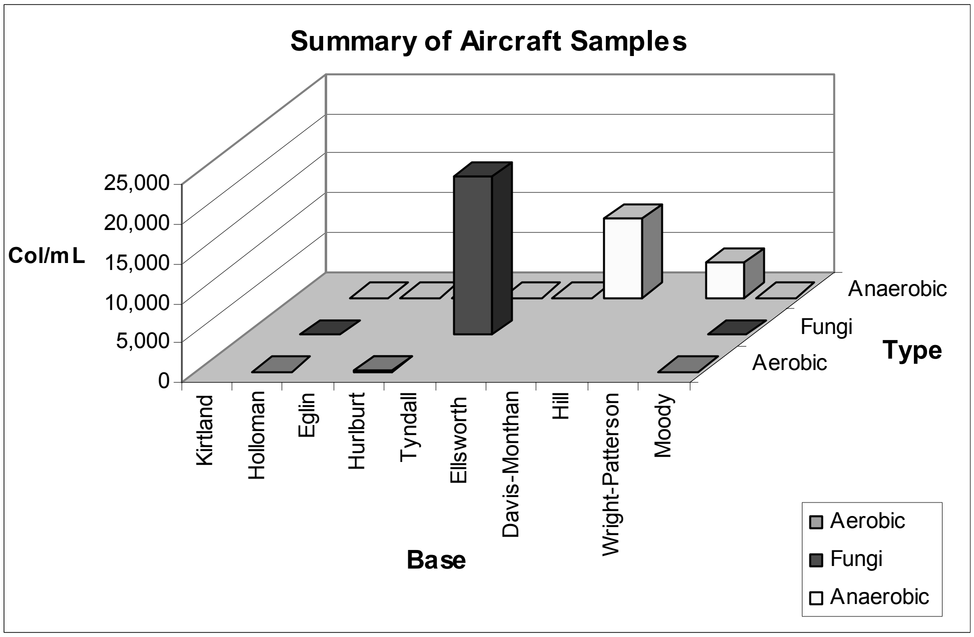


Figure 32. Summary of Microorganism Types Isolated From All Sample Aircraft

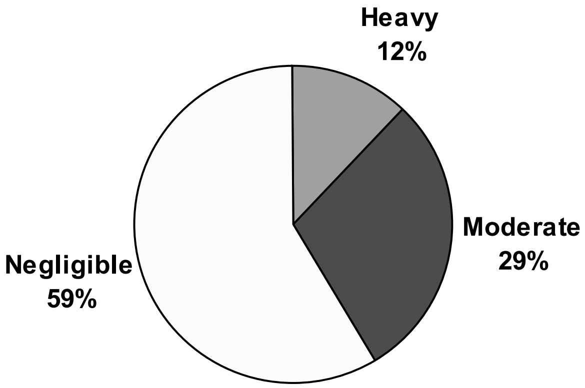
Significant Levels of Microorganisms

Table 32 shows the levels of contamination for each sample that produced a visible microbial colony.

Table 32. Summary of Levels of Contamination for All Samples

Base	Sample	Type	Level of Contamination
Kirtland	Truck	Aerobic	Moderate
Kirtland	Truck	Fungi	Moderate
Kirtland	Truck	Anaerobic	Moderate
Holloman	Tank	Aerobic	Negligible
Holloman	Tank	Fungi	Negligible
Holloman	Tank	Anaerobic	Negligible
Holloman	Truck	Aerobic	Negligible
Holloman	Truck	Fungi	Negligible
Holloman	Truck	Anaerobic	Negligible
Holloman	Aircraft	Aerobic	Negligible
Holloman	Aircraft	Fungi	Negligible
Holloman	Aircraft	Anaerobic	Negligible
Eglin	Tank	Aerobic	Moderate
Eglin	Tank	Anaerobic	Heavy
Eglin	Truck	Aerobic	Negligible
Eglin	Truck	Fungi	Moderate
Eglin	Truck	Anaerobic	Negligible
Eglin	Aircraft	Anaerobic	Negligible
Hurlburt	Tank	Aerobic	Moderate
Hurlburt	Tank	Fungi	Negligible
Hurlburt	Tank	Anaerobic	Negligible
Hurlburt	Truck	Aerobic	Negligible
Hurlburt	Truck	Anaerobic	Negligible
Hurlburt	Aircraft	Aerobic	Moderate
Hurlburt	Aircraft	Anaerobic	Negligible
Tyndall	Tank	Anaerobic	Negligible
Tyndall	Truck	Aerobic	Moderate
Tyndall	Truck	Anaerobic	Negligible
Tyndall	Aircraft	Fungi	Moderate
Tyndall	Aircraft	Anaerobic	Negligible
Ellsworth	Tank	Aerobic	Negligible
Ellsworth	Tank	Anaerobic	Negligible
Ellsworth	Truck	Anaerobic	Negligible
Ellsworth	Aircraft	Anaerobic	Negligible
Edwards	Tank	Aerobic	Heavy
Edwards	Tank	Fungi	Heavy
Edwards	Tank	Anaerobic	Heavy
Edwards	Truck 1	Aerobic	Negligible
Edwards	Truck 1	Anaerobic	Moderate
Edwards	Truck 2	Aerobic	Heavy
Edwards	Truck 2	Fungi	Heavy
Middle Eastern	Bladder	Aerobic	Negligible
Middle Eastern	Bladder	Anaerobic	Heavy
Middle Eastern	Liner	Aerobic	Negligible
Middle Eastern	Liner	Anaerobic	Moderate
Middle Eastern	Drain	Aerobic	Negligible
Middle Eastern	Drain	Anaerobic	Heavy
Middle Eastern	Vent	Aerobic	Negligible
Middle Eastern	Vent	Anaerobic	Heavy
Davis-Monthan	Tank	Anaerobic	Negligible
Davis-Monthan	Truck	Aerobic	Negligible
Davis-Monthan	Truck	Anaerobic	Negligible
Davis-Monthan	Aircraft	Anaerobic	Moderate
Hill	Tank	Anaerobic	Negligible
Hill	Truck	Anaerobic	Negligible
Wright-Patterson	Tank 1	Anaerobic	Heavy
Wright-Patterson	Tank 2	Anaerobic	Heavy
Wright-Patterson	Tank 3	Aerobic	Moderate
Wright-Patterson	Tank 3	Anaerobic	Heavy
Wright-Patterson	Tank 4	Aerobic	Moderate
Wright-Patterson	Tank 4	Anaerobic	Heavy
Wright-Patterson	Truck	Anaerobic	Moderate
Wright-Patterson	Aircraft	Anaerobic	Moderate
Moody	Tank	Aerobic	Moderate
Moody	Tank	Fungi	Moderate
Moody	Tank	Anaerobic	Moderate
Moody	Aircraft	Aerobic	Negligible
Moody	Aircraft	Fungi	Negligible
Moody	Aircraft	Anaerobic	Negligible

Although many serial dilutions displayed the presence of microorganisms, more than half (72 of 123) fell within the negligible level of contamination. However, 51 of 123 serial dilutions produced microorganisms that were labeled as moderately or heavily contaminated (Figure 33).



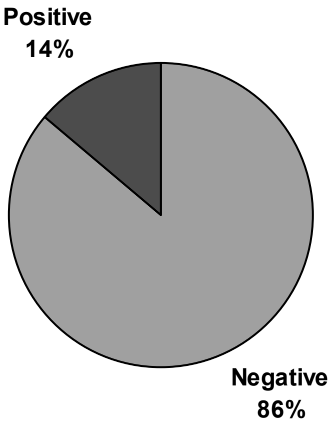
Frequencies

Level	Number
Heavy	15
Moderate	36
Negligible	72
Total	**123**

Figure 33. Summary of Microbial Contamination Isolated From Serial Dilutions

Descriptions of Microorganisms Found

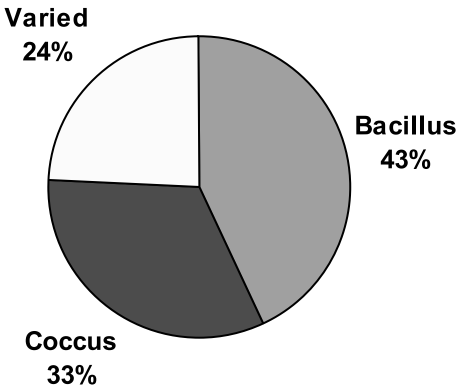
The microorganisms isolated were overwhelmingly Gram negative, anaerobic, bacillus bacteria (Figures 10, 34, and 35).



Frequencies

Gram	Number
Negative	80
Positive	13
Total	**93**

Figure 34. Gram Stain Summary of Colonies Isolated



Frequencies

Shape	Number
Bacillus	53
Coccus	40
Varied	30
Total	**93**

Figure 35. Organism Shape Summary of Colonies Isolated

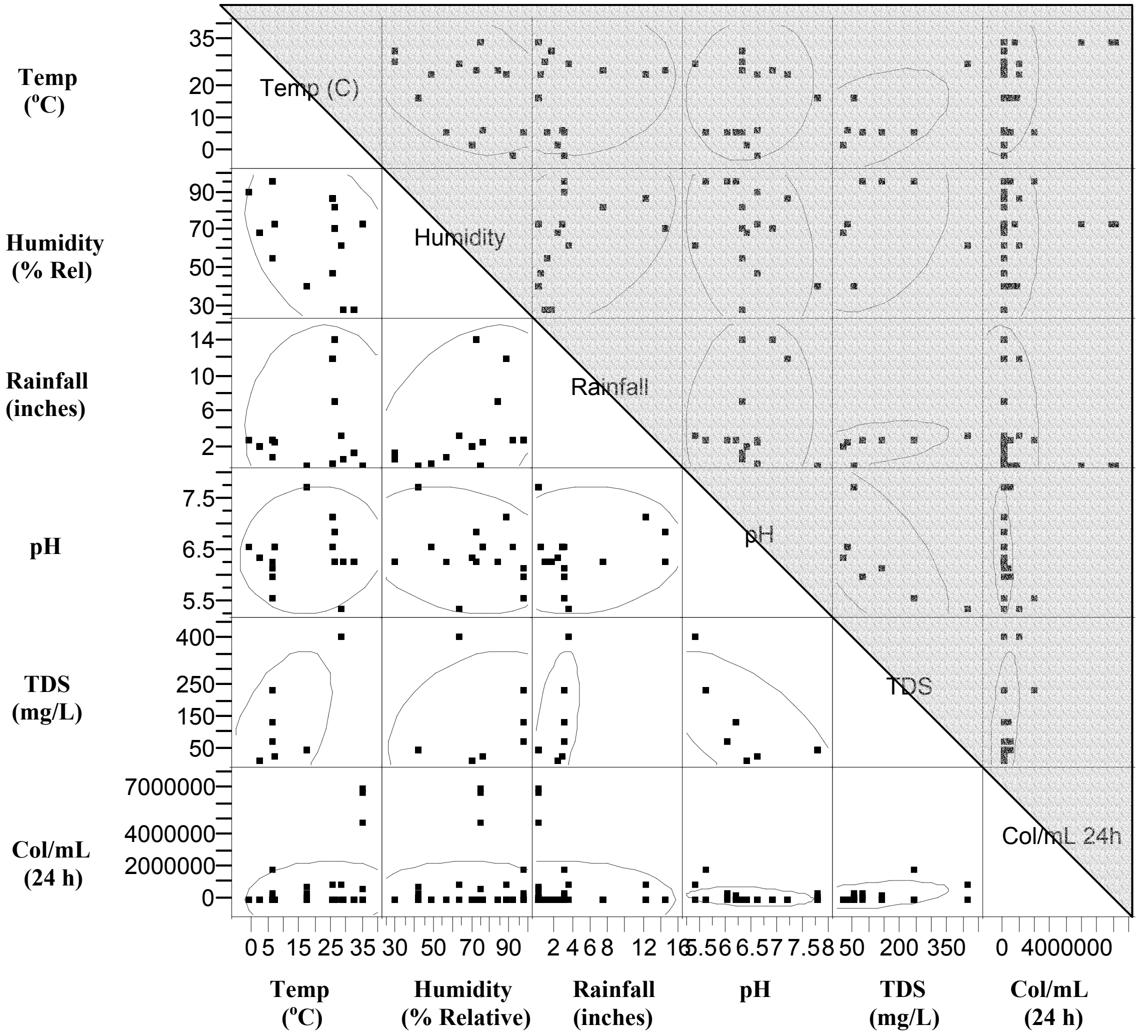
Potential Common Variables

Significant levels of microorganisms have been isolated from military aviation fuel systems. The next step is to determine if there are any common variables linking the contamination.

The numerical data gathered in this research was analyzed using the JMP™ Release 5.0 statistical software. An attempt was made to find a correlation between the site's environmental conditions (temperature, relative humidity, and average rainfall last 30 days) and the pH, Total Dissolved Solids (TDS), and quantities of microorganisms isolated. Figure 36 displays a graphical depiction of the correlations between variables. In the upper segment of the figure, the ellipses highlight a pattern. Both the vertical and horizontal axis contains the same six variables mentioned above. None of the relationships are confidently characterized as correlated with respect to predicting microbial contamination (the shaded portion of the figure is simply an inverse representation of the unshaded portion).

As seen in the pairwise correlations (lower segment of figure), some of the relationships have a correlation factor that is statistically relevant. Although the correlations are statistically significant based on large sample sizes, there is no managerial significance to the data. For example, determining that as the amount of rainfall increases so does the percent relative humidity (positively correlated at 0.4351) does not lead to useful predictions of the amount of contamination (Col/mL).

It is with this data that a conclusion is drawn that the weather data, pH, and TDS do not significantly contribute to the level of microbial contamination.



Pairwise Correlations

Variable	by Variable	Correlation	Count	Plot Correlation
Humidity	Temp (C)	-0.3453	123	
Rainfall	Temp (C)	0.0691	123	
Rainfall	Humidity	0.4351	123	
pH	Temp (C)	0.1029	62	
pH	Humidity	-0.2468	62	
pH	Rainfall	0.0956	62	
TDS	Temp (C)	0.4675	26	
TDS	Humidity	0.2892	26	
TDS	Rainfall	0.4802	26	
TDS	pH	-0.6698	26	
Col/mL 24h	Temp (C)	0.1545	123	
Col/mL 24h	Humidity	0.0919	123	
Col/mL 24h	Rainfall	-0.1405	123	
Col/mL 24h	pH	-0.2333	62	
Col/mL 24h	TDS	0.3933	26	

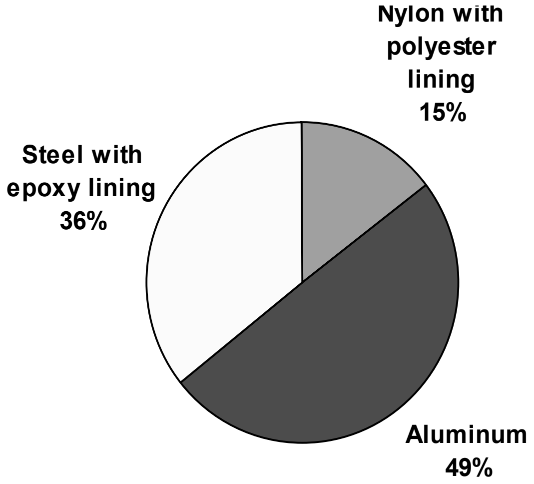
Figure 36. Summary of Correlation Analysis

Physical Characteristics among No Match Found Organisms

Analyzing only the 72 serial dilutions which produced the GC identification of No Match Found, similar characteristics were found as those mentioned above for the named organisms. The No Match Found organisms were overwhelmingly Gram negative, anaerobic, bacillus bacteria.

Noteworthy Facts

There are two noteworthy facts from analyzing the data. First, this research drew samples from only three types of tank compositions: steel with epoxy lining, aluminum, and nylon with polyester lining (expeditionary fuel storage tank). As seen in Figure 37, most of the organisms isolated were from aluminum tanks (trucks and aircraft). No strong correlation was found in this study to suggest that tank composition increases or decreases the chances of microbial growth within the tank.

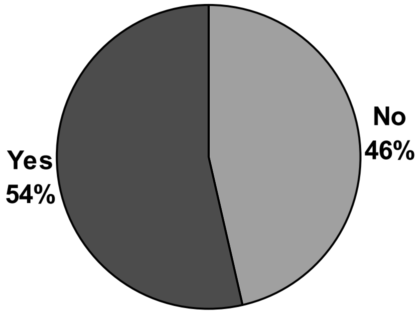


Frequencies

Tank Composition	Number
Nylon with polyester lining	18
Aluminum	61
Steel with epoxy lining	44
Total	**123**

Figure 37. Summary of Tank Compositions for Visible Colonies from All Bases

The second noteworthy fact is that of the fuel samples that produced visible microbial colonies, nearly half did not contain any free phase water (Figure 38). A basic theory is challenged by this finding. Conventional wisdom dictates that the microorganisms are nourished by the hydrocarbons in the fuel, yet this “feeding” takes place at the fuel/water interface because water is also a necessary for the microorganisms to survive. This suggests that certain microorganisms can survive and even thrive in environments that contain only a miniscule amount of water.



Frequencies

Free Phase Water	Number
No	57
Yes	66
Total	**123**

Figure 38. Number of GC Identified Samples with Free Phase Water

V. Conclusions and Recommendations for Further Study

Overall

It is clear from the analysis of fuel samples from 12 military bases that there are many microorganisms present in military aviation fuel systems. The results from 40 fuel samples provide a much more complete picture of the microbial presence than could be accomplished through any single sample.

The main focus of this research was to determine whether significant levels of microorganisms are growing in military aviation fuel systems.

Using the criteria in Table 6 (page 51) to define significance, there were significant levels of microorganisms growing in military aviation fuel systems. Whether looking at bases, individual fuel samples, serial dilutions, or contamination level; all had a large percentage of microbial contamination (Table 33).

Table 33. Summary of Microbial Contamination

Category	Number Contaminated	Percent Contaminated
Military Base	12 of 12	100 %
Fuel Samples	36 of 40	90 %
Serial Dilutions	123 of 480	25.6 %
Moderate or Heavy Contamination	51 of 123	41.5 %

In addition to quantifying the extent of the microbial contamination, physical characteristics of the organisms were also determined. The overwhelming majority of microorganisms isolated were similar in physical characteristics in that most were Gram negative, anaerobic, bacillus bacteria. Although many organisms were identified at the genus level, gas chromatograph identifications with high confidence were sporadic.

Altogether, this work is a first step in providing cleaner and safer aviation fuel to the U.S. military's operating forces.

Research Objectives

The four primary objectives of this research were:

1. To determine the types and quantities of microorganisms, if any, present in U.S. military aviation fuel storage tanks, aircraft refueling trucks, and aircraft fuel tanks.

Gram negative, anaerobic, bacillus bacteria were common in microorganisms isolated. Although slightly more than half the microbially contaminated samples were labeled as negligible, over 40% were labeled as moderately or heavily contaminated.

2. To determine the characteristic conditions in which microorganisms thrive in U.S. military aviation fuel systems.

No obvious characteristic conditions were found in this study. Samples from across the continental United States, as well as abroad, all produced visible colonies with roughly the same frequency and type. Weather conditions, personnel training and experience, season, and housekeeping techniques all varied base by base, yet no common characteristic conditions were consistently found in this study.

3. To determine if current microbial minimization methods are appropriate.

Current microbial minimization methods are not entirely effective. Although most of the samples produced a negligible amount of contamination, 41% produced moderate or heavy contamination levels. These higher levels of microbial contamination may be a cause of concern to military officials and worthy of closer examination.

4. To determine if U.S. military aviation fuel systems are vulnerable to intentional microbial contamination.

Although this research placed little emphasis on this objective, some observations were noted. It is difficult to determine the root cause of any microbial contamination outbreak due to the frequent transfer of fuel from one tank to another. With that as a reference, intentional contamination could spread quickly and leave officials unsure as to the source of the contamination (pipeline, storage tank, refueling truck, or aircraft). In this era of heightened security measures, there are still many types of personnel with total access to the fuel storage facilities (military and civilian employees). Transient personnel, such as researchers in this study, gained complete unaccompanied access to storage tanks, trucks, and aircraft without any hesitation or being asked for identification of any kind. Treating the fuel program with the same level of security awareness as the flight line is worthy of attention.

Research Strengths

The strength of this research lies in three parts. First, this research included a large number of samples from all the major regions of the continental United States and selected samples abroad. Twelve bases with a total of 40 samples provided a good representation of fuel and fuel contamination currently used in the military on a daily basis.

Second, the research timeframe and locations covered both warm and cold environmental conditions. Again, this presented a reasonable representation of aviation fuel currently used.

Third, only a limited number of personnel were allowed to participate in this study. This provided an atmosphere in which sampling techniques, laboratory analysis, and interpretation variances were kept to a minimum.

Research Limitations

As with any research, limitations exist. Although the gas chromatograph analysis provided insight into identifying the microorganisms isolated in this study, the lack of sufficient libraries for environmental organisms created an abundance of samples in which no matches were found.

Another limitation of this study derived from the lack of an "industry standard" for the definition of significant microbial contamination. Although commercial guidelines exist, there is no indication they are based on solid scientific research. The potential exists for these commercial guidelines to be based more on sales than science.

Recommendations for Further Study

The results of this work, if nothing else, illustrate the fact that microorganisms abound in military aviation fuel systems. Recommendations for follow-on research fall into two categories: (1) microbial identification method and (2) number of samples.

First, the gas chromatograph method of identifying microorganisms in military fuel systems proved to be disappointing. Suitable libraries for environmental purposes are lacking. Until more suitable libraries become available, it is recommended all future microbial identification to be done by using DNA gene sequencing, which has the potential of providing a more accurate best match with a higher level of confidence.

Second, although the numbers of samples in this study were adequate to draw basic conclusions about microbial contamination, these conclusions may not be expanded to include all the climates in which the military routinely finds itself. This thesis is applicable to the mainstream military unit, yet may not be as useful to units serving in more austere environments such as the Antarctic. Analysis of more samples taken from remote environments is recommended.

Appendix A: Worksheet / Sample Data – Kirtland AFB (KIKR)

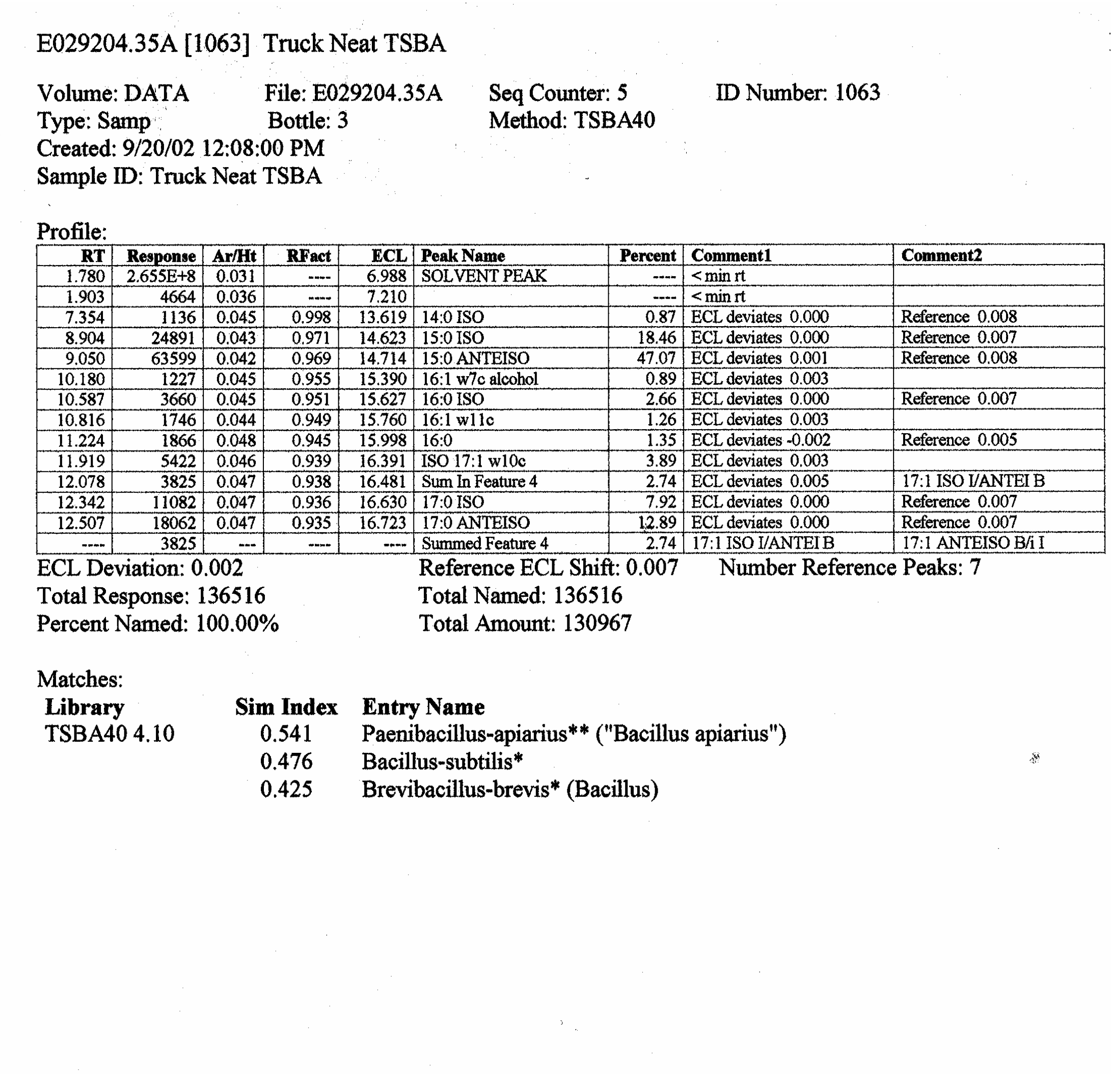
	Site Information									
#	Sample	Type / Number	Date Sampled	Dilution	Medium	Temp °C	% Relative Humidity	Inches Rainfall (last 30 days)	Fuel Type	Sample Method
1	Neg Control	—	—	—	—	—	—	—	—	—
2	Pos Control	—	—	—	—	—	—	—	—	—
3	Tank	#23	9/3/2002	Neat	TSBA	32	29	1.59	JP-8	gravity
4	Tank	#23	9/3/2002	1:10	TSBA	32	29	1.59	JP-8	gravity
5	Tank	#23	9/3/2002	1:100	TSBA	32	29	1.59	JP-8	gravity
6	Tank	#23	9/3/2002	1:1000	TSBA	32	29	1.59	JP-8	gravity
7	Tank	#23	9/3/2002	Neat	SDA	32	29	1.59	JP-8	gravity
8	Tank	#23	9/3/2002	1:10	SDA	32	29	1.59	JP-8	gravity
9	Tank	#23	9/3/2002	1:100	SDA	32	29	1.59	JP-8	gravity
10	Tank	#23	9/3/2002	1:1000	SDA	32	29	1.59	JP-8	gravity
11	Tank	#23	9/3/2002	Neat	BHIBLA	32	29	1.59	JP-8	gravity
12	Tank	#23	9/3/2002	1:10	BHIBLA	32	29	1.59	JP-8	gravity
13	Tank	#23	9/3/2002	1:100	BHIBLA	32	29	1.59	JP-8	gravity
14	Tank	#23	9/3/2002	1:1000	BHIBLA	32	29	1.59	JP-8	gravity
15	Truck	N/A	9/3/2002	Neat	TSBA	32	29	1.59	JP-8	gravity
16	Truck	N/A	9/3/2002	1:10	TSBA	32	29	1.59	JP-8	gravity
17	Truck	N/A	9/3/2002	1:100	TSBA	32	29	1.59	JP-8	gravity
18	Truck	N/A	9/3/2002	1:1000	TSBA	32	29	1.59	JP-8	gravity
19	Truck	N/A	9/3/2002	Neat	SDA	32	29	1.59	JP-8	gravity
20	Truck	N/A	9/3/2002	1:10	SDA	32	29	1.59	JP-8	gravity
21	Truck	N/A	9/3/2002	1:100	SDA	32	29	1.59	JP-8	gravity
22	Truck	N/A	9/3/2002	1:1000	SDA	32	29	1.59	JP-8	gravity
23	Truck	N/A	9/3/2002	Neat	BHIBLA	32	29	1.59	JP-8	gravity
24	Truck	N/A	9/3/2002	1:10	BHIBLA	32	29	1.59	JP-8	gravity
25	Truck	N/A	9/3/2002	1:100	BHIBLA	32	29	1.59	JP-8	gravity
26	Truck	N/A	9/3/2002	1:1000	BHIBLA	32	29	1.59	JP-8	gravity
27	Aircraft	C-130	9/3/2002	Neat	TSBA	32	29	1.59	JP-8 +100	gravity
28	Aircraft	C-130	9/3/2002	1:10	TSBA	32	29	1.59	JP-8 +100	gravity
29	Aircraft	C-130	9/3/2002	1:100	TSBA	32	29	1.59	JP-8 +100	gravity
30	Aircraft	C-130	9/3/2002	1:1000	TSBA	32	29	1.59	JP-8 +100	gravity
31	Aircraft	C-130	9/3/2002	Neat	SDA	32	29	1.59	JP-8 +100	gravity
32	Aircraft	C-130	9/3/2002	1:10	SDA	32	29	1.59	JP-8 +100	gravity
33	Aircraft	C-130	9/3/2002	1:100	SDA	32	29	1.59	JP-8 +100	gravity
34	Aircraft	C-130	9/3/2002	1:1000	SDA	32	29	1.59	JP-8 +100	gravity
35	Aircraft	C-130	9/3/2002	Neat	BHIBLA	32	29	1.59	JP-8 +100	gravity
36	Aircraft	C-130	9/3/2002	1:10	BHIBLA	32	29	1.59	JP-8 +100	gravity
37	Aircraft	C-130	9/3/2002	1:100	BHIBLA	32	29	1.59	JP-8 +100	gravity
38	Aircraft	C-130	9/3/2002	1:1000	BHIBLA	32	29	1.59	JP-8 +100	gravity

— Indicates analysis omitted

		Sample Description					
#	Tank Composition	pH	Sample Color	General Description	Obvious Solids	TDS (mg/L)	Free Phase Water
1	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—
3	steel w/ epoxy lining	6.3	light yellow	turbid	no	30	yes
4	steel w/ epoxy lining	6.3	light yellow	turbid	no	30	yes
5	steel w/ epoxy lining	6.3	light yellow	turbid	no	30	yes
6	steel w/ epoxy lining	6.3	light yellow	turbid	no	30	yes
7	steel w/ epoxy lining	6.3	light yellow	turbid	no	30	yes
8	steel w/ epoxy lining	6.3	light yellow	turbid	no	30	yes
9	steel w/ epoxy lining	6.3	light yellow	turbid	no	30	yes
10	steel w/ epoxy lining	6.3	light yellow	turbid	no	30	yes
11	steel w/ epoxy lining	6.3	light yellow	turbid	no	30	yes
12	steel w/ epoxy lining	6.3	light yellow	turbid	no	30	yes
13	steel w/ epoxy lining	6.3	light yellow	turbid	no	30	yes
14	steel w/ epoxy lining	6.3	light yellow	turbid	no	30	yes
15	aluminum	6.3	light yellow	clear	yes	—	yes
16	aluminum	6.3	light yellow	clear	yes	—	yes
17	aluminum	6.3	light yellow	clear	yes	—	yes
18	aluminum	6.3	light yellow	clear	yes	—	yes
19	aluminum	6.3	light yellow	clear	yes	—	yes
20	aluminum	6.3	light yellow	clear	yes	—	yes
21	aluminum	6.3	light yellow	clear	yes	—	yes
22	aluminum	6.3	light yellow	clear	yes	—	yes
23	aluminum	6.3	light yellow	clear	yes	—	yes
24	aluminum	6.3	light yellow	clear	yes	—	yes
25	aluminum	6.3	light yellow	clear	yes	—	yes
26	aluminum	6.3	light yellow	clear	yes	—	yes
27	aluminum	—	light yellow	clear	no	—	no
28	aluminum	—	light yellow	clear	no	—	no
29	aluminum	—	light yellow	clear	no	—	no
30	aluminum	—	light yellow	clear	no	—	no
31	aluminum	—	light yellow	clear	no	—	no
32	aluminum	—	light yellow	clear	no	—	no
33	aluminum	—	light yellow	clear	no	—	no
34	aluminum	—	light yellow	clear	no	—	no
35	aluminum	—	light yellow	clear	no	—	no
36	aluminum	—	light yellow	clear	no	—	no
37	aluminum	—	light yellow	clear	no	—	no
38	aluminum	—	light yellow	clear	no	—	no

	Light Microscopy				Colony Description		Quantity Data		
#	Gram +/-	Organism Shape	Length of Cells (μm)	Width of Cells (μm)	Colony Type	Colony Color	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours
1	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	0	0	0
4	—	—	—	—	—	—	0	0	0
5	—	—	—	—	—	—	0	0	0
6	—	—	—	—	—	—	0	0	0
7	—	—	—	—	—	—	0	0	0
8	—	—	—	—	—	—	0	0	0
9	—	—	—	—	—	—	0	0	0
10	—	—	—	—	—	—	0	0	0
11	—	—	—	—	—	—	0	0	0
12	—	—	—	—	—	—	0	0	0
13	—	—	—	—	—	—	0	0	0
14	—	—	—	—	—	—	0	0	0
15	neg	bacillus	1	0.5	irregular (leaf-like)	white	23	32	230
16	neg	bacillus	1	0.5	irregular (leaf-like)	white	4	5	400
17	—	—	—	—	—	—	0	0	0
18	—	—	—	—	—	—	0	0	0
19	neg	bacillus	1	0.5	round	opaque white	10	18	100
20	neg	bacillus	1	0.5	round	opaque white	1	2	100
21	—	—	—	—	—	—	0	0	0
22	—	—	—	—	—	—	0	0	0
23	neg	bacillus	1	0.5	round	pale white	23	39	230
24	neg	bacillus	1	0.5	round	pale white	3	6	300
25	—	—	—	—	—	—	0	0	0
26	—	—	—	—	—	—	0	0	0
27	—	—	—	—	—	—	0	0	0
28	—	—	—	—	—	—	0	0	0
29	—	—	—	—	—	—	0	0	0
30	—	—	—	—	—	—	0	0	0
31	—	—	—	—	—	—	0	0	0
32	—	—	—	—	—	—	0	0	0
33	—	—	—	—	—	—	0	0	0
34	—	—	—	—	—	—	0	0	0
35	—	—	—	—	—	—	0	0	0
36	—	—	—	—	—	—	0	0	0
37	—	—	—	—	—	—	0	0	0
38	—	—	—	—	—	—	0	0	0

				Identification Data				
				FAME analysis			DNA gene sequence	
#	Bacteria vs. Fungi	Spores	Digital Photo	Similarity Index	> .1 S. I. Separation	GC Best Match	DNA % ID	DNA Best Match
1	—	—	—	—	—	Library match not attempted	—	—
2	—	—	—	0.769	yes	*Stenotrophomonas-maltophilia*	—	—
3	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—
13	—	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—	—
15	bacteria	no	IKRTRNEATTSBA	0.541	no	*Paenibacillus-apiarius*	99.81	*Bacillus mojavensis*
16	bacteria	no	—	0.678	no	*Paenibacillus-apiarius*	99	*Bacillus endophyticus*
17	—	—	—	—	—	—	—	—
18	—	—	—	—	—	—	—	—
19	bacteria	no	—	—	—	No match found	—	—
20	bacteria	no	—	—	—	No match found	—	—
21	—	—	—	—	—	—	—	—
22	—	—	—	—	—	—	—	—
23	bacteria	no	—	—	—	No match found	—	—
24	bacteria	no	IKRTR10BHIBLA	—	—	No match found	—	—
25	—	—	—	—	—	—	—	—
26	—	—	—	—	—	—	—	—
27	—	—	—	—	—	—	—	—
28	—	—	—	—	—	—	—	—
29	—	—	—	—	—	—	—	—
30	—	—	—	—	—	—	—	—
31	—	—	—	—	—	—	—	—
32	—	—	—	—	—	—	—	—
33	—	—	—	—	—	—	—	—
34	—	—	—	—	—	—	—	—
35	—	—	—	—	—	—	—	—
36	—	—	—	—	—	—	—	—
37	—	—	—	—	—	—	—	—
38	—	—	—	—	—	—	—	—



E029204.35A [1063] Truck Neat TSBA

Volume: DATA File: E029204.35A Seq Counter: 5 ID Number: 1063
Type: Samp Bottle: 3 Method: TSBA40
Created: 9/20/02 12:08:00 PM
Sample ID: Truck Neat TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.780	2.655E+8	0.031	----	6.988	SOLVENT PEAK	----	< min rt	
1.903	4664	0.036	----	7.210		----	< min rt	
7.354	1136	0.045	0.998	13.619	14:0 ISO	0.87	ECL deviates 0.000	Reference 0.008
8.904	24891	0.043	0.971	14.623	15:0 ISO	18.46	ECL deviates 0.000	Reference 0.007
9.050	63599	0.042	0.969	14.714	15:0 ANTEISO	47.07	ECL deviates 0.001	Reference 0.008
10.180	1227	0.045	0.955	15.390	16:1 w7c alcohol	0.89	ECL deviates 0.003	
10.587	3660	0.045	0.951	15.627	16:0 ISO	2.66	ECL deviates 0.000	Reference 0.007
10.816	1746	0.044	0.949	15.760	16:1 w11c	1.26	ECL deviates 0.003	
11.224	1866	0.048	0.945	15.998	16:0	1.35	ECL deviates -0.002	Reference 0.005
11.919	5422	0.046	0.939	16.391	ISO 17:1 w10c	3.89	ECL deviates 0.003	
12.078	3825	0.047	0.938	16.481	Sum In Feature 4	2.74	ECL deviates 0.005	17:1 ISO I/ANTEI B
12.342	11082	0.047	0.936	16.630	17:0 ISO	7.92	ECL deviates 0.000	Reference 0.007
12.507	18062	0.047	0.935	16.723	17:0 ANTEISO	12.89	ECL deviates 0.000	Reference 0.007
----	3825	---	----	----	Summed Feature 4	2.74	17:1 ISO I/ANTEI B	17:1 ANTEISO B/i I

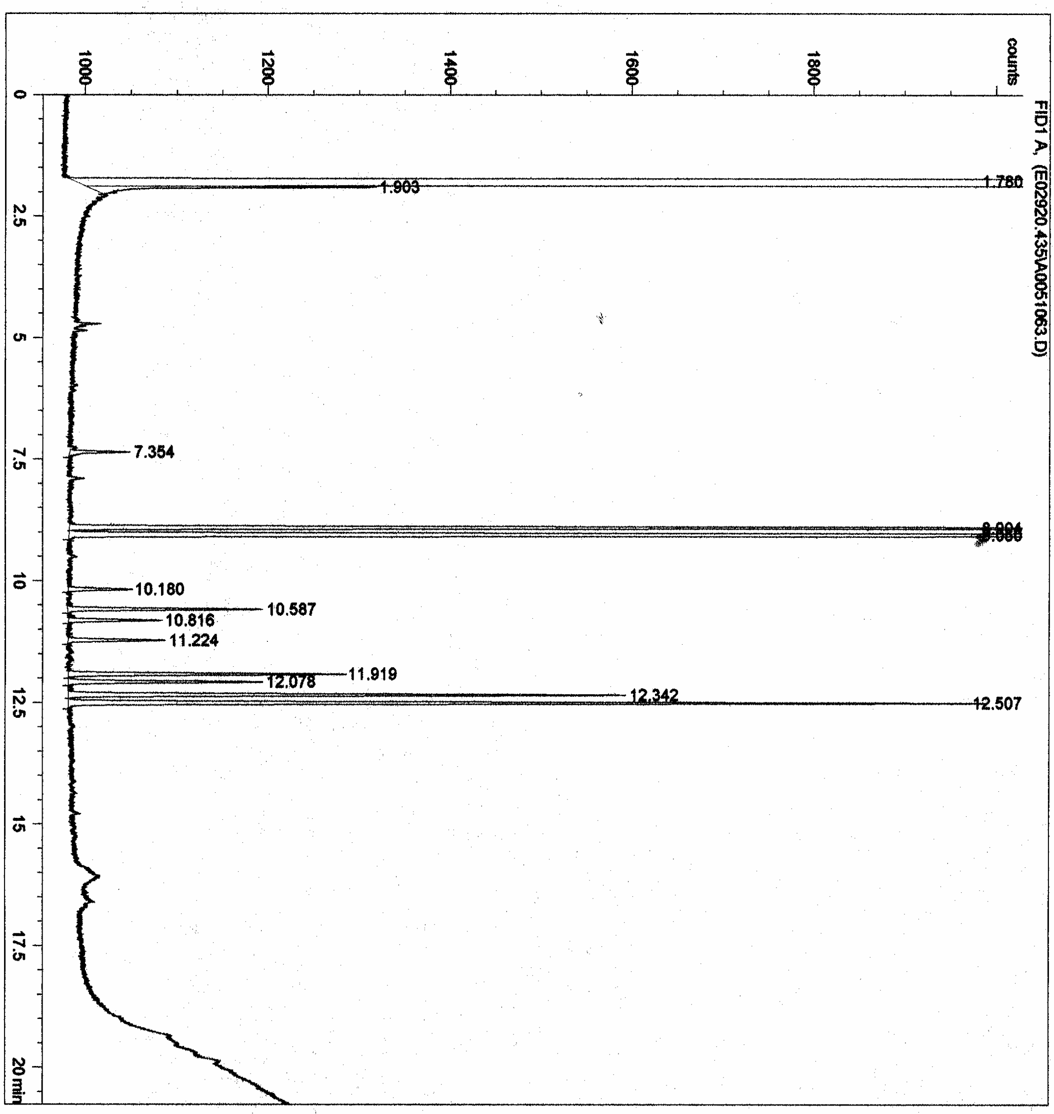
ECL Deviation: 0.002 Reference ECL Shift: 0.007 Number Reference Peaks: 7
Total Response: 136516 Total Named: 136516
Percent Named: 100.00% Total Amount: 130967

Matches:

Library	Sim Index	Entry Name
TSBA40 4.10	0.541	Paenibacillus-apiarius** ("Bacillus apiarius")
	0.476	Bacillus-subtilis*
	0.425	Brevibacillus-brevis* (Bacillus)

```
Data File C:\SHERLOCK\RAW\E02920.435\A0051063.D                    Sample Name:
    Sherlock Id: Truck Neat TSBA

    ===================================================================
    Injection Date  : 9/20/02 12:12:10 PM
    Sample Name     :  1063                          Location : Vial 3
    Acq. Operator   :
                                                   Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 9/20/02 12:08:00 PM
    ===================================================================
```



*** End of Report ***

E029204.35A [1057] Truck 1:10 TSBA

Volume: DATA	File: E029204.35A	Seq Counter: 4	ID Number: 1057
Type: Samp	Bottle: 4	Method: TSBA40	

Created: 9/20/02 11:42:22 AM
Sample ID: Truck 1:10 TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.778	2.67E+8	0.031	----	6.990	SOLVENT PEAK	----	< min rt	
1.901	4449	0.043	----	7.210		----	< min rt	
7.347	1402	0.042	0.998	13.618	14:0 ISO	1.06	ECL deviates -0.001	Reference 0.003
8.898	22487	0.043	0.971	14.623	15:0 ISO	16.56	ECL deviates 0.000	Reference 0.003
9.044	66248	0.042	0.969	14.714	15:0 ANTEISO	48.68	ECL deviates 0.001	Reference 0.004
10.173	1501	0.044	0.955	15.391	16:1 w7c alcohol	1.09	ECL deviates 0.004	
10.579	3950	0.044	0.951	15.627	16:0 ISO	2.85	ECL deviates 0.000	Reference 0.002
10.808	1884	0.046	0.949	15.760	16:1 w11c	1.36	ECL deviates 0.003	
11.216	1958	0.046	0.945	15.998	16:0	1.40	ECL deviates -0.002	Reference 0.000
11.911	5352	0.048	0.939	16.391	ISO 17:1 w10c	3.81	ECL deviates 0.003	
12.071	4492	0.048	0.938	16.482	Sum In Feature 4	3.19	ECL deviates -0.004	17:1 ANTEISO B/i I
12.334	9322	0.047	0.936	16.630	17:0 ISO	6.61	ECL deviates 0.000	Reference 0.003
12.499	18882	0.045	0.935	16.724	17:0 ANTEISO	13.38	ECL deviates 0.001	Reference 0.003
16.583	4506	0.134	----	19.022		----	> max ar/ht	
17.261	3997	0.130	----	19.416		----	> max ar/ht	
19.202	365	0.041	----	20.542		----	> max rt	
19.872	5468	0.045	----	20.932		----	> max rt	
19.953	1392	0.048	----	20.979		----	> max rt	
----	4492	---	----	----	Summed Feature 4	3.19	17:1 ISO I/ANTEI B	17:1 ANTEISO B/i I

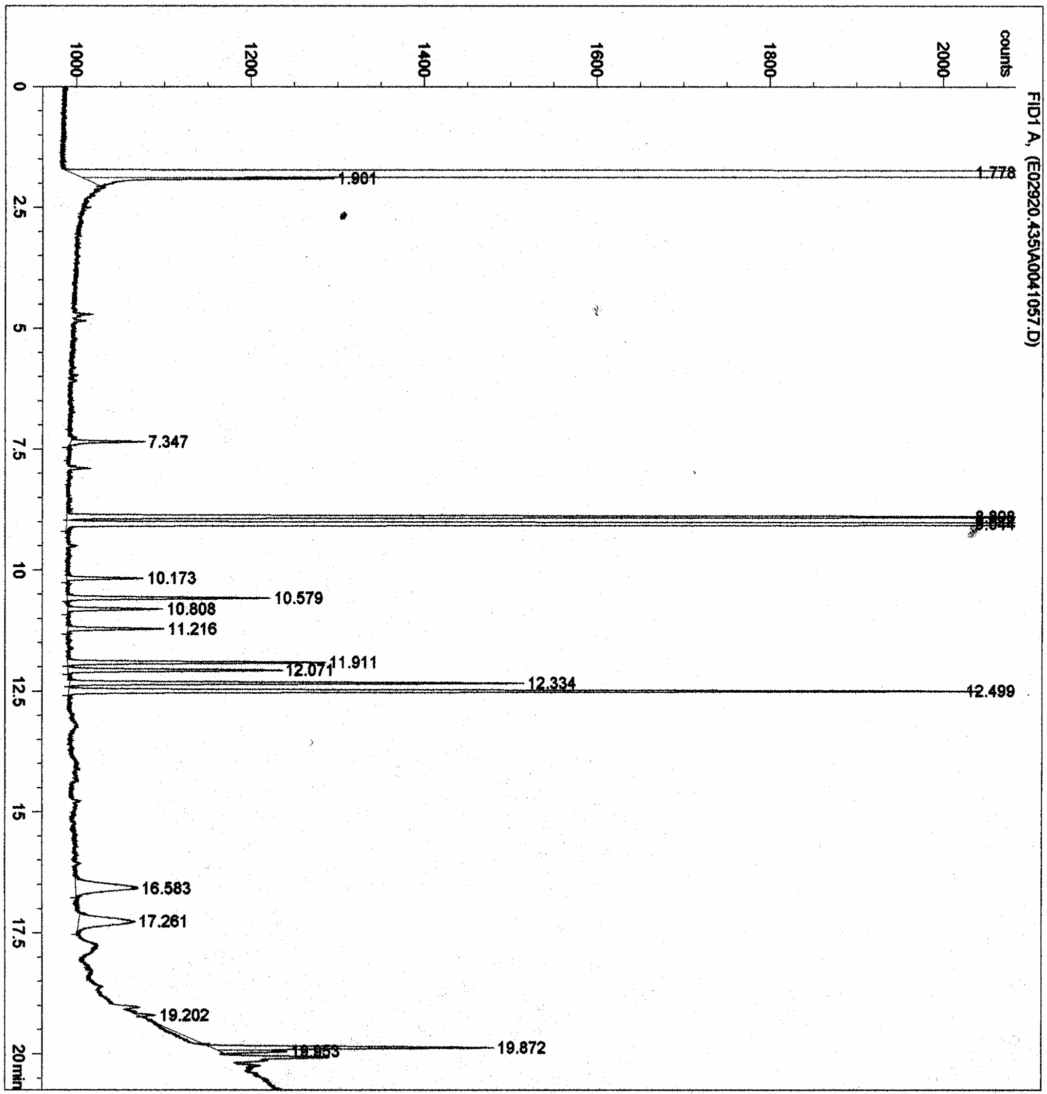
ECL Deviation: 0.002	Reference ECL Shift: 0.003	Number Reference Peaks: 7
Total Response: 145982	Total Named: 137479	
Percent Named: 94.18%	Total Amount: 131900	

Matches:

Library	Sim Index	Entry Name
TSBA40 4.10	0.678	Paenibacillus-apiarius** ("Bacillus apiarius")
	0.589	Brevibacillus-brevis* (Bacillus)
	0.386	Bacillus-subtilis*

```
Data File C:\SHERLOCK\RAW\E02920.435\A0041057.D                          Sample Name:
    Sherlock Id: Truck 1:10 TSBA

    ====================================================================
    Injection Date  : 9/20/02 11:46:34 AM
    Sample Name     :  1057                          Location : Vial 4
    Acq. Operator   :
                                                   Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 9/20/02 11:42:22 AM
    ====================================================================
```



*** End of Report ***

Appendix B: Worksheet / Sample Data – Holloman AFB (KHMN)

	Site Information									
#	Sample	Type / Number	Date Sampled	Dilution	Medium	Temp °C	% Relative Humidity	Inches Rainfall (last 30 days)	Fuel Type	Sample Method
1	Neg Control	—	—	—	—	—	—	—	—	—
2	Pos Control	—	—	—	—	—	—	—	—	—
3	Tank	recovery	9/9/2002	Neat	TSBA	29	29	0.95	JP-8	gravity
4	Tank	recovery	9/9/2002	1:10	TSBA	29	29	0.95	JP-8	gravity
5	Tank	recovery	9/9/2002	1:100	TSBA	29	29	0.95	JP-8	gravity
6	Tank	recovery	9/9/2002	1:1000	TSBA	29	29	0.95	JP-8	gravity
7	Tank	recovery	9/9/2002	Neat	SDA	29	29	0.95	JP-8	gravity
8	Tank	recovery	9/9/2002	1:10	SDA	29	29	0.95	JP-8	gravity
9	Tank	recovery	9/9/2002	1:100	SDA	29	29	0.95	JP-8	gravity
10	Tank	recovery	9/9/2002	1:1000	SDA	29	29	0.95	JP-8	gravity
11	Tank	recovery	9/9/2002	Neat	BHIBLA	29	29	0.95	JP-8	gravity
12	Tank	recovery	9/9/2002	1:10	BHIBLA	29	29	0.95	JP-8	gravity
13	Tank	recovery	9/9/2002	1:100	BHIBLA	29	29	0.95	JP-8	gravity
14	Tank	recovery	9/9/2002	1:1000	BHIBLA	29	29	0.95	JP-8	gravity
15	Truck	F-34 #375	9/9/2002	Neat	TSBA	29	29	0.95	JP-8	gravity
16	Truck	F-34 #375	9/9/2002	1:10	TSBA	29	29	0.95	JP-8	gravity
17	Truck	F-34 #375	9/9/2002	1:100	TSBA	29	29	0.95	JP-8	gravity
18	Truck	F-34 #375	9/9/2002	1:1000	TSBA	29	29	0.95	JP-8	gravity
19	Truck	F-34 #375	9/9/2002	Neat	SDA	29	29	0.95	JP-8	gravity
20	Truck	F-34 #375	9/9/2002	1:10	SDA	29	29	0.95	JP-8	gravity
21	Truck	F-34 #375	9/9/2002	1:100	SDA	29	29	0.95	JP-8	gravity
22	Truck	F-34 #375	9/9/2002	1:1000	SDA	29	29	0.95	JP-8	gravity
23	Truck	F-34 #375	9/9/2002	Neat	BHIBLA	29	29	0.95	JP-8	gravity
24	Truck	F-34 #375	9/9/2002	1:10	BHIBLA	29	29	0.95	JP-8	gravity
25	Truck	F-34 #375	9/9/2002	1:100	BHIBLA	29	29	0.95	JP-8	gravity
26	Truck	F-34 #375	9/9/2002	1:1000	BHIBLA	29	29	0.95	JP-8	gravity
27	Aircraft	F-117	9/9/2002	Neat	TSBA	29	29	0.95	JP-8	gravity
28	Aircraft	F-117	9/9/2002	1:10	TSBA	29	29	0.95	JP-8	gravity
29	Aircraft	F-117	9/9/2002	1:100	TSBA	29	29	0.95	JP-8	gravity
30	Aircraft	F-117	9/9/2002	1:1000	TSBA	29	29	0.95	JP-8	gravity
31	Aircraft	F-117	9/9/2002	Neat	SDA	29	29	0.95	JP-8	gravity
32	Aircraft	F-117	9/9/2002	1:10	SDA	29	29	0.95	JP-8	gravity
33	Aircraft	F-117	9/9/2002	1:100	SDA	29	29	0.95	JP-8	gravity
34	Aircraft	F-117	9/9/2002	1:1000	SDA	29	29	0.95	JP-8	gravity
35	Aircraft	F-117	9/9/2002	Neat	BHIBLA	29	29	0.95	JP-8	gravity
36	Aircraft	F-117	9/9/2002	1:10	BHIBLA	29	29	0.95	JP-8	gravity
37	Aircraft	F-117	9/9/2002	1:100	BHIBLA	29	29	0.95	JP-8	gravity
38	Aircraft	F-117	9/9/2002	1:1000	BHIBLA	29	29	0.95	JP-8	gravity

— Indicates analysis omitted

		Sample Description					
#	Tank Composition	pH	Sample Color	General Description	Obvious Solids	TDS (mg/L)	Free Phase Water
1	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—
3	steel w/ epoxy lining	—	light yellow	clear	no	—	no
4	steel w/ epoxy lining	—	light yellow	clear	no	—	no
5	steel w/ epoxy lining	—	light yellow	clear	no	—	no
6	steel w/ epoxy lining	—	light yellow	clear	no	—	no
7	steel w/ epoxy lining	—	light yellow	clear	no	—	no
8	steel w/ epoxy lining	—	light yellow	clear	no	—	no
9	steel w/ epoxy lining	—	light yellow	clear	no	—	no
10	steel w/ epoxy lining	—	light yellow	clear	no	—	no
11	steel w/ epoxy lining	—	light yellow	clear	no	—	no
12	steel w/ epoxy lining	—	light yellow	clear	no	—	no
13	steel w/ epoxy lining	—	light yellow	clear	no	—	no
14	steel w/ epoxy lining	—	light yellow	clear	no	—	no
15	aluminum	6.3	light yellow	clear	no	—	yes
16	aluminum	6.3	light yellow	clear	no	—	yes
17	aluminum	6.3	light yellow	clear	no	—	yes
18	aluminum	6.3	light yellow	clear	no	—	yes
19	aluminum	6.3	light yellow	clear	no	—	yes
20	aluminum	6.3	light yellow	clear	no	—	yes
21	aluminum	6.3	light yellow	clear	no	—	yes
22	aluminum	6.3	light yellow	clear	no	—	yes
23	aluminum	6.3	light yellow	clear	no	—	yes
24	aluminum	6.3	light yellow	clear	no	—	yes
25	aluminum	6.3	light yellow	clear	no	—	yes
26	aluminum	6.3	light yellow	clear	no	—	yes
27	aluminum	—	dark yellow	clear	yes	—	no
28	aluminum	—	dark yellow	clear	yes	—	no
29	aluminum	—	dark yellow	clear	yes	—	no
30	aluminum	—	dark yellow	clear	yes	—	no
31	aluminum	—	dark yellow	clear	yes	—	no
32	aluminum	—	dark yellow	clear	yes	—	no
33	aluminum	—	dark yellow	clear	yes	—	no
34	aluminum	—	dark yellow	clear	yes	—	no
35	aluminum	—	dark yellow	clear	yes	—	no
36	aluminum	—	dark yellow	clear	yes	—	no
37	aluminum	—	dark yellow	clear	yes	—	no
38	aluminum	—	dark yellow	clear	yes	—	no

	Light Microscopy				**Colony Description**		**Quantity Data**		
#	**Gram +/-**	**Organism Shape**	**Length of Cells (μm)**	**Width of Cells (μm)**	**Colony Type**	**Colony Color**	**Col/0.1 mL 24 hours**	**Col/0.1 mL 48 hours**	**Col/mL 24 hours**
1	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—
3	neg	bacillus	1	0.5	round	yellow	0	0	5 days to grow
4	—	—	—	—	—	—	0	0	0
5	—	—	—	—	—	—	0	0	0
6	neg	coccus	1 diameter	—	round	white	0	0	5 days to grow
7	—	—	—	—	—	—	0	0	0
8	—	—	—	—	—	—	0	0	0
9	—	—	—	—	—	—	0	0	0
10	neg	coccus	1 diameter	—	round	white	0	0	5 days to grow
11	—	—	—	—	—	—	0	0	0
12	—	—	—	—	—	—	0	0	0
13	neg	coccus	1 diameter	—	round	white	0	0	5 days to grow
14	—	—	—	—	—	—	0	0	0
15	—	—	—	—	—	—	0	0	0
16	neg	bacillus	1	0.5	round	yellow	0	0	5 days to grow
17	neg	bacillus	1	0.5	round	yellow	0	0	5 days to grow
18	neg	coccus	1 diameter	—	round	white	0	0	5 days to grow
19	—	—	—	—	—	—	0	0	0
20	neg	bacillus	1	0.5	round	yellow	0	0	5 days to grow
21	neg	bacillus	1	0.5	round	yellow	0	0	5 days to grow
22	neg	coccus	1 diameter	—	round	white	0	0	5 days to grow
23	—	—	—	—	—	—	0	0	0
24	neg	coccus	1 diameter		round	white	0	0	5 days to grow
25	—	—	—	—	—	—	0	0	0
26	—	—	—	—	—	—	0	0	0
27	neg	bacillus	1	0.5	round	yellow	0	0	5 days to grow
28	neg	bacillus	1	0.5	round	yellow	0	0	5 days to grow
29	neg	bacillus	1	0.5	round	yellow	0	0	5 days to grow
30	neg	coccus	1 diameter	—	round	white	0	0	5 days to grow
31	—	—	—	—	—	—	0	0	0
32	—	—	—	—	—	—	0	0	0
33	neg	coccus	1 diameter	—	round	white	0	0	5 days to grow
34	neg	coccus	1 diameter	—	round	white	0	0	5 days to grow
35	—	—	—	—	—	—	0	0	0
36	neg	coccus	1 diameter	—	round	white	0	0	5 days to grow
37	—	—	—	—	—	—	0	0	0
38	—	—	—	—	—	—	0	0	0

				Identification Data				
				FAME analysis			DNA gene sequence	
#	**Bacteria vs. Fungi**	**Spores**	**Digital Photo**	**Sim Index**	**> .1 S. I. Separation**	**GC Best Match**	**DNA % ID**	**DNA Best Match**
1	—	—	—	—	—	Library match not attempted	—	—
2	—	—	—	0.425	yes	*Stenotrophomonas-maltophilia*	—	—
3	bacteria	no	—	0.016	no	*Brevundimonas-vesicularis (Pseudomanas-vesicularis)*	99	*Sphingomonas sanguinis*
4	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—
6	bacteria	no	—	—	—	No match found	—	—
7	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—
10	bacteria	no	—	—	—	No match found	—	—
11	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—
13	bacteria	no	—	—	—	No match found	—	—
14	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—
16	bacteria	no	HMNTR10TSBA	0.144	no	*Brevundimonas-vesicularis (Pseudomanas-vesicularis)*	99.91	*Bacillus licheniformis*
17	bacteria	no	—	—	—	No match found	—	—
18	bacteria	no	—	—	—	No match found	—	—
19	—	—	—	—	—	—	—	—
20	bacteria	no	—	—	—	No match found	—	—
21	bacteria	no	—	—	—	No match found	—	—
22	bacteria	no	—	—	—	Library match not attempted	—	—
23	—	—	—	—	—	—	—	—
24	bacteria	no	HMNTR10BHI			No match found	—	—
25	—	—	—	—	—	—	—	—
26	—	—	—	—	—	—	—	—
27	bacteria	no	—	0.027	no	*Brevundimonas-vesicularis (Pseudomanas-vesicularis)*	99.91	*Bacillus licheniformis*
28	bacteria	no	—	0.011	no	*Brevundimonas-vesicularis (Pseudomanas-vesicularis)*	99	*Sphingomonas sanguinis*
29	bacteria	no	—	0.368	yes	*Cellulomonas-flavigena*	99.81	*Bacillus pumilus*
30	bacteria	no	—	—	—	No match found	—	—
31	—	—	—	—	—	—	—	—
32	—	—	—	—	—	—	—	—
33	bacteria	no	—	—	—	No match found	—	—
34	bacteria	no	—	—	—	No match found	—	—
35	—	—	—	—	—	—	—	—
36	bacteria	no	HMNAC10BHI	—	—	No match found	—	—
37	—	—	—	—	—	—	—	—
38	—	—	—	—	—	—	—	—

E029306.38A [1072] Holloman tank neat TSBA

Volume: DATA File: E029306.38A Seq Counter: 5 ID Number: 1072
Type: Samp Bottle: 3 Method: TSBA40
Created: 9/30/02 5:03:13 PM
Sample ID: Holloman tank neat TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.777	2.286E+8	0.030	----	7.004	SOLVENT PEAK	----	< min rt	
1.890	6256	0.033	----	7.209		----	< min rt	
1.989	869	0.033	----	7.386		----	< min rt	
7.888	535	0.041	0.981	14.001	14:0	0.63	ECL deviates 0.001	Reference 0.013
9.845	7611	0.045	0.943	15.205	14:0 2OH	8.65	ECL deviates 0.002	
10.900	1870	0.047	0.929	15.820	Sum In Feature 3	2.09	ECL deviates -0.002	16:1 w7c/15 iso 2OH
11.207	27269	0.044	0.925	15.999	16:0	30.42	ECL deviates -0.001	Reference 0.010
14.453	44807	0.048	0.899	17.825	18:1 w7c	48.58	ECL deviates 0.002	
14.766	697	0.042	0.898	18.001	18:0	0.75	ECL deviates 0.001	Reference 0.012
14.910	6426	0.049	0.897	18.082	11 methyl 18:1 w7c	6.95	ECL deviates 0.001	
16.369	1792	0.051	0.889	18.906	19:0 CYCLO w8c	1.92	ECL deviates 0.004	
16.584	808	0.048	----	19.028		----		
----	1870	---	----	----	Summed Feature 3	2.09	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c

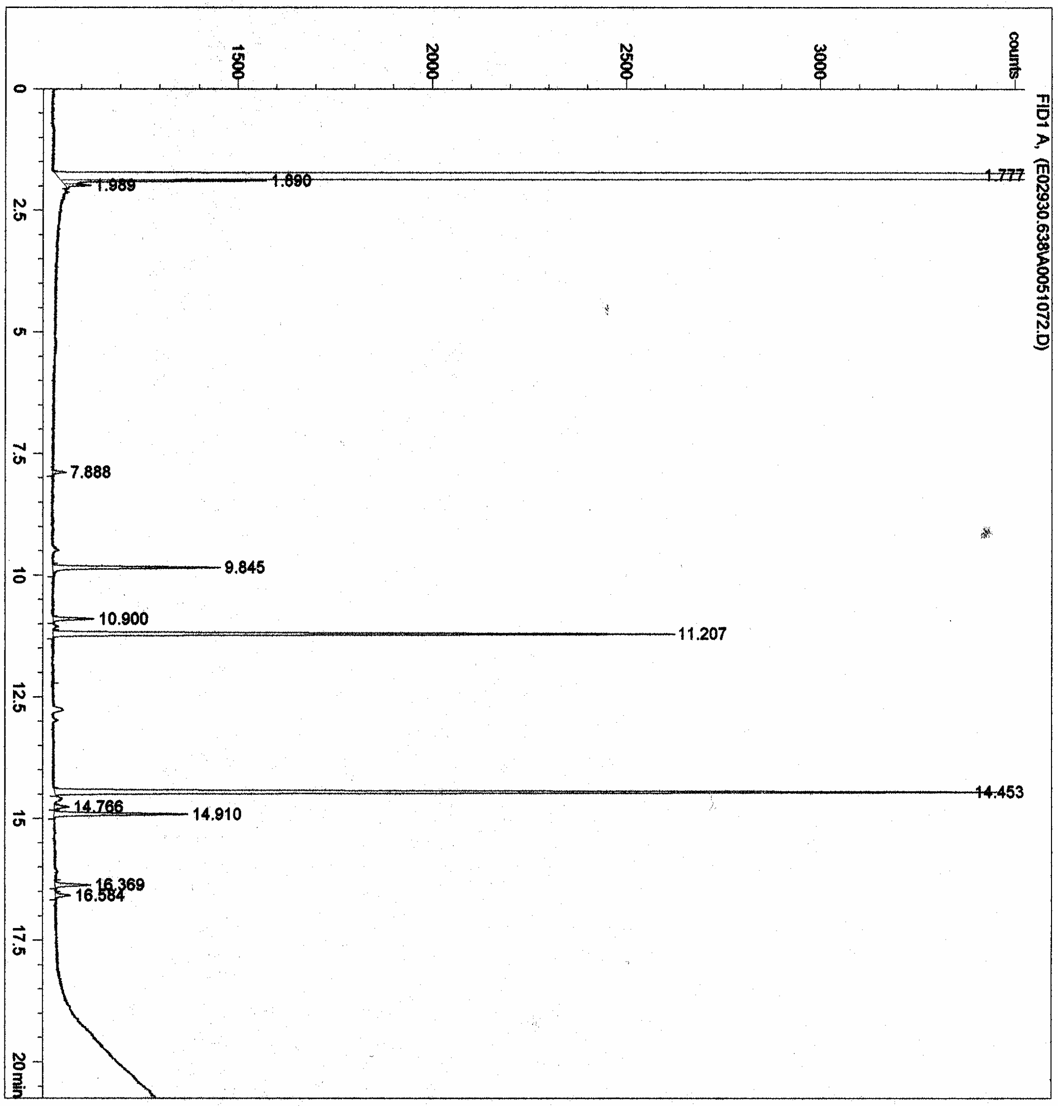
ECL Deviation: 0.002 Reference ECL Shift: 0.012 Number Reference Peaks: 3
Total Response: 91817 Total Named: 91009
Percent Named: 99.12% Total Amount: 82946

Matches:

Library	**Sim Index**	**Entry Name**
TSBA40 4.10	0.016	Brevundimonas-vesicularis* (Pseudomonas vesicularis)
	0.011	Brevundimonas-diminuta* (Pseudomonas diminuta)

```
Data File C:\SHERLOCK\RAW\E02930.638\A0051072.D                                 Sample Name:
   Sherlock Id: Holloman tank neat TSBA

   =====================================================================
   Injection Date  : 9/30/02 5:07:21 PM
   Sample Name     :  1072                           Location : Vial 3
   Acq. Operator   :
                                                   Inj Volume : 2 µl
   Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
   Last changed    : 9/30/02 5:03:13 PM
   =====================================================================
```



*** End of Report ***

E029306.38A [1074] truck 1:10 TSBA

Volume: DATA File: E029306.38A Seq Counter: 7 ID Number: 1074
Type: Samp Bottle: 5 Method: TSBA40
Created: 9/30/02 5:54:25 PM
Sample ID: truck 1:10 TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.778	2.174E+8	0.030	----	6.993	SOLVENT PEAK	----	< min rt	
1.891	7141	0.031	----	7.197		----	< min rt	
1.990	1179	0.032	----	7.375		----	< min rt	
7.891	1762	0.039	0.981	13.998	14:0	1.22	ECL deviates -0.002	Reference 0.014
9.497	1691	0.045	0.948	15.003	15:0	1.14	ECL deviates 0.003	Reference 0.014
9.851	7307	0.046	0.943	15.210	14:0 2OH	4.88	ECL deviates 0.007	
10.904	8131	0.045	0.929	15.825	Sum In Feature 3	5.35	ECL deviates 0.003	16:1 w7c/15 iso 2OH
11.213	44916	0.044	0.925	16.005	16:0	29.46	ECL deviates 0.005	Reference 0.014
12.748	1460	0.073	0.911	16.874	17:0 CYCLO	0.94	ECL deviates -0.014	Reference -0.006
12.981	708	0.046	0.909	17.005	17:0	0.46	ECL deviates 0.005	Reference 0.013
14.459	73579	0.049	0.899	17.831	18:1 w7c	46.90	ECL deviates 0.008	
14.767	1143	0.045	0.898	18.003	18:0	0.73	ECL deviates 0.003	Reference 0.013
14.916	9795	0.048	0.897	18.086	11 methyl 18:1 w7c	6.23	ECL deviates 0.005	
16.371	4272	0.053	0.889	18.905	19:0 CYCLO w8c	2.69	ECL deviates 0.003	
----	8131	---	----	----	Summed Feature 3	5.35	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c

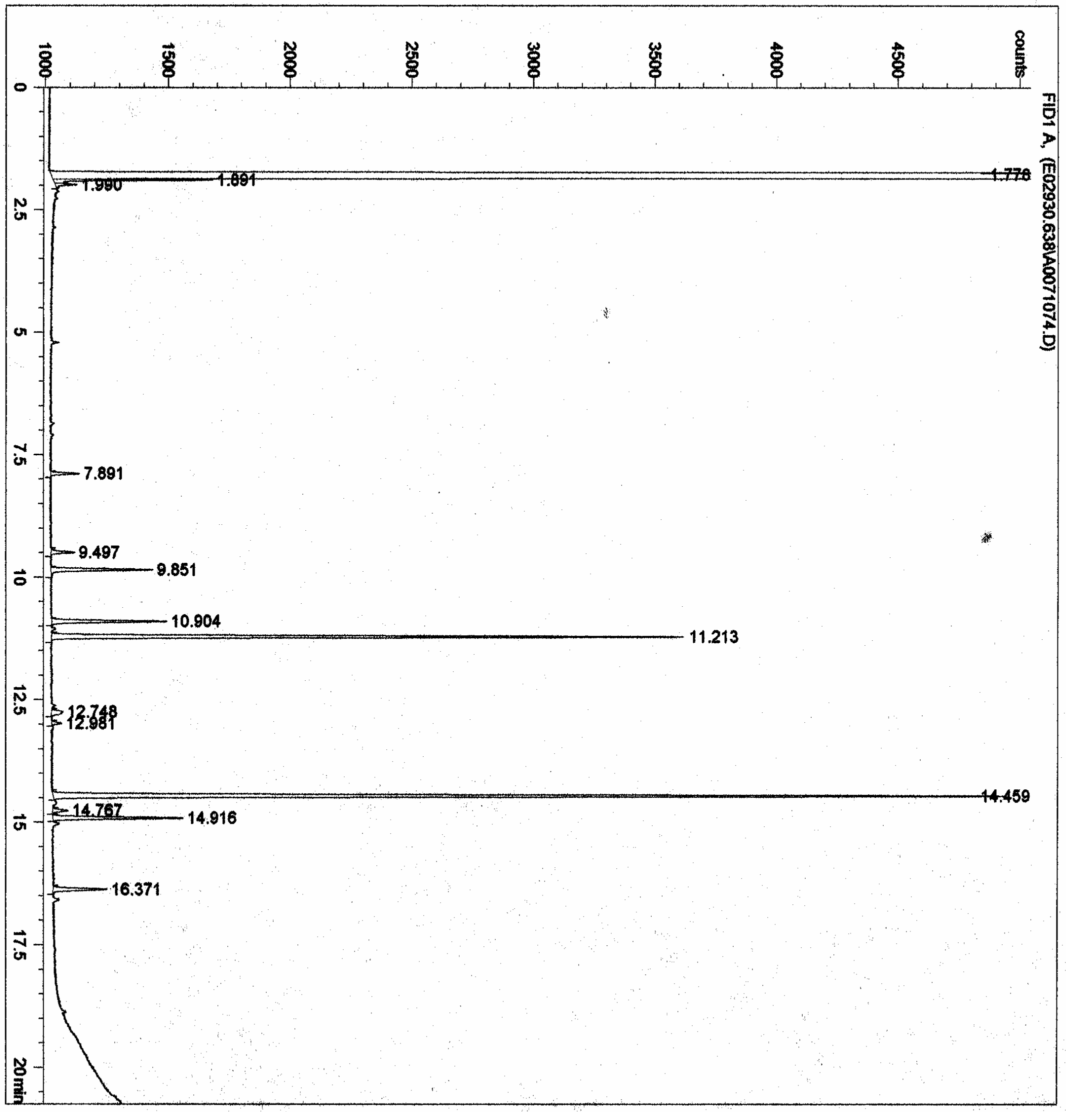
ECL Deviation: 0.006 Reference ECL Shift: 0.013 Number Reference Peaks: 6
Total Response: 154763 Total Named: 154763
Percent Named: 100.00% Total Amount: 141080

Matches:

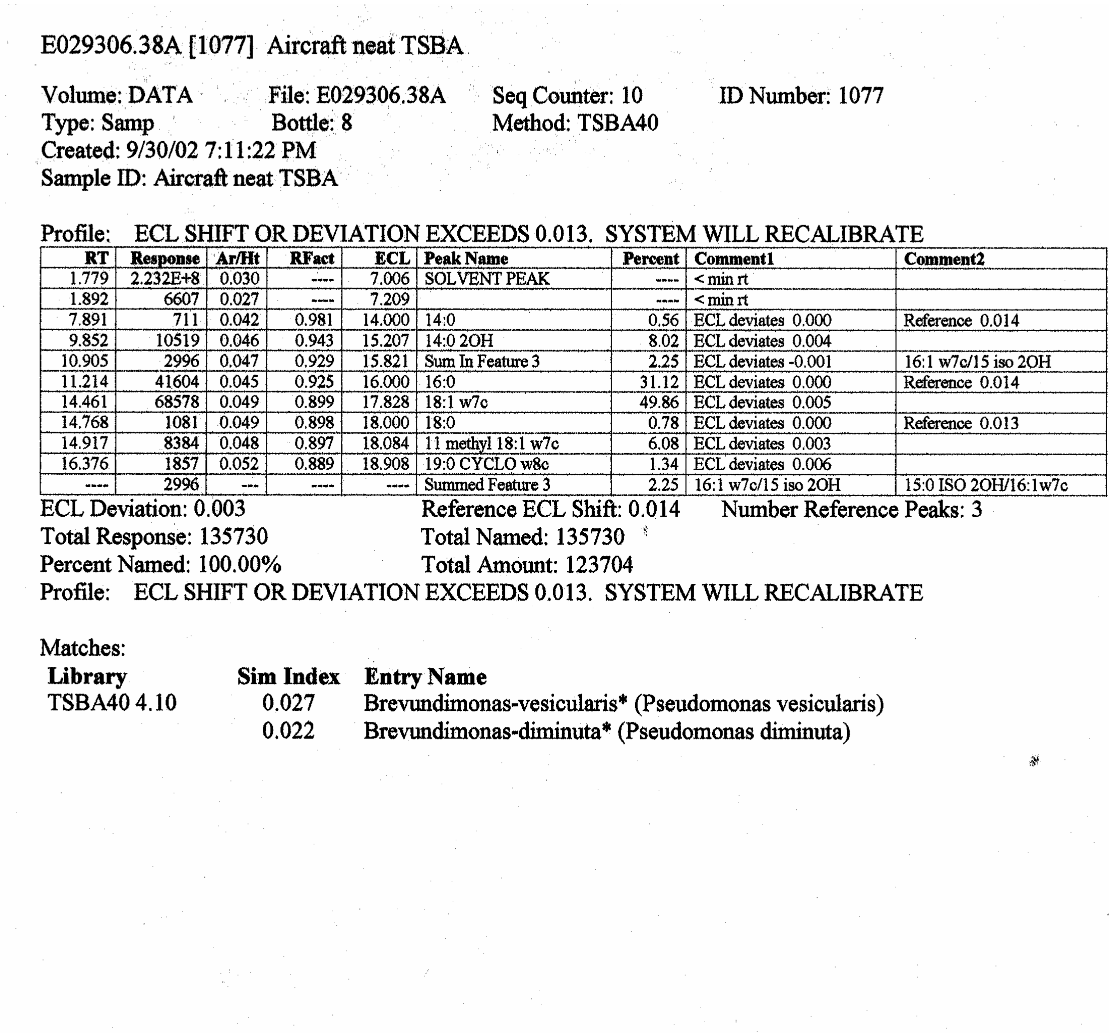
Library	Sim Index	Entry Name
TSBA40 4.10	0.144	Brevundimonas-vesicularis* (Pseudomonas vesicularis)
	0.078	Brevundimonas-diminuta* (Pseudomonas diminuta)

```
Data File C:\SHERLOCK\RAW\E02930.638\A0071074.D                                    Sample Name:
    Sherlock Id: truck 1:10 TSBA

    =====================================================================
    Injection Date   : 9/30/02 5:58:36 PM
    Sample Name      :  1074                              Location : Vial 5
    Acq. Operator    :
                                                        Inj Volume : 2 µl
    Method           : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed     : 9/30/02 5:54:25 PM
    =====================================================================
```



*** End of Report ***



E029306.38A [1077] Aircraft neat TSBA

Volume: DATA File: E029306.38A Seq Counter: 10 ID Number: 1077
Type: Samp Bottle: 8 Method: TSBA40
Created: 9/30/02 7:11:22 PM
Sample ID: Aircraft neat TSBA

Profile: ECL SHIFT OR DEVIATION EXCEEDS 0.013. SYSTEM WILL RECALIBRATE

RT	**Response**	**Ar/Ht**	**RFact**	**ECL**	**Peak Name**	**Percent**	**Comment1**	**Comment2**
1.779	2.232E+8	0.030	----	7.006	SOLVENT PEAK	----	< min rt	
1.892	6607	0.027	----	7.209		----	< min rt	
7.891	711	0.042	0.981	14.000	14:0	0.56	ECL deviates 0.000	Reference 0.014
9.852	10519	0.046	0.943	15.207	14:0 2OH	8.02	ECL deviates 0.004	
10.905	2996	0.047	0.929	15.821	Sum In Feature 3	2.25	ECL deviates -0.001	16:1 w7c/15 iso 2OH
11.214	41604	0.045	0.925	16.000	16:0	31.12	ECL deviates 0.000	Reference 0.014
14.461	68578	0.049	0.899	17.828	18:1 w7c	49.86	ECL deviates 0.005	
14.768	1081	0.049	0.898	18.000	18:0	0.78	ECL deviates 0.000	Reference 0.013
14.917	8384	0.048	0.897	18.084	11 methyl 18:1 w7c	6.08	ECL deviates 0.003	
16.376	1857	0.052	0.889	18.908	19:0 CYCLO w8c	1.34	ECL deviates 0.006	
----	2996	---	----	----	Summed Feature 3	2.25	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c

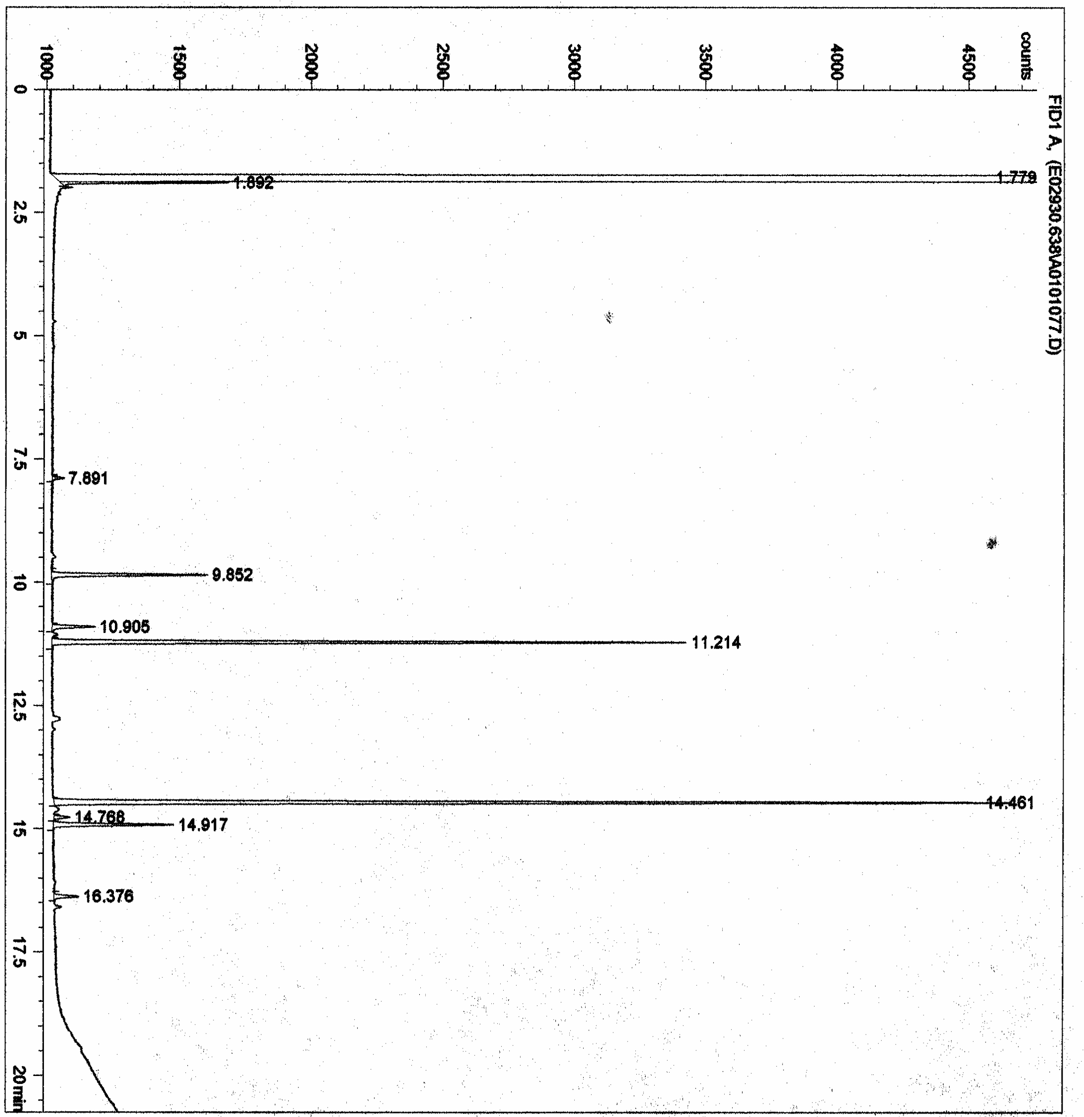
ECL Deviation: 0.003 Reference ECL Shift: 0.014 Number Reference Peaks: 3
Total Response: 135730 Total Named: 135730
Percent Named: 100.00% Total Amount: 123704
Profile: ECL SHIFT OR DEVIATION EXCEEDS 0.013. SYSTEM WILL RECALIBRATE

Matches:

Library	**Sim Index**	**Entry Name**
TSBA40 4.10	0.027	Brevundimonas-vesicularis* (Pseudomonas vesicularis)
	0.022	Brevundimonas-diminuta* (Pseudomonas diminuta)

```
Data File C:\SHERLOCK\RAW\E02930.638\A0101077.D                          Sample Name:
    Sherlock Id: Aircraft heat TSBA

    =====================================================================
    Injection Date  : 9/30/02 7:15:31 PM
    Sample Name     :  1077                          Location : Vial 8
    Acq. Operator   :
                                                   Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 9/30/02 7:11:22 PM
    =====================================================================
```



```
*** End of Report ***
```

E02A013.61A [1078] Aircraft 1:10 TSBA

Volume: DATA File: E02A013.61A Seq Counter: 4 ID Number: 1078
Type: Samp Bottle: 9 Method: TSBA40
Created: 10/1/02 9:55:12 AM
Sample ID: Aircraft 1:10 TSBA

Profile:

RT	**Response**	**Ar/Ht**	**RFact**	**ECL**	**Peak Name**	**Percent**	**Comment1**	**Comment2**
1.778	2.35E+8	0.030	----	7.010	SOLVENT PEAK	----	< min rt	
1.894	5028	0.032	----	7.217		----	< min rt	
1.992	911	0.032	----	7.394		----	< min rt	
7.889	769	0.041	0.983	13.999	14:0	0.71	ECL deviates -0.001	Reference 0.005
9.848	9842	0.045	0.944	15.213	14:0 2OH	8.74	ECL deviates 0.010	
10.902	2096	0.046	0.929	15.829	Sum In Feature 3	1.83	ECL deviates 0.007	16:1 w7c/15 iso 2OH
11.209	38534	0.045	0.925	16.008	16:0	33.54	ECL deviates 0.008	Reference 0.004
12.752	1180	0.077	0.910	16.880	17:0 CYCLO	1.01	ECL deviates -0.008	Reference -0.013
14.457	51952	0.049	0.899	17.831	18:1 w7c	43.92	ECL deviates 0.008	
14.764	1200	0.050	0.897	18.001	18:0	1.01	ECL deviates 0.001	Reference 0.002
14.912	8422	0.050	0.896	18.084	11 methyl 18:1 w7c	7.10	ECL deviates 0.003	
16.373	2544	0.049	0.890	18.903	19:0 CYCLO w8c	2.13	ECL deviates 0.001	Reference 0.009
----	2096	---	----	----	Summed Feature 3	1.83	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c

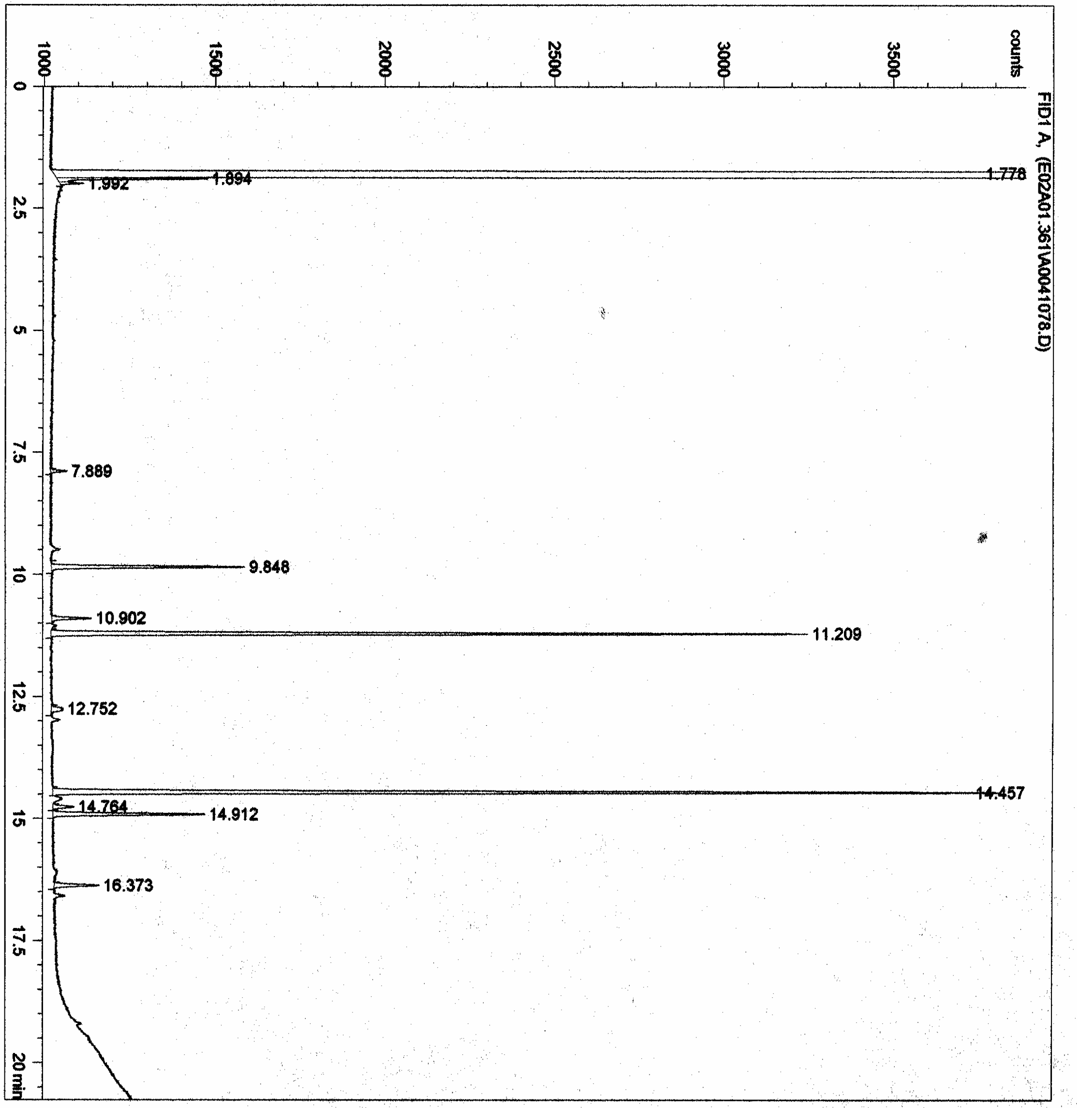
ECL Deviation: 0.006 Reference ECL Shift: 0.007 Number Reference Peaks: 5
Total Response: 116539 Total Named: 116539
Percent Named: 100.00% Total Amount: 106306

Matches:

Library	**Sim Index**	**Entry Name**
TSBA40 4.10	0.011	Brevundimonas-vesicularis* (Pseudomonas vesicularis)
	0.007	Brevundimonas-diminuta* (Pseudomonas diminuta)

```
Data File C:\SHERLOCK\RAW\E02A01.361\A0041078.D                                    Sample Name:
    Sherlock Id: Aircraft 1:10 TSBA

    =====================================================================
    Injection Date   : 10/1/02 9:59:24 AM
    Sample Name      :  1078                          Location : Vial 9
    Acq. Operator    :
                                                    Inj Volume : 2 µl
    Method           : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed     : 10/1/02 9:55:12 AM
    =====================================================================
```



*** End of Report ***

E02A013.61A [1079] Aircraft 1:100 TSBA

Volume: DATA File: E02A013.61A Seq Counter: 5 ID Number: 1079
Type: Samp Bottle: 10 Method: TSBA40
Created: 10/1/02 10:20:51 AM
Sample ID: Aircraft 1:100 TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.779	2.303E+8	0.030	----	7.001	SOLVENT PEAK	----	< min rt	
1.899	3177	0.030	----	7.217		----	< min rt	
1.959	986	0.038	----	7.324		----	< min rt	
7.342	9052	0.040	0.998	13.619	14:0 ISO	2.02	ECL deviates 0.000	Reference 0.008
7.891	1165	0.041	0.983	13.998	14:0	0.26	ECL deviates -0.002	Reference 0.006
8.894	9333	0.042	0.961	14.623	15:0 ISO	2.00	ECL deviates 0.000	Reference 0.008
9.043	177813	0.042	0.958	14.716	15:0 ANTEISO	38.08	ECL deviates 0.003	Reference 0.011
9.494	1319	0.048	0.949	14.997	15:0	0.28	ECL deviates -0.003	Reference 0.005
9.850	7262	0.044	0.944	15.205	14:0 2OH	1.53	ECL deviates 0.002	
10.579	123569	0.044	0.933	15.629	16:0 ISO	25.79	ECL deviates 0.002	Reference 0.010
10.906	1344	0.049	0.929	15.820	Sum In Feature 3	0.28	ECL deviates -0.002	16:1 w7c/15 iso 2OH
11.211	12924	0.046	0.925	15.997	16:0	2.67	ECL deviates -0.003	Reference 0.004
12.329	2040	0.045	0.914	16.630	17:0 ISO	0.42	ECL deviates 0.000	Reference 0.007
12.497	111633	0.048	0.913	16.724	17:0 ANTEISO	22.78	ECL deviates 0.001	Reference 0.008
14.457	17494	0.048	0.899	17.823	18:1 w7c	3.52	ECL deviates 0.000	
14.915	1890	0.048	0.896	18.080	11 methyl 18:1 w7c	0.38	ECL deviates -0.001	
----	1344	---	----	----	Summed Feature 3	0.28	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c

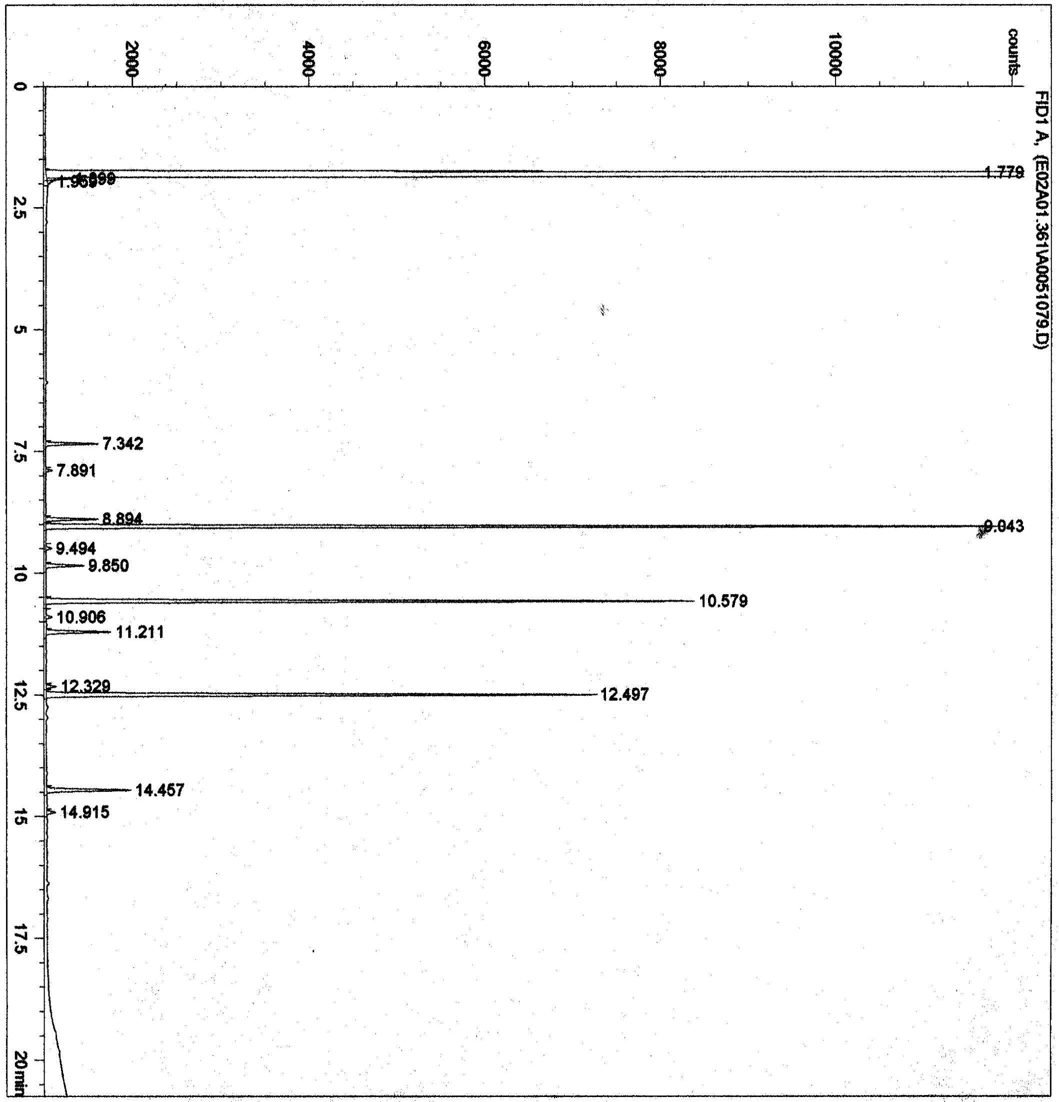
ECL Deviation: 0.002 Reference ECL Shift: 0.008 Number Reference Peaks: 9
Total Response: 476837 Total Named: 476837
Percent Named: 100.00% Total Amount: 447294

Matches:

Library	Sim Index	Entry Name
TSBA40 4.10	0.368	Cellulomonas-flavigena*
	0.214	Paenibacillus-macerans-GC subgroup B* (Bacillus macerans)

```
Data File C:\SHERLOCK\RAW\E02A01.361\A0051079.D                 Sample Name:
    Sherlock Id: Aircraft 1:100 TSBA

    =====================================================================
    Injection Date   : 10/1/02 10:25:00 AM
    Sample Name      :  1079                       Location : Vial 10
    Acq. Operator    :
                                                 Inj Volume : 2 µl
    Method           : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed     : 10/1/02 10:20:51 AM
    =====================================================================
```



```
*** End of Report ***
```

Appendix C: Worksheet / Sample Data – Eglin AFB (KVPS)

	Site Information									
#	Sample	Type / Number	Date Sampled	Dilution	Medium	Temp °C	% Relative Humidity	Inches Rainfall (last 30 days)	Fuel Type	Sample Method
1	Neg Control	—	—	—	—	—	—	—	—	—
2	Pos Control	—	—	—	—	—	—	—	—	—
3	Tank	29	9/30/2002	Neat	TSBA	25	88	12.25	JP-8	gravity
4	Tank	29	9/30/2002	1:10	TSBA	25	88	12.25	JP-8	gravity
5	Tank	29	9/30/2002	1:100	TSBA	25	88	12.25	JP-8	gravity
6	Tank	29	9/30/2002	1:1000	TSBA	25	88	12.25	JP-8	gravity
7	Tank	29	9/30/2002	Neat	SDA	25	88	12.25	JP-8	gravity
8	Tank	29	9/30/2002	1:10	SDA	25	88	12.25	JP-8	gravity
9	Tank	29	9/30/2002	1:100	SDA	25	88	12.25	JP-8	gravity
10	Tank	29	9/30/2002	1:1000	SDA	25	88	12.25	JP-8	gravity
11	Tank	29	9/30/2002	Neat	BHIBLA	25	88	12.25	JP-8	gravity
12	Tank	29	9/30/2002	1:10	BHIBLA	25	88	12.25	JP-8	gravity
13	Tank	29	9/30/2002	1:100	BHIBLA	25	88	12.25	JP-8	gravity
14	Tank	29	9/30/2002	1:1000	BHIBLA	25	88	12.25	JP-8	gravity
15	Truck	89L545	9/30/2002	Neat	TSBA	25	88	12.25	JP-8	gravity
16	Truck	89L545	9/30/2002	1:10	TSBA	25	88	12.25	JP-8	gravity
17	Truck	89L545	9/30/2002	1:100	TSBA	25	88	12.25	JP-8	gravity
18	Truck	89L545	9/30/2002	1:1000	TSBA	25	88	12.25	JP-8	gravity
19	Truck	89L545	9/30/2002	Neat	SDA	25	88	12.25	JP-8	gravity
20	Truck	89L545	9/30/2002	1:10	SDA	25	88	12.25	JP-8	gravity
21	Truck	89L545	9/30/2002	1:100	SDA	25	88	12.25	JP-8	gravity
22	Truck	89L545	9/30/2002	1:1000	SDA	25	88	12.25	JP-8	gravity
23	Truck	89L545	9/30/2002	Neat	BHIBLA	25	88	12.25	JP-8	gravity
24	Truck	89L545	9/30/2002	1:10	BHIBLA	25	88	12.25	JP-8	gravity
25	Truck	89L545	9/30/2002	1:100	BHIBLA	25	88	12.25	JP-8	gravity
26	Truck	89L545	9/30/2002	1:1000	BHIBLA	25	88	12.25	JP-8	gravity
27	Aircraft	F-15	9/30/2002	Neat	TSBA	25	88	12.25	JP-8 +100	gravity
28	Aircraft	F-15	9/30/2002	1:10	TSBA	25	88	12.25	JP-8 +100	gravity
29	Aircraft	F-15	9/30/2002	1:100	TSBA	25	88	12.25	JP-8 +100	gravity
30	Aircraft	F-15	9/30/2002	1:1000	TSBA	25	88	12.25	JP-8 +100	gravity
31	Aircraft	F-15	9/30/2002	Neat	SDA	25	88	12.25	JP-8 +100	gravity
32	Aircraft	F-15	9/30/2002	1:10	SDA	25	88	12.25	JP-8 +100	gravity
33	Aircraft	F-15	9/30/2002	1:100	SDA	25	88	12.25	JP-8 +100	gravity
34	Aircraft	F-15	9/30/2002	1:1000	SDA	25	88	12.25	JP-8 +100	gravity
35	Aircraft	F-15	9/30/2002	Neat	BHIBLA	25	88	12.25	JP-8 +100	gravity
36	Aircraft	F-15	9/30/2002	1:10	BHIBLA	25	88	12.25	JP-8 +100	gravity
37	Aircraft	F-15	9/30/2002	1:100	BHIBLA	25	88	12.25	JP-8 +100	gravity
38	Aircraft	F-15	9/30/2002	1:1000	BHIBLA	25	88	12.25	JP-8 +100	gravity

— Indicates analysis omitted

		Sample Description					
#	Tank Composition	pH	Sample Color	General Description	Obvious Solids	TDS (mg/L)	Free Phase Water
1	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—
3	steel w/ epoxy lining	—	light yellow	clear	no	—	no
4	steel w/ epoxy lining	—	light yellow	clear	no	—	no
5	steel w/ epoxy lining	—	light yellow	clear	no	—	no
6	steel w/ epoxy lining	—	light yellow	clear	no	—	no
7	steel w/ epoxy lining	—	light yellow	clear	no	—	no
8	steel w/ epoxy lining	—	light yellow	clear	no	—	no
9	steel w/ epoxy lining	—	light yellow	clear	no	—	no
10	steel w/ epoxy lining	—	light yellow	clear	no	—	no
11	steel w/ epoxy lining	—	light yellow	clear	no	—	no
12	steel w/ epoxy lining	—	light yellow	clear	no	—	no
13	steel w/ epoxy lining	—	light yellow	clear	no	—	no
14	steel w/ epoxy lining	—	light yellow	clear	no	—	no
15	aluminum	7.2	light yellow	clear	no	—	yes
16	aluminum	7.2	light yellow	clear	no	—	yes
17	aluminum	7.2	light yellow	clear	no	—	yes
18	aluminum	7.2	light yellow	clear	no	—	yes
19	aluminum	7.2	light yellow	clear	no	—	yes
20	aluminum	7.2	light yellow	clear	no	—	yes
21	aluminum	7.2	light yellow	clear	no	—	yes
22	aluminum	7.2	light yellow	clear	no	—	yes
23	aluminum	7.2	light yellow	clear	no	—	yes
24	aluminum	7.2	light yellow	clear	no	—	yes
25	aluminum	7.2	light yellow	clear	no	—	yes
26	aluminum	7.2	light yellow	clear	no	—	yes
27	aluminum	—	light yellow	clear	no	—	no
28	aluminum	—	light yellow	clear	no	—	no
29	aluminum	—	light yellow	clear	no	—	no
30	aluminum	—	light yellow	clear	no	—	no
31	aluminum	—	light yellow	clear	no	—	no
32	aluminum	—	light yellow	clear	no	—	no
33	aluminum	—	light yellow	clear	no	—	no
34	aluminum	—	light yellow	clear	no	—	no
35	aluminum	—	light yellow	clear	no	—	no
36	aluminum	—	light yellow	clear	no	—	no
37	aluminum	—	light yellow	clear	no	—	no
38	aluminum	—	light yellow	clear	no	—	no

	Light Microscopy				Colony Description		Quantity Data		
#	Gram +/-	Organism Shape	Length of Cells (μm)	Width of Cells (μm)	Colony Type	Colony Color	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours
1	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	0	0	0
4	neg	coccus	1 diameter	—	round	yellow	24	150	2,400
5	—	—	—	—	—	—	0	0	0
6	—	—	—	—	—	—	0	0	0
7	—	—	—	—	—	—	0	0	0
8	—	—	—	—	—	—	0	0	0
9	—	—	—	—	—	—	0	0	0
10	—	—	—	—	—	—	0	0	0
11	—	varied	varied	varied	round	white	12	30	120
12	neg	bacillus	2	0.5	round	white	50	190	5,000
13	neg	bacillus	2	0.5	irregular	white	910	3,000	910,000
14	—	—	—	—	—	—	0	0	0
15	—	—	—	—	—	—	0	0	0
16		varied	varied	varied	round	white	0	0	5 days to grow
17	—	—	—	—	—	—	0	0	0
18	—	—	—	—	—	—	0	0	0
19	—	—	—	—	—	—	0	0	0
20	—	varied	varied	varied	round	white	29	185	2,900
21	—	—	—	—	—	—	0	0	0
22	—	—	—	—	—	—	0	0	0
23	—	—	—	—	—	—	0	0	0
24	—	—	—	—	—	—	0	0	0
25	neg	bacillus	2	0.5	irregular	white	0	0	5 days to grow
26	neg	bacillus	2	0.5	irregular	white	0	0	5 days to grow
27	—	—	—	—	—	—	0	0	0
28	—	—	—	—	—	—	0	0	0
29	—	—	—	—	—	—	0	0	0
30	—	—	—	—	—	—	0	0	0
31	—	—	—	—	—	—	0	0	0
32	—	—	—	—	—	—	0	0	0
33	—	—	—	—	—	—	0	0	0
34	—	—	—	—	—	—	0	0	0
35	neg	bacillus	2	0.5	irregular	white	0	0	5 days to grow
36	—	—	—	—	—	—	0	0	0
37	neg	bacillus	2	0.5	irregular	white	0	0	5 days to grow
38	—	—	—	—	—	—	0	0	0

				Identification Data				
				FAME analysis			DNA gene sequence	
#	Bacteria vs. Fungi	Spores	Digital Photo	Sim Index	> .1 S. I. Separation	GC Best Match	DNA % ID	DNA Best Match
1	—	—	—	—	—	Library match not attempted	—	—
2	—	—	—	0.808	yes	*Stenotrophomonas-maltophilia*	—	—
3	—	—	—	—	—	—	—	—
4	bacteria	no	VPSTA10TSBA	0.654	yes	*Micrococcus-luteus-GC subgroup C*	—	—
5	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—
11	fungi	no		—	—	No match found	—	—
12	bacteria	no	VPSTA10BHI	—	—	No match found	—	—
13	bacteria	no	—	—	—	Library match not attempted	—	—
14	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—
16	fungi	no	VPSTR10TSBA	0.018	yes	*Actinomadura-yumaensis (72h)*	—	—
17	—	—	—	—	—	—	—	—
18	—	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—
20	fungi	no	VPSTR10SDA	—	—	No match found		
21	—	—	—	—	—	—	—	—
22	—	—	—	—	—	—	—	—
23	—	—	—	—	—	—	—	—
24	—	—	—	—	—	—	—	—
25	bacteria	no	VPSTR100BHI	—	—	No match found	—	—
26	bacteria	no	VPSTR1000BHI	—	—	No match found	—	—
27	—	—	—	—	—	—	—	—
28	—	—	—	—	—	—	—	—
29	—	—	—	—	—	—	—	—
30	—	—	—	—	—	—	—	—
31	—	—	—	—	—	—	—	—
32	—	—	—	—	—	—	—	—
33	—	—	—	—	—	—	—	—
34	—	—	—	—	—	—	—	—
35	bacteria	no	—	—	—	No match found	—	—
36	—	—	—	—	—	—	—	—
37	bacteria	no	—	—	—	No match found	—	—
38	—	—	—	—	—	—	—	—

E02A303.86A [1098] VPS Tank 1:10 TSBA

Volume: DATA File: E02A303.86A Seq Counter: 6 ID Number: 1098
Type: Samp Bottle: 5 Method: TSBA40
Created: 10/30/02 11:23:09 AM
Sample ID: VPS Tank 1:10 TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.778	2.16E+8	0.028	----	7.017	SOLVENT PEAK	----	< min rt	
2.494	691	0.030	----	8.361		----	< min rt	
5.796	5356	0.035	1.045	12.613	13:0 ISO	1.39	ECL deviates -0.001	Reference 0.000
5.902	2506	0.036	1.041	12.702	13:0 ANTEISO	0.65	ECL deviates 0.000	Reference 0.001
7.131	9026	0.039	0.999	13.618	14:0 ISO	2.24	ECL deviates -0.001	Reference 0.000
7.667	8591	0.040	0.985	13.999	14:0	2.10	ECL deviates -0.001	Reference -0.001
8.652	58455	0.042	0.963	14.624	15:0 ISO	14.01	ECL deviates 0.001	Reference 0.001
8.801	255774	0.043	0.961	14.718	15:0 ANTEISO	61.12	ECL deviates 0.005	Reference 0.006
9.242	2575	0.045	0.952	14.998	15:0	0.61	ECL deviates -0.002	Reference -0.001
10.306	24137	0.044	0.937	15.627	16:0 ISO	5.62	ECL deviates 0.000	Reference 0.000
10.632	925	0.043	0.932	15.819	Sum In Feature 3	0.21	ECL deviates -0.003	16:1 w7c/15 iso 2OH
10.934	6145	0.045	0.929	15.998	16:0	1.42	ECL deviates -0.002	Reference -0.001
12.041	2603	0.049	0.917	16.630	17:0 ISO	0.59	ECL deviates 0.000	Reference 0.001
12.205	41867	0.046	0.916	16.723	17:0 ANTEISO	9.54	ECL deviates 0.000	Reference 0.002
13.586	2915	0.246	----	17.505		----	> max ar/ht	
13.782	852	0.116	----	17.616		----	> max ar/ht	
13.969	416	0.056	0.904	17.721	Sum In Feature 5	0.09	ECL deviates 0.001	18:2 w6,9c/18:0 ANTE
14.146	459	0.063	0.903	17.820	18:1 w7c	0.10	ECL deviates -0.003	
15.701	1695	0.035	----	18.701		----		
15.874	1391	0.051	----	18.800		----		
16.055	1298	0.068	0.895	18.902	19:0 CYCLO w8c	0.29	ECL deviates 0.000	Reference 0.003
16.344	1035	0.049	----	19.068		----		
17.417	8297	0.053	----	19.685		----		
17.591	13071	0.051	----	19.785		----		
18.048	1190	0.035	----	20.048		----	> max rt	
18.211	4532	0.045	----	20.141		----	> max rt	
18.845	12252	0.022	----	20.508		----	> max rt	
18.954	43079	0.031	----	20.571		----	> max rt	
19.056	44699	0.031	----	20.630		----	> max rt	
19.769	28326	0.250	----	21.041		----	> max rt	
19.865	8371	0.034	----	21.096		----	> max rt	
----	925	---	----	----	Summed Feature 3	0.21	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c
----	416	---	----	----	Summed Feature 5	0.09	18:2 w6,9c/18:0 ANTE	18:0 ANTE/18:2 w6,9c

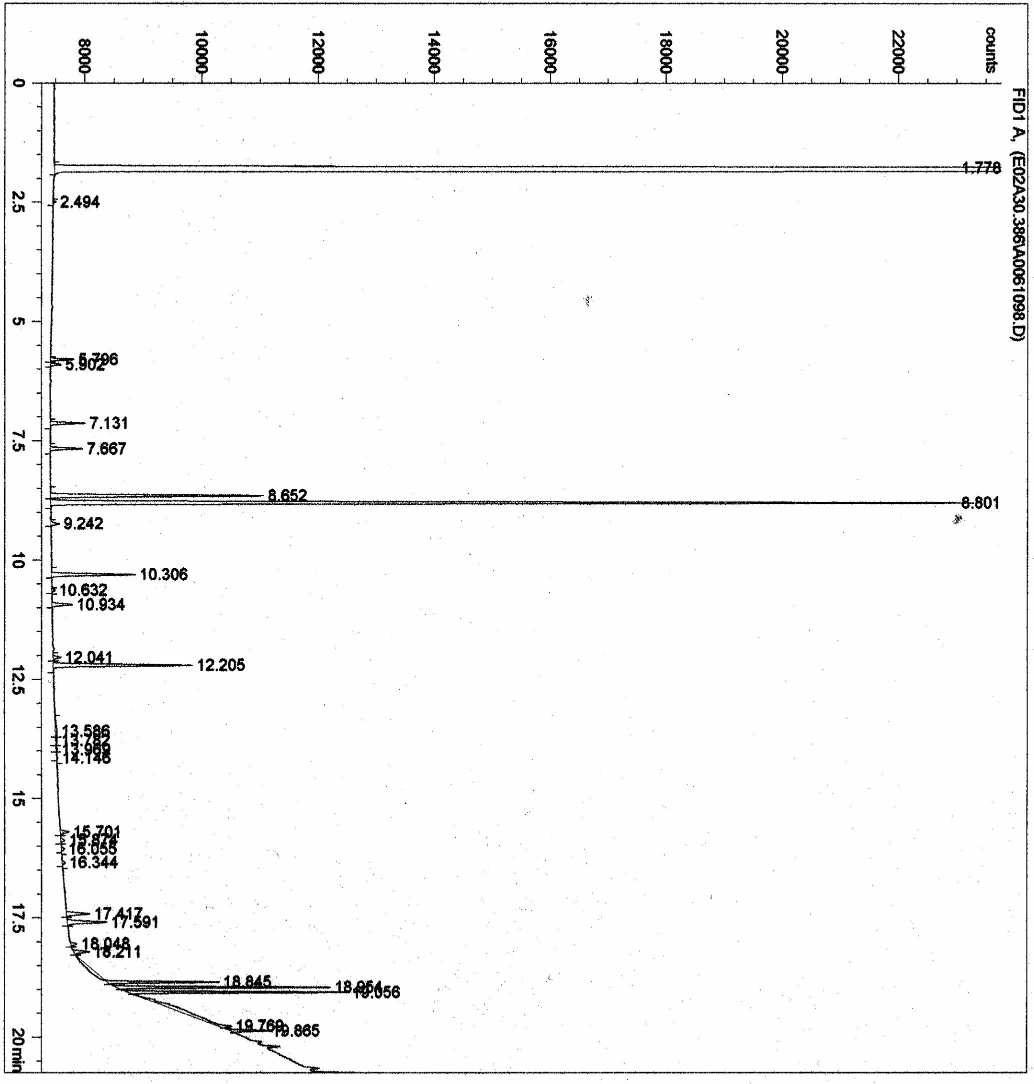
ECL Deviation: 0.002 Reference ECL Shift: 0.002 Number Reference Peaks: 12
Total Response: 449388 Total Named: 420133
Percent Named: 93.49% Total Amount: 402052

Matches:

Library	**Sim Index**	**Entry Name**
TSBA40 4.10	0.654	Micrococcus-luteus-GC subgroup C*
	0.498	Micrococcus-luteus-GC subgroup B*
	0.463	Arthrobacter-atrocyaneus
	0.434	Brevibacillus-brevis* (Bacillus)
	0.431	Kocuria-varians* (Micrococcus)
	0.350	Cellulomonas-fimi*
	0.335	Bacillus-megaterium-GC subgroup B
	0.329	Brochothrix-thermosphacta

```
Data File C:\SHERLOCK\RAW\E02A30.386\A0061098.D                              Sample Name:
    Sherlock Id: VPS Tank 1:10 TSBA

    =====================================================================
    Injection Date  : 10/30/02 11:27:20 AM
    Sample Name     :  1098                          Location : Vial 5
    Acq. Operator   :
                                                   Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 10/30/02 11:23:09 AM
    =====================================================================
```



*** End of Report ***

E02A303.86A [1097] VPS Truck 1:10 TSBA

Volume: DATA File: E02A303.86A Seq Counter: 5 ID Number: 1097
Type: Samp Bottle: 4 Method: TSBA40
Created: 10/30/02 10:57:34 AM
Sample ID: VPS Truck 1:10 TSBA

Profile: PERCENT NAMED IS LESS THAN 85.00. CHECK FOR CONTAMINATION.

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.777	2.22E+8	0.029	----	7.015	SOLVENT PEAK	----	< min rt	
2.493	546	0.035	----	8.358		----	< min rt	
5.446	671	0.035	----	12.323		----		
10.630	976	0.045	0.932	15.820	Sum In Feature 3	0.90	ECL deviates -0.002	16:1 w7c/15 iso 2OH
10.933	8739	0.047	0.929	15.999	16:0	8.05	ECL deviates -0.001	Reference -0.002
13.977	72634	0.052	0.904	17.728	Sum In Feature 5	65.12	ECL deviates 0.001	18:0 ANTE/18:2 w6,9c
14.060	27849	0.055	0.903	17.775	18:1 w9c	24.96	ECL deviates 0.006	
15.537	7829	0.161	----	18.612		----	> max ar/ht	
16.327	7487	0.165	----	19.062		----	> max ar/ht	
16.540	94	0.033	----	19.184		----		
16.601	162	0.042	----	19.219		----		
16.734	635	0.055	----	19.296		----		
16.924	1841	0.106	0.893	19.406	20:4 w6,9,12,15c	----	> max ar/ht	
17.021	762	0.091	----	19.461		----		
17.072	257	0.038	----	19.491		----		
17.289	3223	0.058	----	19.616		----		
17.501	843	0.053	0.892	19.738	20:2 w6,9c	0.75	ECL deviates 0.006	
17.573	254	0.043	0.892	19.780	20:1 w9c	0.22	ECL deviates 0.010	
19.647	1947	0.040	----	20.974		----	> max rt	
19.752	4098	0.044	----	21.035		----	> max rt	
19.839	968	0.099	----	21.085		----	> max rt	
----	976	---	----	----	Summed Feature 3	0.90	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c
----	72634	---	----	----	Summed Feature 5	65.12	18:2 w6,9c/18:0 ANTE	18:0 ANTE/18:2 w6,9c

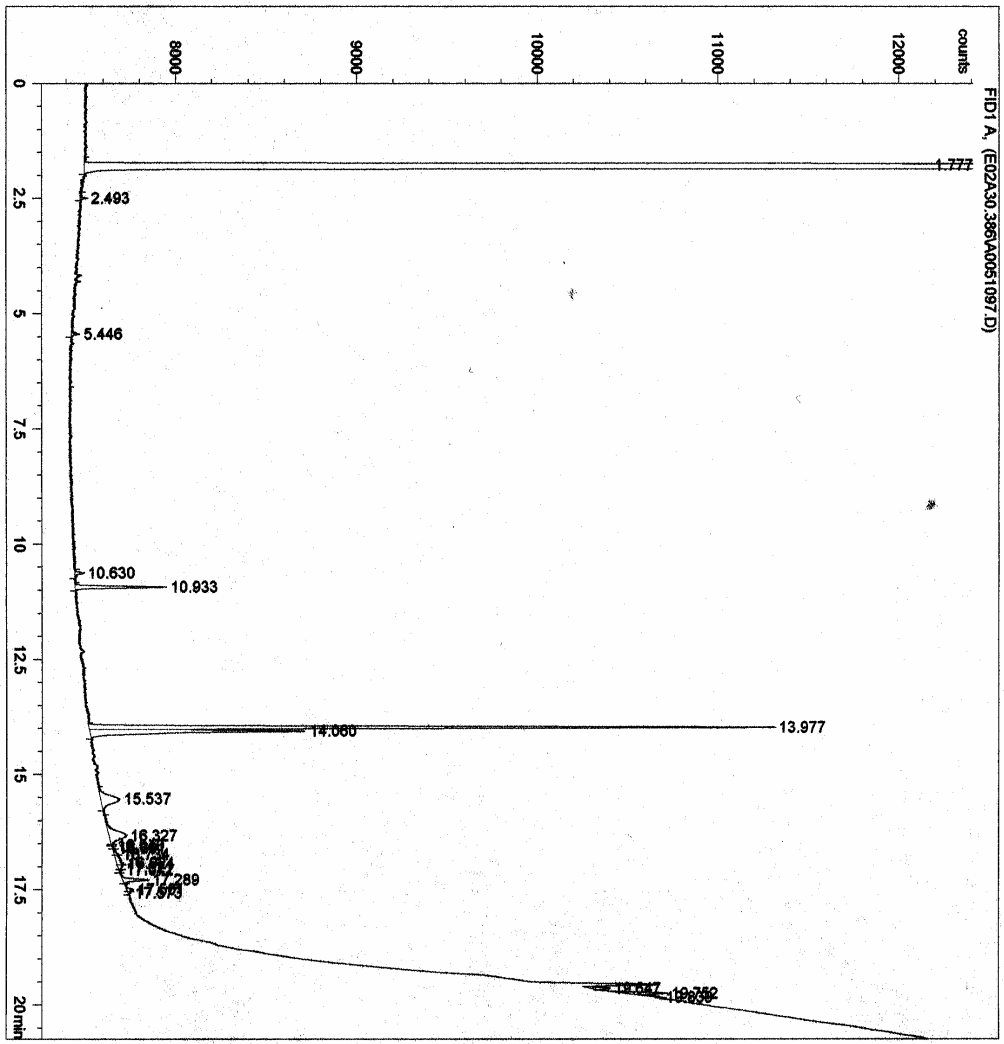
ECL Deviation: 0.006 Reference ECL Shift: 0.002 Number Reference Peaks: 1
Total Response: 134255 Total Named: 111294
Percent Named: 82.90% Total Amount: 102433
Profile: PERCENT NAMED IS LESS THAN 85.00. CHECK FOR CONTAMINATION.

Matches:

Library	Sim Index	Entry Name
TSBA40 4.10	0.018	Actinomadura-yumaensis* (72h)

```
Data File C:\SHERLOCK\RAW\E02A30.386\A0051097.D                          Sample Name:
    Sherlock Id: VPS Truck 1:10 TSBA

    =====================================================================
    Injection Date  : 10/30/02 11:01:41 AM
    Sample Name     :  1097                       Location : Vial 4
    Acq. Operator   :
                                                Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 10/30/02 10:57:34 AM
    =====================================================================
```



*** End of Report ***

Appendix D: Worksheet / Sample Data – Hurlburt AFB (KHRT)

	Site Information									
#	Sample	Type / Number	Date Sampled	Dilution	Medium	Temp °C	% Relative Humidity	Inches Rainfall (last 30 days)	Fuel Type	Sample Method
1	Neg Control	—	—	—	—	—	—	—	—	—
2	Pos Control	—	—	—	—	—	—	—	—	—
3	Tank	TK-2	10/1/2002	Neat	TSBA	26	72	14.34	JP-8	Bacon bomb
4	Tank	TK-2	10/1/2002	1:10	TSBA	26	72	14.34	JP-8	Bacon bomb
5	Tank	TK-2	10/1/2002	1:100	TSBA	26	72	14.34	JP-8	Bacon bomb
6	Tank	TK-2	10/1/2002	1:1000	TSBA	26	72	14.34	JP-8	Bacon bomb
7	Tank	TK-2	10/1/2002	Neat	SDA	26	72	14.34	JP-8	Bacon bomb
8	Tank	TK-2	10/1/2002	1:10	SDA	26	72	14.34	JP-8	Bacon bomb
9	Tank	TK-2	10/1/2002	1:100	SDA	26	72	14.34	JP-8	Bacon bomb
10	Tank	TK-2	10/1/2002	1:1000	SDA	26	72	14.34	JP-8	Bacon bomb
11	Tank	TK-2	10/1/2002	Neat	BHIBLA	26	72	14.34	JP-8	Bacon bomb
12	Tank	TK-2	10/1/2002	1:10	BHIBLA	26	72	14.34	JP-8	Bacon bomb
13	Tank	TK-2	10/1/2002	1:100	BHIBLA	26	72	14.34	JP-8	Bacon bomb
14	Tank	TK-2	10/1/2002	1:1000	BHIBLA	26	72	14.34	JP-8	Bacon bomb
15	Truck	90L-510	10/1/2002	Neat	TSBA	26	72	14.34	JP-8	gravity
16	Truck	90L-510	10/1/2002	1:10	TSBA	26	72	14.34	JP-8	gravity
17	Truck	90L-510	10/1/2002	1:100	TSBA	26	72	14.34	JP-8	gravity
18	Truck	90L-510	10/1/2002	1:1000	TSBA	26	72	14.34	JP-8	gravity
19	Truck	90L-510	10/1/2002	Neat	SDA	26	72	14.34	JP-8	gravity
20	Truck	90L-510	10/1/2002	1:10	SDA	26	72	14.34	JP-8	gravity
21	Truck	90L-510	10/1/2002	1:100	SDA	26	72	14.34	JP-8	gravity
22	Truck	90L-510	10/1/2002	1:1000	SDA	26	72	14.34	JP-8	gravity
23	Truck	90L-510	10/1/2002	Neat	BHIBLA	26	72	14.34	JP-8	gravity
24	Truck	90L-510	10/1/2002	1:10	BHIBLA	26	72	14.34	JP-8	gravity
25	Truck	90L-510	10/1/2002	1:100	BHIBLA	26	72	14.34	JP-8	gravity
26	Truck	90L-510	10/1/2002	1:1000	BHIBLA	26	72	14.34	JP-8	gravity
27	Aircraft	AC-130	10/1/2002	Neat	TSBA	26	72	14.34	JP-8	gravity
28	Aircraft	AC-130	10/1/2002	1:10	TSBA	26	72	14.34	JP-8	gravity
29	Aircraft	AC-130	10/1/2002	1:100	TSBA	26	72	14.34	JP-8	gravity
30	Aircraft	AC-130	10/1/2002	1:1000	TSBA	26	72	14.34	JP-8	gravity
31	Aircraft	AC-130	10/1/2002	Neat	SDA	26	72	14.34	JP-8	gravity
32	Aircraft	AC-130	10/1/2002	1:10	SDA	26	72	14.34	JP-8	gravity
33	Aircraft	AC-130	10/1/2002	1:100	SDA	26	72	14.34	JP-8	gravity
34	Aircraft	AC-130	10/1/2002	1:1000	SDA	26	72	14.34	JP-8	gravity
35	Aircraft	AC-130	10/1/2002	Neat	BHIBLA	26	72	14.34	JP-8	gravity
36	Aircraft	AC-130	10/1/2002	1:10	BHIBLA	26	72	14.34	JP-8	gravity
37	Aircraft	AC-130	10/1/2002	1:100	BHIBLA	26	72	14.34	JP-8	gravity
38	Aircraft	AC-130	10/1/2002	1:1000	BHIBLA	26	72	14.34	JP-8	gravity

— Indicates analysis omitted

		Sample Description					
#	Tank Composition	pH	Sample Color	General Description	Obvious Solids	TDS (mg/L)	Free Phase Water
1	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—
3	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
4	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
5	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
6	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
7	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
8	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
9	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
10	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
11	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
12	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
13	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
14	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
15	aluminum	6.3	dark yellow	clear	no	—	yes
16	aluminum	6.3	dark yellow	clear	no	—	yes
17	aluminum	6.3	dark yellow	clear	no	—	yes
18	aluminum	6.3	dark yellow	clear	no	—	yes
19	aluminum	6.3	dark yellow	clear	no	—	yes
20	aluminum	6.3	dark yellow	clear	no	—	yes
21	aluminum	6.3	dark yellow	clear	no	—	yes
22	aluminum	6.3	dark yellow	clear	no	—	yes
23	aluminum	6.3	dark yellow	clear	no	—	yes
24	aluminum	6.3	dark yellow	clear	no	—	yes
25	aluminum	6.3	dark yellow	clear	no	—	yes
26	aluminum	6.3	dark yellow	clear	no	—	yes
27	aluminum	6.9	dark yellow	clear	no	—	yes
28	aluminum	6.9	dark yellow	clear	no	—	yes
29	aluminum	6.9	dark yellow	clear	no	—	yes
30	aluminum	6.9	dark yellow	clear	no	—	yes
31	aluminum	6.9	dark yellow	clear	no	—	yes
32	aluminum	6.9	dark yellow	clear	no	—	yes
33	aluminum	6.9	dark yellow	clear	no	—	yes
34	aluminum	6.9	dark yellow	clear	no	—	yes
35	aluminum	6.9	dark yellow	clear	no	—	yes
36	aluminum	6.9	dark yellow	clear	no	—	yes
37	aluminum	6.9	dark yellow	clear	no	—	yes
38	aluminum	6.9	dark yellow	clear	no	—	yes

	Light Microscopy				Colony Description		Quantity Data		
#	Gram +/-	Organism Shape	Length of Cells (μm)	Width of Cells (μm)	Colony Type	Colony Color	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours
1	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	0	0	0
4	neg	coccus	1 diameter	—	round	yellow	7	12	700
5	neg	coccus	1 diameter	—	round	white	0	1	0
6	—	—	—	—	—	—	0	0	0
7	pos	coccus	1 diameter	—	irregular	white	0	1	0
8	—	—	—	—	—	—	0	0	0
9	neg	coccus	1 diameter	—	round	yellow	0	0	4 days to grow
10	—	—	—	—	—	—	0	0	0
11	—	—	—	—	—	—	0	0	0
12	—	—	—	—	—	—	0	0	0
13	pos	coccus	1 diameter	—	round	white	0	0	4 days to grow
14	neg	coccus	1 diameter	—	irregular	white	0	0	4 days to grow
15	pos	coccus	1 diameter	—	round	white	1	2	10
16	—	—	—	—	—	—	0	0	0
17	pos	coccus	1 diameter		round	white	0	0	4 days to grow
18	—	—	—	—	—	—	0	0	0
19	—	—	—	—	—	—	0	0	0
20	—	—	—	—	—	—	0	0	0
21	—	—	—	—	—	—	0	0	0
22	—	—	—	—	—	—	0	0	0
23	neg	bacillus	1	0.5	irregular	white	0	0	4 days to grow
24	neg	bacillus	1	0.5	irregular	white	0	0	4 days to grow
25	neg	bacillus	1	0.5	irregular	white	0	0	4 days to grow
26	neg	bacillus	1	0.5	irregular	white	0	0	4 days to grow
27	neg	coccus	1 diameter	—	round	yellow	0	1	4 days to grow
28	pos	coccus	1 diameter	—	round	white	1	2	100
29	—	—	—	—	—	—	0	0	0
30	—	—	—	—	—	—	0	0	0
31	—	—	—	—	—	—	0	0	0
32	—	—	—	—	—	—	0	0	0
33	—	—	—	—	—	—	0	0	0
34	—	—	—	—	—	—	0	0	0
35	neg	bacillus	1	0.5	irregular	white	0	0	4 days to grow
36	pos	coccus	1 diameter	—	round	white	0	0	4 days to grow
37	neg	bacillus	1	0.5	irregular	white	0	0	4 days to grow
38	pos	coccus	1 diameter	—	round	white	0	0	4 days to grow

				Identification Data				
				FAME analysis			DNA gene sequence	
#	Bacteria vs. Fungi	Spores	Digital Photo	Sim Index	> .1 S. I. Separation	GC Best Match	DNA % ID	DNA Best Match
1	—	—	—	—	—	Library match not attempted	—	—
2	—	—	—	0.808	yes	*Stenotrophomonas-maltophilia*	—	—
3	—	—	—	—	—	—	—	—
4	bacteria	no	—	0.238	no	*Staphylococcus-warneri*	—	—
5	bacteria	no	—	0.299	no	*Staphylococcus-cohnii-cohnii*	—	—
6	—	—	—	—	—	—	—	—
7	bacteria	no	HRTTANEATSDA	—	—	Library match not attempted	—	—
8	—	—	—	—	—	—	—	—
9	bacteria	no	HRTTA100SDA	—	—	No match found	—	—
10	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—
13	bacteria	no	—	—	—	No match found	—	—
14	bacteria	no	HRTTA1000BHI	—	—	No match found	—	—
15	bacteria	no	—	0.780	no	*Bacillus-pasteurii*	—	—
16	—	—	—	—	—	—	—	—
17	bacteria	no	—	0.292	no	*Staphylococcus-warneri*	—	—
18	—	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—
20	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—
22	—	—	—	—	—	—	—	—
23	bacteria	no	—	—	—	Library match not attempted	—	—
24	bacteria	no	—	—	—	No match found	—	—
25	bacteria	no	—	—	—	Library match not attempted	—	—
26	bacteria	no	—	—	—	No match found	—	—
27	bacteria	no	HRTACNEATTSBA	0.248	no	*Staphylococcus-warneri*	—	—
28	bacteria	no	—	0.325	no	*Staphylococcus-cohnii-cohnii*	—	—
29	—	—	—	—	—	—	—	—
30	—	—	—	—	—	—	—	—
31	—	—	—	—	—	—	—	—
32	—	—	—	—	—	—	—	—
33	—	—	—	—	—	—	—	—
34	—	—	—	—	—	—	—	—
35	bacteria	no	HRTACNEATBHI	—	—	Library match not attempted	—	—
36	bacteria	no	HRTAC10BHI	—	—	No match found	—	—
37	bacteria	no	—	—	—	No match found	—	—
38	bacteria	no	—	—	—	No match found	—	—

E02A303.86A [1104] HRT Truck Neat TSBA

Volume: DATA File: E02A303.86A Seq Counter: 12 ID Number: 1104
Type: Samp Bottle: 11 Method: TSBA40
Created: 10/30/02 1:56:59 PM
Sample ID: HRT Truck Neat TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.775	2.217E+8	0.029	----	7.013	SOLVENT PEAK	----	< min rt	
1.882	15805	0.025	----	7.214		----	< min rt	
1.975	1294	0.029	----	7.389		----	< min rt	
3.809	603	0.031	1.174	10.606	11:0 ISO	0.32	ECL deviates 0.000	Reference -0.004
4.681	2050	0.033	1.103	11.609	12:0 ISO	1.01	ECL deviates 0.000	Reference -0.004
5.057	1096	0.032	1.079	11.999	12:0	0.53	ECL deviates -0.001	Reference -0.005
5.790	23694	0.034	1.045	12.614	13:0 ISO	11.08	ECL deviates 0.000	Reference -0.004
5.896	3002	0.036	1.041	12.703	13:0 ANTEISO	1.40	ECL deviates 0.001	Reference -0.004
7.124	12515	0.039	0.999	13.620	14:0 ISO	5.59	ECL deviates 0.001	Reference -0.004
7.659	6978	0.041	0.985	13.998	14:0	3.07	ECL deviates -0.002	Reference -0.007
8.644	75549	0.041	0.963	14.624	15:0 ISO	32.56	ECL deviates 0.001	Reference -0.004
8.785	14147	0.043	0.961	14.714	15:0 ANTEISO	6.08	ECL deviates 0.001	Reference -0.004
9.087	619	0.050	0.955	14.906	15:1 w5c	0.26	ECL deviates 0.003	
9.234	1279	0.048	0.952	14.999	15:0	0.54	ECL deviates -0.001	Reference -0.006
9.896	2354	0.045	0.942	15.391	16:1 w7c alcohol	0.99	ECL deviates 0.004	
10.057	6796	0.044	0.940	15.486	Sum In Feature 2	2.86	ECL deviates -0.002	14:0 3OH/16:1 ISO I
10.296	14621	0.046	0.937	15.627	16:0 ISO	6.13	ECL deviates 0.000	Reference -0.006
10.524	764	0.042	0.934	15.761	16:1 w11c	0.32	ECL deviates 0.004	
10.691	21415	0.048	0.932	15.860	Sum In Feature 3	8.93	ECL deviates 0.008	15:0 ISO 2OH/16:1w7c
10.925	9004	0.044	0.929	15.999	16:0	3.74	ECL deviates -0.001	Reference -0.007
11.315	951	0.040	0.924	16.221	15:0 2OH	0.39	ECL deviates 0.002	
11.610	5313	0.052	0.921	16.390	ISO 17:1 w10c	2.19	ECL deviates 0.002	
11.741	8622	0.053	0.920	16.465	ISO 17:1 w5c	3.55	ECL deviates 0.004	
11.882	2216	0.045	0.919	16.545	17:1 ANTEISO A	0.91	ECL deviates 0.005	
12.030	14663	0.047	0.917	16.630	17:0 ISO	6.02	ECL deviates 0.000	Reference -0.005
12.194	3388	0.049	0.916	16.724	17:0 ANTEISO	1.39	ECL deviates 0.001	Reference -0.004
15.345	3294	0.050	----	18.508		----		
15.517	544	0.047	----	18.606		----		
15.987	324	0.076	0.895	18.873	Sum In Feature 7	0.13	ECL deviates 0.006	19:0 CYCLO w10c/19w6
17.122	621	0.087	----	19.525		----		
17.890	675	0.162	----	19.968		----	> max ar/ht	
----	6796	---	----	----	Summed Feature 2	2.86	12:0 ALDE ?	unknown 10.928
----	-----	---	----	----		----	16:1 ISO I/14:0 3OH	14:0 3OH/16:1 ISO I
----	21415	---	----	----	Summed Feature 3	8.93	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c
----	324	---	----	----	Summed Feature 7	0.13	un 18.846/19:1 w6c	19:1 w6c/.846/19cy
----	-----	---	----	----		----	19:0 CYCLO w10c/19w6	

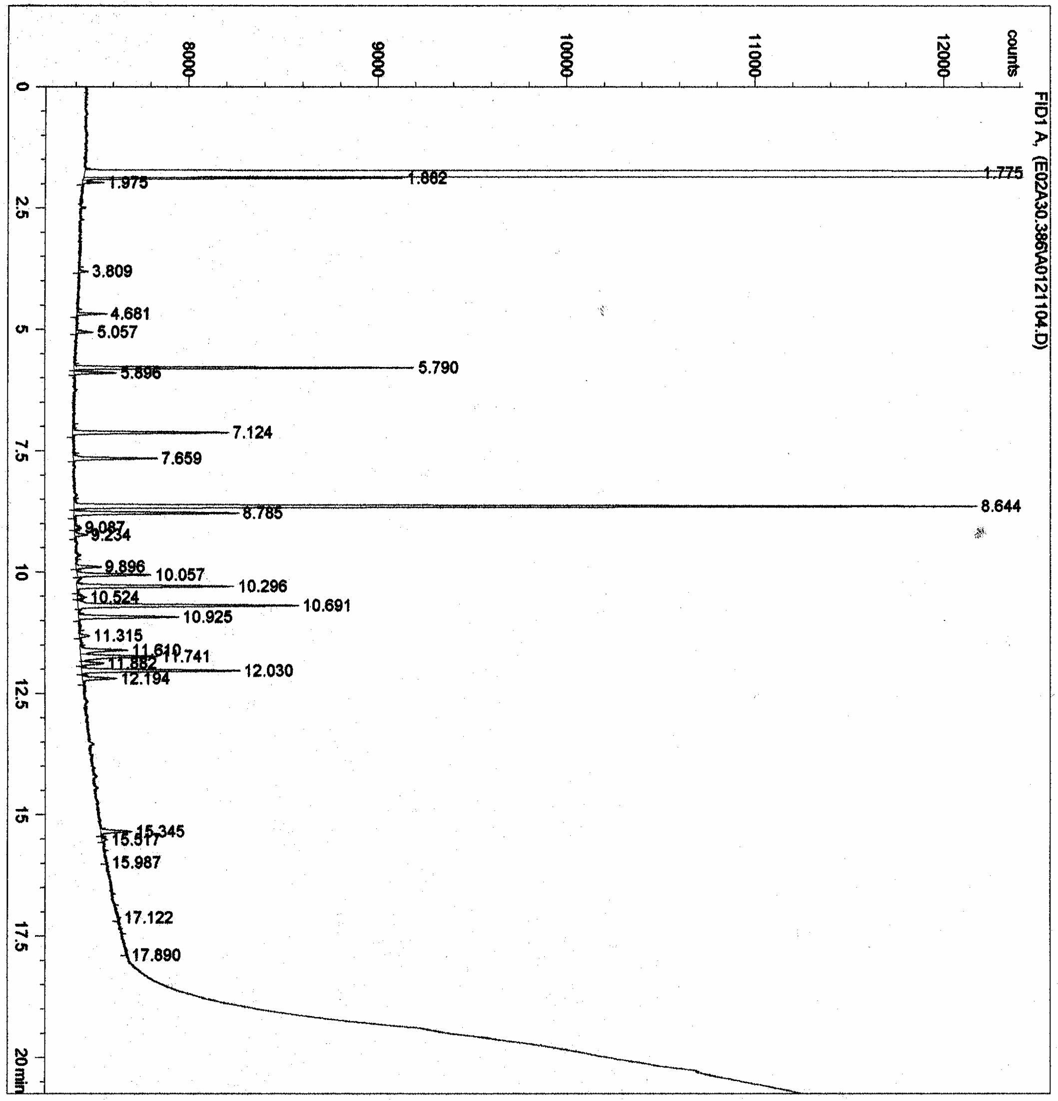
ECL Deviation: 0.003 Reference ECL Shift: 0.005 Number Reference Peaks: 14
Total Response: 237097 Total Named: 231963
Percent Named: 97.83% Total Amount: 223534

Matches:

Library	**Sim Index**	**Entry Name**
TSBA40 4.10	0.780	Bacillus-pasteurii*
	0.724	Bacillus-thuringiensis kurstakii**
	0.717	Bacillus-thuringiensis kenyae sv.**
	0.712	Bacillus-dipsosauri**
	0.689	Bacillus-thuringiensis dendrolimus sv.**
	0.660	Bacillus-cereus-GC subgroup A*
	0.500	Bacillus-thuringiensis canadensis sv.**
	0.397	Bacillus-thuringiensis aizawai sv.**
	0.397	Bacillus-thuringiensis israelensis**

```
Data File C:\SHERLOCK\RAW\E02A30.386\A0121104.D                      Sample Name:
    Sherlock Id: HRT Truck Neat TSBA

    =====================================================================
    Injection Date  : 10/30/02 2:01:11 PM
    Sample Name     :  1104                         Location : Vial 11
    Acq. Operator   :
                                                  Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 10/30/02 1:56:59 PM
    =====================================================================
```



```
*** End of Report ***
```

E02A303.86A [1103] HRT Truck 1:1000 TSBA

Volume: DATA File: E02A303.86A Seq Counter: 11 ID Number: 1103
Type: Samp Bottle: 10 Method: TSBA40
Created: 10/30/02 1:31:21 PM
Sample ID: HRT Truck 1:1000 TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.776	2.205E+8	0.028	----	7.010	SOLVENT PEAK	----	< min rt	
1.889	1973	0.026	----	7.222		----	< min rt	
5.793	1167	0.034	1.045	12.614	13:0 ISO	1.04	ECL deviates 0.000	Reference -0.002
7.126	7917	0.039	0.999	13.618	14:0 ISO	6.73	ECL deviates -0.001	Reference -0.004
8.644	10746	0.042	0.963	14.623	15:0 ISO	8.81	ECL deviates 0.000	Reference -0.004
8.788	51814	0.041	0.961	14.714	15:0 ANTEISO	42.35	ECL deviates 0.001	Reference -0.003
10.297	2637	0.045	0.937	15.627	16:0 ISO	2.10	ECL deviates 0.000	Reference -0.005
10.927	2309	0.047	0.929	15.999	16:0	1.82	ECL deviates -0.001	Reference -0.006
12.031	7069	0.047	0.917	16.631	17:0 ISO	5.52	ECL deviates 0.001	Reference -0.004
12.193	6157	0.046	0.916	16.724	17:0 ANTEISO	4.80	ECL deviates 0.001	Reference -0.005
13.797	2346	0.050	0.905	17.632	18:0 ISO	1.81	ECL deviates 0.000	Reference -0.006
14.450	12254	0.049	0.901	18.000	18:0	9.39	ECL deviates 0.000	Reference -0.007
15.565	3865	0.048	0.897	18.634	19:0 ISO	2.95	ECL deviates 0.000	Reference -0.007
15.736	2091	0.050	0.896	18.731	19:0 ANTEISO	1.59	ECL deviates 0.000	
16.926	226	0.084	----	19.413		----		
17.310	756	0.088	0.892	19.635	20:0 ISO	0.57	ECL deviates 0.000	Reference -0.008
17.944	13866	0.052	0.891	20.000	20:0	10.52	ECL deviates 0.000	Reference -0.008

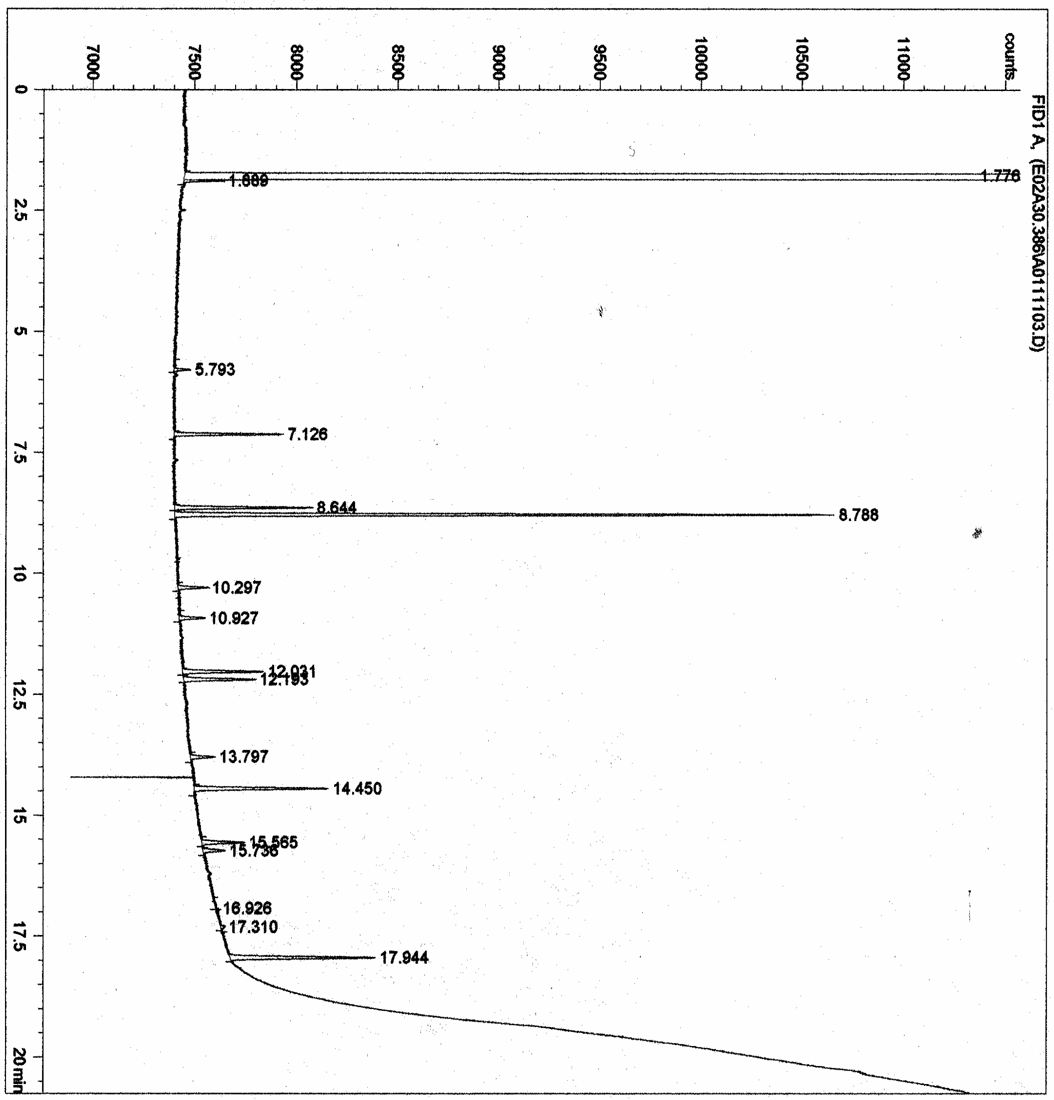
ECL Deviation: 0.001 Reference ECL Shift: 0.006 Number Reference Peaks: 13
Total Response: 125221 Total Named: 124995
Percent Named: 99.82% Total Amount: 117544

Matches:

Library	Sim Index	Entry Name
TSBA40 4.10	0.292	Staphylococcus-warneri*
	0.217	Staphylococcus-aureus-GC subgroup C*
	0.181	Staphylococcus-epidermidis*
	0.166	Staphylococcus-hominis*
	0.152	Staphylococcus-xylosus
	0.147	Staphylococcus-cohnii-cohnii

```
Data File C:\SHERLOCK\RAW\E02A30.386\A0111103.D                   Sample Name:
   Sherlock Id: HRT Truck 1:1000 TSBA

   ===================================================================
   Injection Date  : 10/30/02 1:35:31 PM
   Sample Name     :  1103                        Location : Vial 10
   Acq. Operator   :
                                                Inj Volume : 2 µl
   Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
   Last changed    : 10/30/02 1:31:21 PM
   ===================================================================
```



*** End of Report ***

E02A303.86A [1102] HRT A/C 1:10 TSBA

Volume: DATA File: E02A303.86A Seq Counter: 10 ID Number: 1102
Type: Samp Bottle: 9 Method: TSBA40
Created: 10/30/02 1:05:37 PM
Sample ID: HRT A/C 1:10 TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.759	2.208E+8	0.028	----	6.988	SOLVENT PEAK	----	< min rt	
1.872	1891	0.030	----	7.200		----	< min rt	
2.478	444	0.024	----	8.336		----	< min rt	
5.784	767	0.034	1.045	12.614	13:0 ISO	0.59	ECL deviates 0.000	Reference -0.010
7.121	5765	0.038	0.999	13.619	14:0 ISO	4.22	ECL deviates 0.000	Reference -0.007
7.656	722	0.039	0.985	13.998	14:0	0.52	ECL deviates -0.002	Reference -0.008
8.641	10904	0.042	0.963	14.623	15:0 ISO	7.70	ECL deviates 0.000	Reference -0.006
8.786	65495	0.042	0.961	14.715	15:0 ANTEISO	46.11	ECL deviates 0.002	Reference -0.004
10.297	2636	0.045	0.937	15.627	16:0 ISO	1.81	ECL deviates 0.000	Reference -0.005
10.928	3587	0.046	0.929	16.000	16:0	2.44	ECL deviates 0.000	Reference -0.005
12.032	6972	0.048	0.917	16.630	17:0 ISO	4.69	ECL deviates 0.000	Reference -0.004
12.194	11067	0.047	0.916	16.723	17:0 ANTEISO	7.43	ECL deviates 0.000	Reference -0.004
13.799	2148	0.054	0.905	17.632	18:0 ISO	1.42	ECL deviates 0.000	Reference -0.005
14.451	14248	0.050	0.901	17.999	18:0	9.41	ECL deviates -0.001	Reference -0.006
15.566	3157	0.050	0.897	18.633	19:0 ISO	2.07	ECL deviates -0.001	Reference -0.006
15.737	2527	0.048	0.896	18.731	19:0 ANTEISO	1.66	ECL deviates 0.000	
16.210	526	0.050	0.895	19.000	19:0	0.34	ECL deviates 0.000	Reference -0.006
17.945	14658	0.052	0.891	20.001	20:0	9.58	ECL deviates 0.001	Reference -0.008

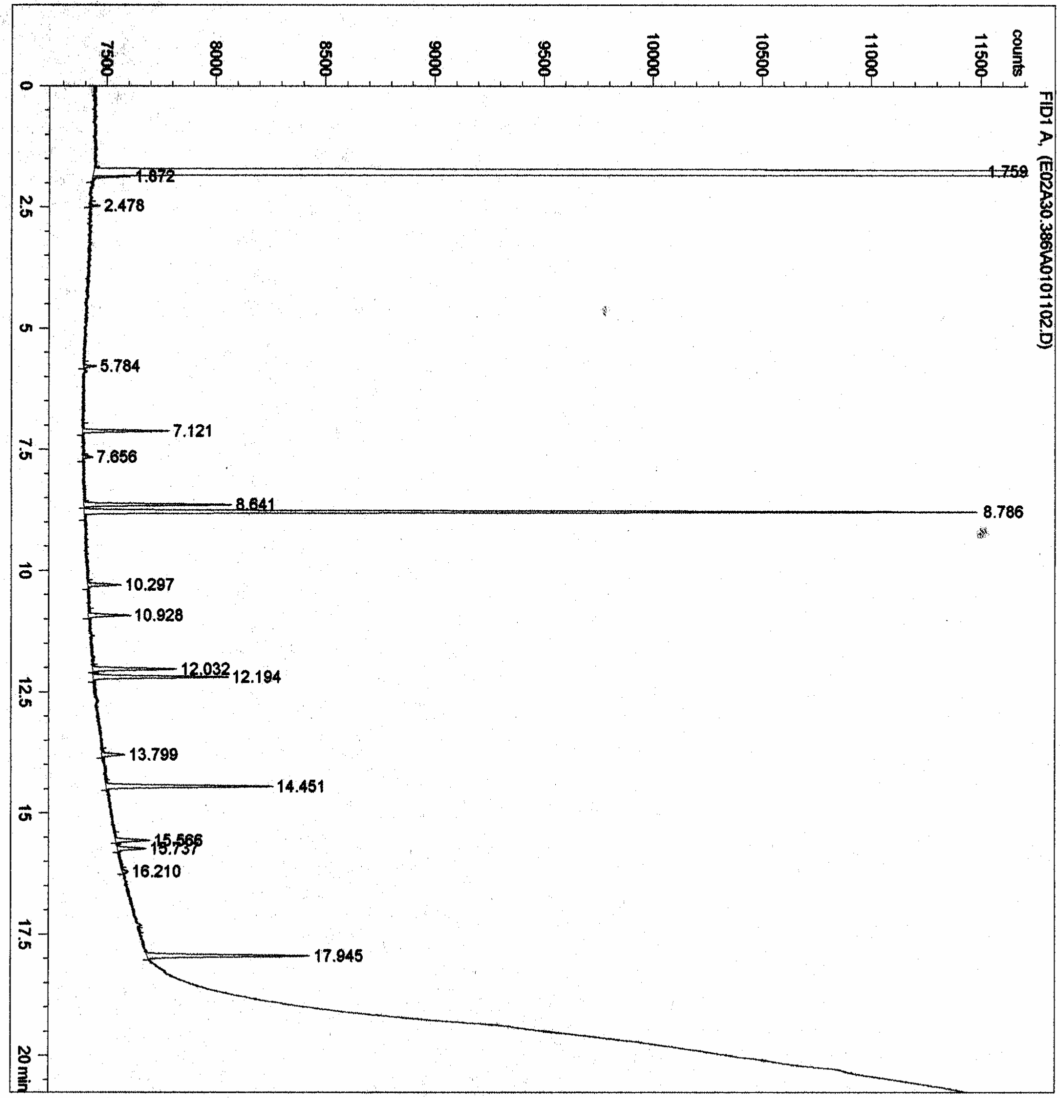
ECL Deviation: 0.001 Reference ECL Shift: 0.006 Number Reference Peaks: 14
Total Response: 145177 Total Named: 145177
Percent Named: 100.00% Total Amount: 136449

Matches:

Library	**Sim Index**	**Entry Name**
TSBA40 4.10	0.325	Staphylococcus-cohnii-cohnii
	0.315	Staphylococcus-xylosus
	0.290	Staphylococcus-simulans*
	0.287	Staphylococcus-warneri*
	0.242	Staphylococcus-lugdunensis*

```
Data File C:\SHERLOCK\RAW\E02A30.386\A0101102.D                           Sample Name:
    Sherlock Id: HRT A/C 1:10 TSBA

    =====================================================================
    Injection Date  : 10/30/02 1:09:54 PM
    Sample Name     :  1102                          Location : Vial 9
    Acq. Operator   :
                                                   Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 10/30/02 1:05:37 PM
    =====================================================================
```



```
*** End of Report ***
```

E02A303.86A [1101] HRT Tank 1:100 TSBA

Volume: DATA File: E02A303.86A Seq Counter: 9 ID Number: 1101
Type: Samp Bottle: 8 Method: TSBA40
Created: 10/30/02 12:40:01 PM
Sample ID: HRT Tank 1:100 TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.778	2.174E+8	0.029	----	7.011	SOLVENT PEAK	----	< min rt	
1.890	3337	0.027	----	7.221		----	< min rt	
5.797	1494	0.035	1.045	12.614	13:0 ISO	1.14	ECL deviates 0.000	Reference 0.001
7.131	8464	0.038	0.999	13.619	14:0 ISO	6.18	ECL deviates 0.000	Reference 0.000
7.667	896	0.046	0.985	13.999	14:0	0.64	ECL deviates -0.001	Reference 0.000
8.651	17603	0.042	0.963	14.623	15:0 ISO	12.39	ECL deviates 0.000	Reference 0.001
8.795	59086	0.042	0.961	14.714	15:0 ANTEISO	41.48	ECL deviates 0.001	Reference 0.002
10.305	3042	0.044	0.937	15.627	16:0 ISO	2.08	ECL deviates 0.000	Reference 0.000
10.936	3080	0.046	0.929	16.000	16:0	2.09	ECL deviates 0.000	Reference -0.001
12.039	7176	0.045	0.917	16.631	17:0 ISO	4.81	ECL deviates 0.001	Reference 0.000
12.201	7269	0.047	0.916	16.724	17:0 ANTEISO	4.87	ECL deviates 0.001	Reference 0.000
13.804	2235	0.046	0.905	17.631	18:0 ISO	1.48	ECL deviates -0.001	Reference -0.002
14.456	11015	0.050	0.901	17.999	18:0	7.25	ECL deviates -0.001	Reference -0.003
15.572	4903	0.051	0.897	18.634	19:0 ISO	3.21	ECL deviates 0.000	Reference -0.003
15.743	2722	0.052	0.896	18.731	19:0 ANTEISO	1.78	ECL deviates 0.000	
17.319	693	0.057	0.892	19.636	20:0 ISO	0.45	ECL deviates 0.001	Reference -0.003
17.664	304	0.096	0.892	19.835	20:1 w7c	0.20	ECL deviates 0.004	
17.951	15239	0.051	0.891	20.000	20:0	9.93	ECL deviates 0.000	Reference -0.004

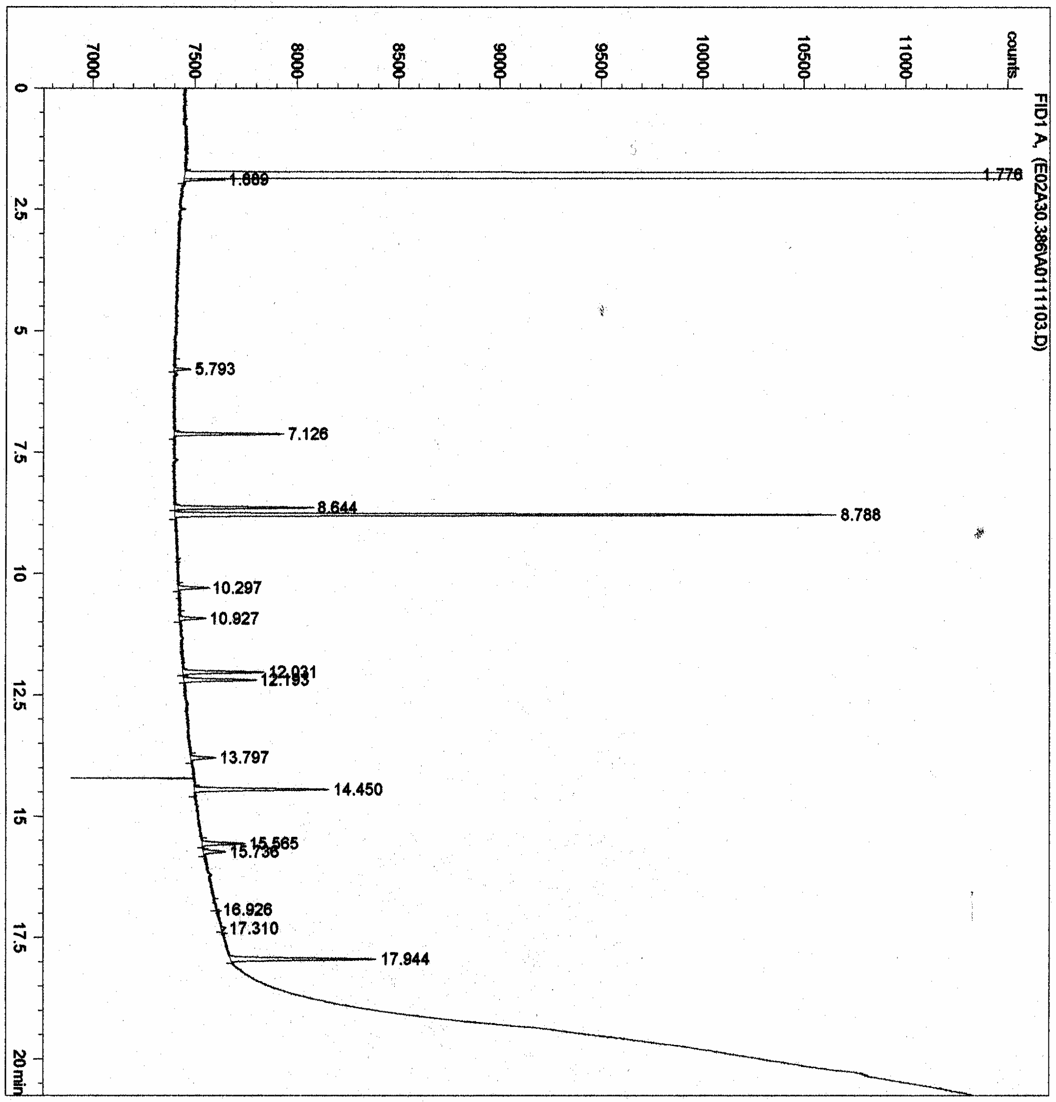
ECL Deviation: 0.001 Reference ECL Shift: 0.002 Number Reference Peaks: 14
Total Response: 145219 Total Named: 145219
Percent Named: 100.00% Total Amount: 136835

Matches:

Library	**Sim Index**	**Entry Name**
TSBA40 4.10	0.299	Staphylococcus-cohnii-cohnii
	0.297	Staphylococcus-warneri*
	0.245	Staphylococcus-xylosus
	0.228	Staphylococcus-aureus-GC subgroup C*
	0.218	Staphylococcus-aureus-GC subroup G*
	0.168	Staphylococcus-epidermidis*

```
Data File C:\SHERLOCK\RAW\E02A30.386\A0111103.D                   Sample Name:
    Sherlock Id: HRT Truck 1:1000 TSBA

    ====================================================================
    Injection Date  : 10/30/02 1:35:31 PM
    Sample Name     :  1103                          Location : Vial 10
    Acq. Operator   :
                                                   Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 10/30/02 1:31:21 PM
    ====================================================================
```



*** End of Report ***

E02A303.86A [1100] HRT Tank 1:10 TSBA

Volume: DATA File: E02A303.86A Seq Counter: 8 ID Number: 1100
Type: Samp Bottle: 7 Method: TSBA40
Created: 10/30/02 12:14:23 PM
Sample ID: HRT Tank 1:10 TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.778	2.183E+8	0.028	----	7.009	SOLVENT PEAK	----	< min rt	
1.890	2396	0.032	----	7.220		----	< min rt	
2.494	576	0.027	----	8.354		----	< min rt	
5.798	1652	0.037	1.045	12.613	13:0 ISO	1.00	ECL deviates -0.001	Reference 0.002
7.132	11718	0.038	0.999	13.618	14:0 ISO	6.77	ECL deviates -0.001	Reference 0.001
7.674	573	0.036	0.985	14.002	14:0	0.33	ECL deviates 0.002	Reference 0.004
8.652	15871	0.041	0.963	14.623	15:0 ISO	8.84	ECL deviates 0.000	Reference 0.001
8.796	82061	0.042	0.961	14.714	15:0 ANTEISO	45.59	ECL deviates 0.001	Reference 0.003
10.306	4230	0.045	0.937	15.626	16:0 ISO	2.29	ECL deviates -0.001	Reference 0.000
10.934	3356	0.047	0.929	15.998	16:0	1.80	ECL deviates -0.002	Reference -0.001
12.040	10950	0.048	0.917	16.630	17:0 ISO	5.81	ECL deviates 0.000	Reference 0.001
12.203	10919	0.047	0.916	16.723	17:0 ANTEISO	5.78	ECL deviates 0.000	Reference 0.000
13.807	3056	0.050	0.905	17.632	18:0 ISO	1.60	ECL deviates 0.000	Reference 0.000
14.459	15085	0.048	0.901	17.999	18:0	7.86	ECL deviates -0.001	Reference -0.002
15.382	1446	0.206	----	18.525		----	> max ar/ht	
15.435	301	0.045	----	18.554		----		
15.576	5761	0.057	0.897	18.635	19:0 ISO	2.99	ECL deviates 0.001	Reference -0.001
15.747	3070	0.055	0.896	18.732	19:0 ANTEISO	1.59	ECL deviates 0.001	
17.205	502	0.113	----	19.569		----	> max ar/ht	
17.318	552	0.055	0.892	19.634	20:0 ISO	0.29	ECL deviates -0.001	Reference -0.003
17.954	14484	0.051	0.891	20.001	20:0	7.47	ECL deviates 0.001	Reference -0.002

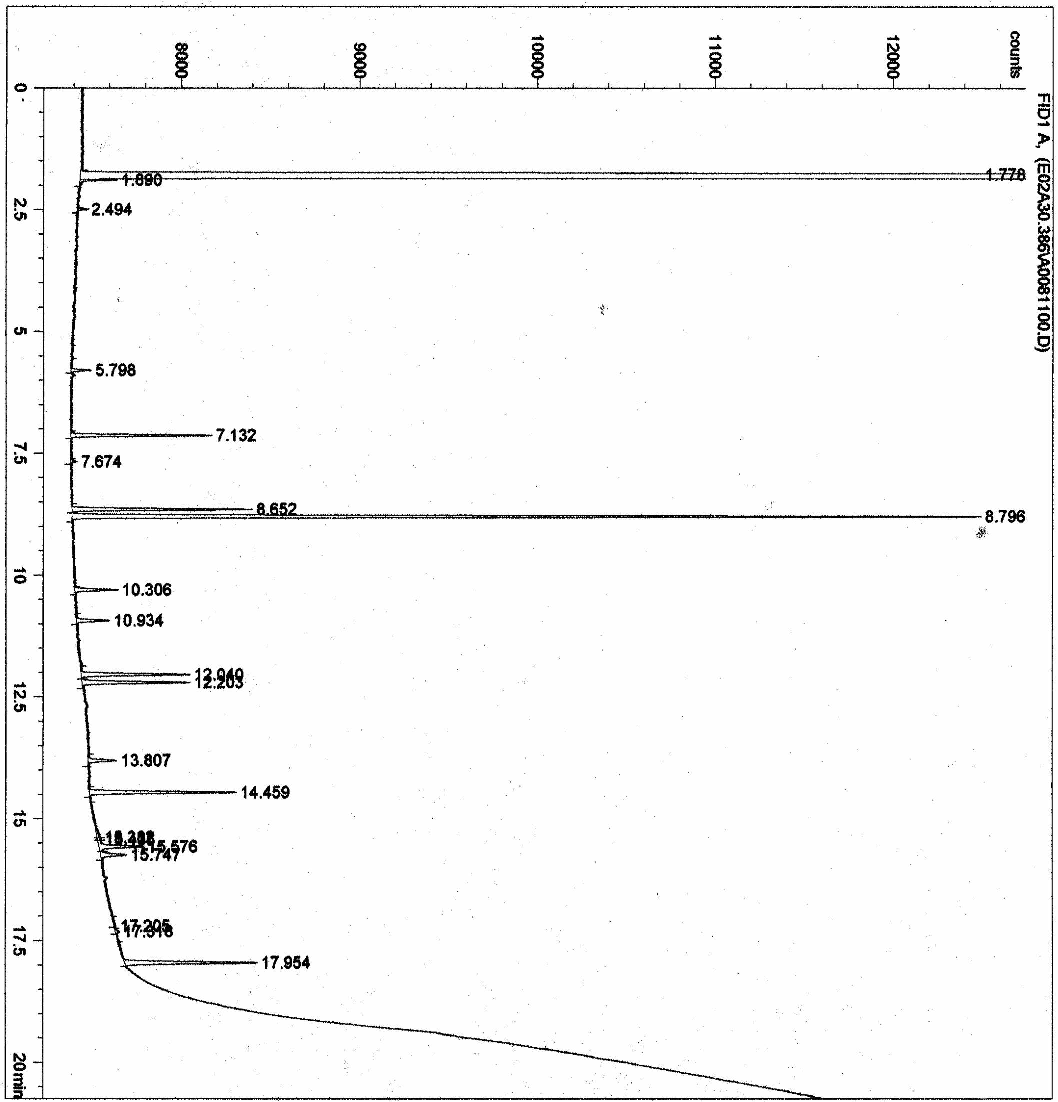
ECL Deviation: 0.001 Reference ECL Shift: 0.002 Number Reference Peaks: 14
Total Response: 185588 Total Named: 183338
Percent Named: 98.79% Total Amount: 172935

Matches:

Library	**Sim Index**	**Entry Name**
TSBA40 4.10	0.238	Staphylococcus-warneri*
	0.156	Staphylococcus-cohnii-cohnii
	0.138	Staphylococcus-aureus-GC subgroup C*
	0.138	Staphylococcus-xylosus

```
Data File C:\SHERLOCK\RAW\E02A30.386\A0081100.D                          Sample Name:
    Sherlock Id: HRT Tank 1:10 TSBA

    =====================================================================
    Injection Date  : 10/30/02 12:18:35 PM
    Sample Name     :  1100                          Location : Vial 7
    Acq. Operator   :
                                                   Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 10/30/02 12:14:23 PM
    =====================================================================
```



*** End of Report ***

E02A303.86A [1099] HRT A/C Neat TSBA

Volume: DATA File: E02A303.86A Seq Counter: 7 ID Number: 1099
Type: Samp Bottle: 6 Method: TSBA40
Created: 10/30/02 11:48:45 AM
Sample ID: HRT A/C Neat TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.779	2.089E+8	0.028	----	7.009	SOLVENT PEAK	----	< min rt	
1.891	2038	0.025	----	7.220		----	< min rt	
2.495	400	0.027	----	8.354		----	< min rt	
5.801	963	0.039	1.045	12.614	13:0 ISO	0.49	ECL deviates 0.000	Reference 0.004
7.134	13875	0.038	0.999	13.619	14:0 ISO	6.70	ECL deviates 0.000	Reference 0.003
7.670	3804	0.039	0.985	13.998	14:0	1.81	ECL deviates -0.002	Reference 0.001
8.655	20161	0.042	0.963	14.624	15:0 ISO	9.38	ECL deviates 0.001	Reference 0.003
8.799	105912	0.042	0.961	14.715	15:0 ANTEISO	49.14	ECL deviates 0.002	Reference 0.004
10.307	4715	0.045	0.937	15.627	16:0 ISO	2.13	ECL deviates 0.000	Reference 0.001
10.937	5501	0.047	0.929	15.999	16:0	2.47	ECL deviates -0.001	Reference 0.000
12.041	6872	0.047	0.917	16.631	17:0 ISO	3.05	ECL deviates 0.001	Reference 0.001
12.203	11704	0.048	0.916	16.723	17:0 ANTEISO	5.18	ECL deviates 0.000	Reference 0.001
13.808	2620	0.049	0.905	17.632	18:0 ISO	1.14	ECL deviates 0.000	Reference 0.000
14.458	15778	0.050	0.901	17.999	18:0	6.87	ECL deviates -0.001	Reference -0.002
15.101	259	0.118	----	18.365		----	> max ar/ht	
15.576	2960	0.059	0.897	18.634	19:0 ISO	1.28	ECL deviates 0.000	Reference -0.001
15.746	2907	0.049	0.896	18.731	19:0 ANTEISO	1.26	ECL deviates 0.000	
16.217	899	0.085	0.895	18.999	19:0	0.39	ECL deviates -0.001	Reference -0.003
17.321	1005	0.066	0.892	19.635	20:0 ISO	0.43	ECL deviates 0.000	Reference -0.002
17.955	19246	0.052	0.891	20.001	20:0	8.29	ECL deviates 0.001	Reference -0.002

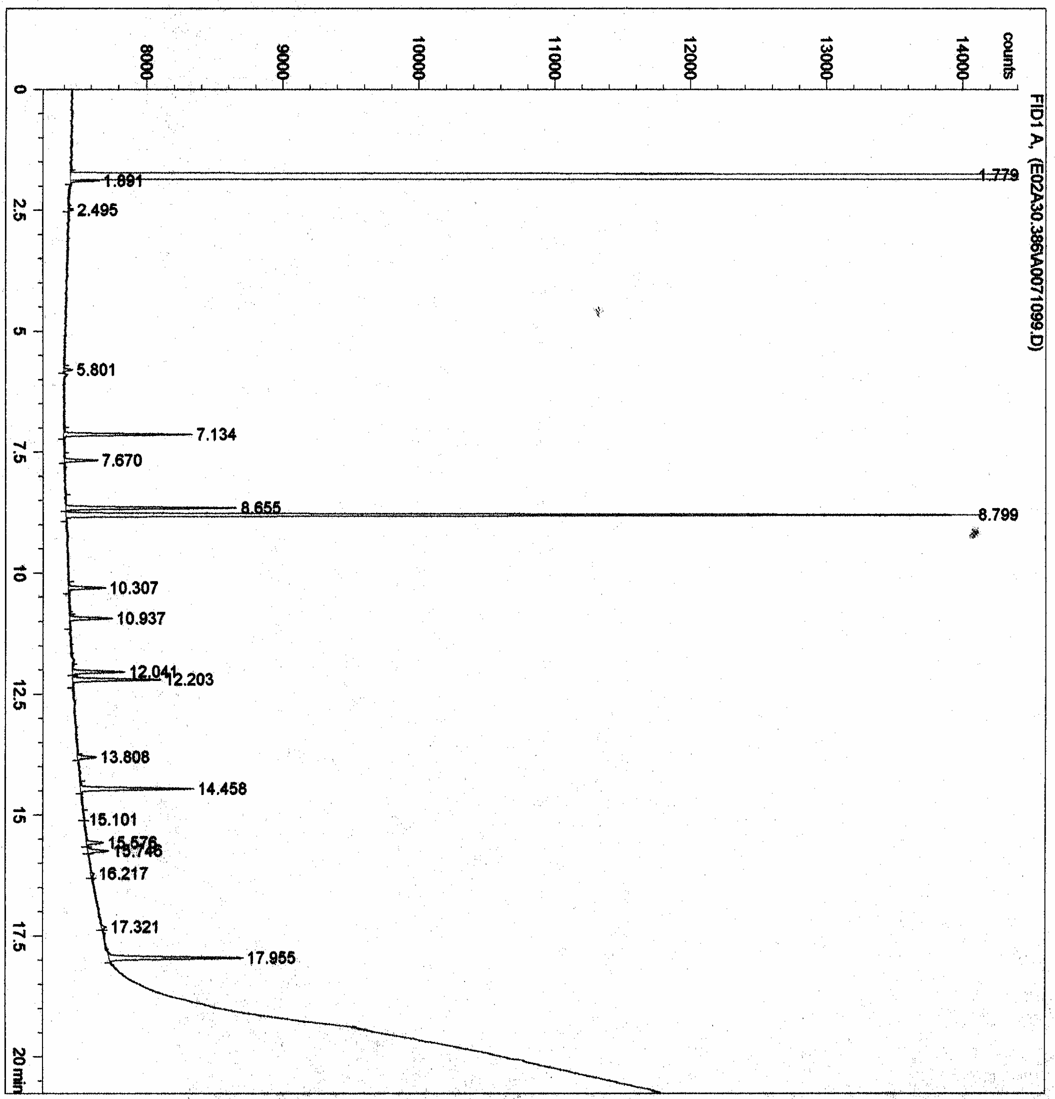
ECL Deviation: 0.001 Reference ECL Shift: 0.002 Number Reference Peaks: 15
Total Response: 219183 Total Named: 218923
Percent Named: 99.88% Total Amount: 207050

Matches:

Library	**Sim Index**	**Entry Name**
TSBA40 4.10	0.248	Staphylococcus-warneri*
	0.183	Staphylococcus-cohnii-cohnii
	0.132	Staphylococcus-xylosus

```
Data File C:\SHERLOCK\RAW\E02A30.386\A0071099.D                              Sample Name:
   Sherlock Id: HRT A/C Neat TSBA

    =====================================================================
    Injection Date  : 10/30/02 11:52:57 AM
    Sample Name     :  1099                          Location : Vial 6
    Acq. Operator   :
                                                   Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 10/30/02 11:48:45 AM
    =====================================================================
```



```
*** End of Report ***
```

Appendix E: Worksheet / Sample Data – Tyndall AFB (KPAM)

	Site Information									
#	Sample	Type / Number	Date Sampled	Dilution	Medium	Temp °C	% Relative Humidity	Inches Rainfall (last 30 days)	Fuel Type	Sample Method
1	Neg Control	—	—	—	—	—	—	—	—	—
2	Pos Control	—	—	—	—	—	—	—	—	—
3	Tank	407	10/2/2002	Neat	TSBA	26	83	7.48	JP-8	gravity
4	Tank	407	10/2/2002	1:10	TSBA	26	83	7.48	JP-8	gravity
5	Tank	407	10/2/2002	1:100	TSBA	26	83	7.48	JP-8	gravity
6	Tank	407	10/2/2002	1:1000	TSBA	26	83	7.48	JP-8	gravity
7	Tank	407	10/2/2002	Neat	SDA	26	83	7.48	JP-8	gravity
8	Tank	407	10/2/2002	1:10	SDA	26	83	7.48	JP-8	gravity
9	Tank	407	10/2/2002	1:100	SDA	26	83	7.48	JP-8	gravity
10	Tank	407	10/2/2002	1:1000	SDA	26	83	7.48	JP-8	gravity
11	Tank	407	10/2/2002	Neat	BHIBLA	26	83	7.48	JP-8	gravity
12	Tank	407	10/2/2002	1:10	BHIBLA	26	83	7.48	JP-8	gravity
13	Tank	407	10/2/2002	1:100	BHIBLA	26	83	7.48	JP-8	gravity
14	Tank	407	10/2/2002	1:1000	BHIBLA	26	83	7.48	JP-8	gravity
15	Truck	124	10/2/2002	Neat	TSBA	26	83	7.48	JP-8 +100	gravity
16	Truck	124	10/2/2002	1:10	TSBA	26	83	7.48	JP-8 +100	gravity
17	Truck	124	10/2/2002	1:100	TSBA	26	83	7.48	JP-8 +100	gravity
18	Truck	124	10/2/2002	1:1000	TSBA	26	83	7.48	JP-8 +100	gravity
19	Truck	124	10/2/2002	Neat	SDA	26	83	7.48	JP-8 +100	gravity
20	Truck	124	10/2/2002	1:10	SDA	26	83	7.48	JP-8 +100	gravity
21	Truck	124	10/2/2002	1:100	SDA	26	83	7.48	JP-8 +100	gravity
22	Truck	124	10/2/2002	1:1000	SDA	26	83	7.48	JP-8 +100	gravity
23	Truck	124	10/2/2002	Neat	BHIBLA	26	83	7.48	JP-8 +100	gravity
24	Truck	124	10/2/2002	1:10	BHIBLA	26	83	7.48	JP-8 +100	gravity
25	Truck	124	10/2/2002	1:100	BHIBLA	26	83	7.48	JP-8 +100	gravity
26	Truck	124	10/2/2002	1:1000	BHIBLA	26	83	7.48	JP-8 +100	gravity
27	Aircraft	F-15	10/2/2002	Neat	TSBA	26	83	7.48	JP-8 +100	gravity
28	Aircraft	F-15	10/2/2002	1:10	TSBA	26	83	7.48	JP-8 +100	gravity
29	Aircraft	F-15	10/2/2002	1:100	TSBA	26	83	7.48	JP-8 +100	gravity
30	Aircraft	F-15	10/2/2002	1:1000	TSBA	26	83	7.48	JP-8 +100	gravity
31	Aircraft	F-15	10/2/2002	Neat	SDA	26	83	7.48	JP-8 +100	gravity
32	Aircraft	F-15	10/2/2002	1:10	SDA	26	83	7.48	JP-8 +100	gravity
33	Aircraft	F-15	10/2/2002	1:100	SDA	26	83	7.48	JP-8 +100	gravity
34	Aircraft	F-15	10/2/2002	1:1000	SDA	26	83	7.48	JP-8 +100	gravity
35	Aircraft	F-15	10/2/2002	Neat	BHIBLA	26	83	7.48	JP-8 +100	gravity
36	Aircraft	F-15	10/2/2002	1:10	BHIBLA	26	83	7.48	JP-8 +100	gravity
37	Aircraft	F-15	10/2/2002	1:100	BHIBLA	26	83	7.48	JP-8 +100	gravity
38	Aircraft	F-15	10/2/2002	1:1000	BHIBLA	26	83	7.48	JP-8 +100	gravity

— Indicates analysis omitted

		Sample Description					
#	Tank Composition	pH	Sample Color	General Description	Obvious Solids	TDS (mg/L)	Free Phase Water
1	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—
3	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
4	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
5	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
6	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
7	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
8	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
9	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
10	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
11	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
12	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
13	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
14	steel w/ epoxy lining	—	dark yellow	clear	no	—	no
15	aluminum	6.3	dark yellow	clear	no	—	yes
16	aluminum	6.3	dark yellow	clear	no	—	yes
17	aluminum	6.3	dark yellow	clear	no	—	yes
18	aluminum	6.3	dark yellow	clear	no	—	yes
19	aluminum	6.3	dark yellow	clear	no	—	yes
20	aluminum	6.3	dark yellow	clear	no	—	yes
21	aluminum	6.3	dark yellow	clear	no	—	yes
22	aluminum	6.3	dark yellow	clear	no	—	yes
23	aluminum	6.3	dark yellow	clear	no	—	yes
24	aluminum	6.3	dark yellow	clear	no	—	yes
25	aluminum	6.3	dark yellow	clear	no	—	yes
26	aluminum	6.3	dark yellow	clear	no	—	yes
27	aluminum	—	dark yellow	clear	no	—	no
28	aluminum	—	dark yellow	clear	no	—	no
29	aluminum	—	dark yellow	clear	no	—	no
30	aluminum	—	dark yellow	clear	no	—	no
31	aluminum	—	dark yellow	clear	no	—	no
32	aluminum	—	dark yellow	clear	no	—	no
33	aluminum	—	dark yellow	clear	no	—	no
34	aluminum	—	dark yellow	clear	no	—	no
35	aluminum	—	dark yellow	clear	no	—	no
36	aluminum	—	dark yellow	clear	no	—	no
37	aluminum	—	dark yellow	clear	no	—	no
38	aluminum	—	dark yellow	clear	no	—	no

	Light Microscopy				Colony Description		Quantity Data		
#	Gram +/-	Organism Shape	Length of Cells (μm)	Width of Cells (μm)	Colony Type	Colony Color	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours
1	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	0	0	0
4	—	—	—	—	—	—	0	0	0
5	—	—	—	—	—	—	0	0	0
6	—	—	—	—	—	—	0	0	0
7	—	—	—	—	—	—	0	0	0
8	—	—	—	—	—	—	0	0	0
9	—	—	—	—	—	—	0	0	0
10	—	—	—	—	—	—	0	0	0
11	—	—	—	—	—	—	0	0	0
12	—	—	—	—	—	—	0	0	0
13	—	—	—	—	—	—	0	0	0
14	neg	coccus	1 diameter	—	irregular	white	0	0	4 days to grow
15	—	—	—	—	—	—	0	0	0
16	—	—	—	—	—	—	0	0	0
17	neg	coccus	1 diameter	—	round	white	1	2	1,000
18	—	—	—	—	—	—	0	0	0
19	—	—	—	—	—	—	0	0	0
20	—	—	—	—	—	—	0	0	0
21	—	—	—	—	—	—	0	0	0
22	—	—	—	—	—	—	0	0	0
23	—	—	—	—	—	—	0	0	0
24	—	—	—	—	—	—	0	0	0
25	neg	coccus	1 diameter	—	round	white	0	0	4 days to grow
26	neg	coccus	1 diameter	—	round	white	0	0	4 days to grow
27	—	—	—	—	—	—	0	0	0
28	—	—	—	—	—	—	0	0	0
29	—	—	—	—	—	—	0	0	0
30	—	—	—	—	—	—	0	0	0
31	—	—	—	—	—	—	0	0	0
32	—	—	—	—	—	—	0	0	0
33	—	—	—	—	—	—	0	0	0
34	pos	coccus	1 diameter	—	round	white	2	7	20,000
35	—	—	—	—	—	—	0	0	0
36	—	—	—	—	—	—	0	0	0
37	neg	bacillus	1	0.5	round	white	0	0	4 days to grow
38	neg	coccus	1 diameter	—	round	white	0	0	4 days to grow

				Identification Data				
				FAME analysis			DNA gene sequence	
#	Bacteria vs. Fungi	Spores	Digital Photo	Sim Index	> .1 S. I. Separation	GC Best Match	DNA % ID	DNA Best Match
1	—	—	—	—	—	Library match not attempted	—	—
2	—	—	—	0.808	yes	*Stenotrophomonas-maltophilia*	—	—
3	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—
13	—	—	—	—	—	—	—	—
14	bacteria	no	—	0.010	no	*Bacteroides-melaninogenicus*	—	—
15	—	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—
17	bacteria	no	PAMTR100TSBA	0.170	no	*Staphylococcus-aureus-GC subgroup C*	—	—
18	—	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—
20	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—
22	—	—	—	—	—	—	—	—
23	—	—	—	—	—	—	—	—
24	—	—	—	—	—	—	—	—
25	bacteria	no	PAMTR100BHI	—	—	No match found	—	—
26	bacteria	no	PAMTR1000BHI	—	—	No match found	—	—
27	—	—	—	—	—	—	—	—
28	—	—	—	—	—	—	—	—
29	—	—	—	—	—	—	—	—
30	—	—	—	—	—	—	—	—
31	—	—	—	—	—	—	—	—
32	—	—	—	—	—	—	—	—
33	—	—	—	—	—	—	—	—
34	bacteria	no	PAMAC1000SDA	—	—	No match found	—	—
35	—	—	—	—	—	—	—	—
36	—	—	—	—	—	—	—	—
37	bacteria	no	PAMAC100BHI	—	—	Library match not attempted	—	—
38	bacteria	no	PAMAC1000BHI	0.027	no	*Propionibacterium-propionicus (Arachnia propionica)*	—	—

E02A303.86A [1105] PAM Truck 1:100 TSBA

Volume: DATA File: E02A303.86A Seq Counter: 13 ID Number: 1105
Type: Samp Bottle: 12 Method: TSBA40
Created: 10/30/02 2:22:36 PM
Sample ID: PAM Truck 1:100 TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.775	2.246E18	0.029	----	7.010	SOLVENT PEAK	----	< min rt	
1.887	1499	0.036	----	7.221		----	< min rt	
2.490	545	0.032	----	8.354		----	< min rt	
5.788	2642	0.037	1.045	12.613	13:0 ISO	1.05	ECL deviates -0.001	Reference -0.006
5.897	715	0.037	1.041	12.704	13:0 ANTEISO	0.28	ECL deviates 0.002	Reference -0.003
7.123	20381	0.038	0.999	13.619	14:0 ISO	7.72	ECL deviates 0.000	Reference -0.005
7.658	8277	0.040	0.985	13.998	14:0	3.09	ECL deviates -0.002	Reference -0.007
8.641	34290	0.041	0.963	14.623	15:0 ISO	12.53	ECL deviates 0.000	Reference -0.006
8.786	124499	0.042	0.961	14.715	15:0 ANTEISO	45.35	ECL deviates 0.002	Reference -0.004
10.295	6546	0.044	0.937	15.627	16:0 ISO	2.32	ECL deviates 0.000	Reference -0.006
10.380	788	0.042	0.936	15.677	unknown 15.669	0.28	ECL deviates 0.008	
10.923	9110	0.047	0.929	15.999	16:0	3.21	ECL deviates -0.001	Reference -0.008
11.111	1309	0.055	----	16.106		----		
11.333	705	0.053	----	16.233		----		
11.430	971	0.051	----	16.288		----		
11.749	1178	0.048	0.920	16.471	Sum In Feature 4	0.41	ECL deviates -0.005	17:1 ISO I/ANTEI B
12.028	8685	0.047	0.917	16.630	17:0 ISO	3.02	ECL deviates 0.000	Reference -0.007
12.191	9426	0.048	0.916	16.724	17:0 ANTEISO	3.27	ECL deviates 0.001	Reference -0.006
13.020	4437	0.049	----	17.195		----		
13.523	1263	0.059	----	17.479		----		
13.794	2558	0.053	0.905	17.632	18:0 ISO	0.88	ECL deviates 0.000	Reference -0.008
14.445	17209	0.049	0.901	17.999	18:0	5.88	ECL deviates -0.001	Reference -0.009
14.804	699	0.057	----	18.203		----		
15.561	3002	0.052	0.897	18.633	19:0 ISO	1.02	ECL deviates -0.001	Reference -0.009
15.731	1824	0.048	0.896	18.730	19:0 ANTEISO	0.62	ECL deviates -0.001	
16.210	850	0.044	0.895	19.003	19:0	0.29	ECL deviates 0.003	Reference -0.006
16.329	872	0.053	----	19.071		----		
16.964	2966	0.062	----	19.437		----		
17.135	131	0.038	----	19.536		----		
17.222	265	0.050	----	19.586		----		
17.305	866	0.050	0.892	19.634	20:0 ISO	0.29	ECL deviates -0.001	Reference -0.011
17.581	696	0.087	----	19.793		----		
17.638	126	0.039	0.892	19.825	20:1 w7c	0.04	ECL deviates -0.006	
17.940	24961	0.049	0.891	20.000	20:0	8.44	ECL deviates 0.000	Reference -0.010
18.036	372	0.037	----	20.055		----	> max rt	
18.198	1213	0.041	----	20.148		----	> max rt	
----	1178	---	----	----	Summed Feature 4	0.41	17:1 ISO I/ANTEI B	17:1 ANTEISO B/i I

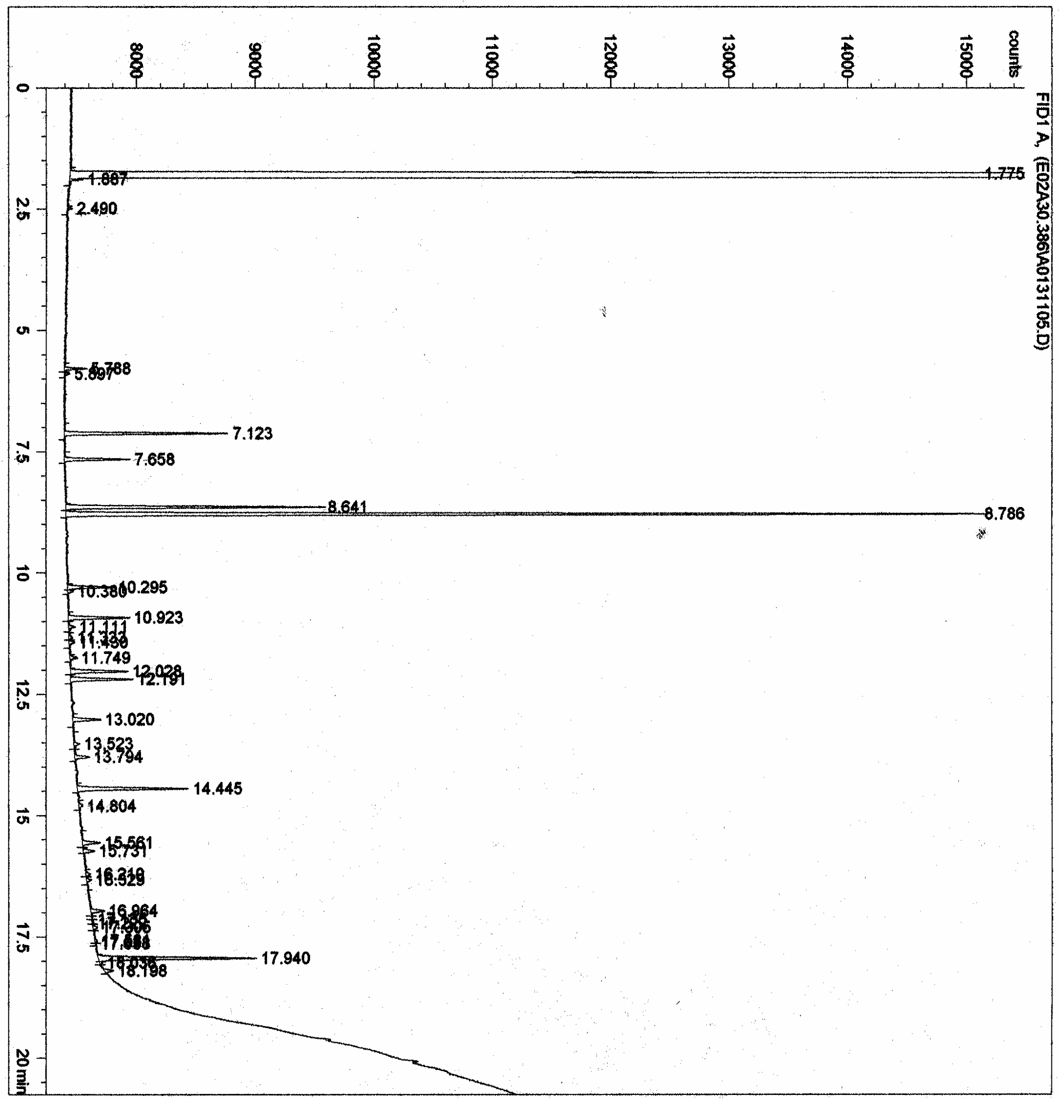
ECL Deviation: 0.003 Reference ECL Shift: 0.007 Number Reference Peaks: 16
Total Response: 292248 Total Named: 277934
Percent Named: 95.10% Total Amount: 263729

Matches:

Library	Sim Index	Entry Name
TSBA40 4.10	0.170	Staphylococcus-aureus-GC subgroup C*
	0.147	Staphylococcus-warneri*
	0.143	Staphylococcus-cohnii-cohnii
	0.105	Staphylococcus-xylosus

```
Data File C:\SHERLOCK\RAW\E02A30.386\A0131105.D                          Sample Name:
    Sherlock Id: PAM Truck 1:100 TSBA

    =====================================================================
    Injection Date  : 10/30/02 2:26:45 PM
    Sample Name     :  1105                        Location : Vial 12
    Acq. Operator   :
                                                 Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 10/30/02 2:22:36 PM
    =====================================================================
```



*** End of Report ***

E02A313.62A [1132] PAM TANK 1:1000 BHI

Volume: DATA File: E02A313.62A Seq Counter: 19 ID Number: 1132
Type: Samp Bottle: 41 Method: ANAEROBE
Created: 10/31/02 4:21:06 PM
Sample ID: PAM TANK 1:1000 BHI

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.775	2.233E+18	0.029	----	7.014	SOLVENT PEAK	----	< min rt	
1.889	1805	0.026	----	7.226		----	< min rt	
2.491	431	0.034	----	8.359		----	< min rt	
2.687	743	0.033	----	8.727		----	< min rt	
3.808	1161	0.028	1.177	10.608	11:0 ISO FAME	0.27	ECL deviates 0.001	Reference -0.003
3.871	756	0.032	1.171	10.695	11:0 ANTEISO FAME	0.17	ECL deviates -0.001	Reference -0.005
5.786	13473	0.035	1.058	12.614	13:0 ISO FAME	2.81	ECL deviates 0.000	Reference -0.004
5.892	7905	0.035	1.053	12.702	13:0 ANTEISO FAME	1.64	ECL deviates -0.001	Reference -0.004
7.119	23568	0.038	1.010	13.618	14:0 ISO FAME	4.69	ECL deviates 0.000	Reference -0.003
7.653	3452	0.041	0.994	13.997	14:0 FAME	0.68	ECL deviates -0.003	Reference -0.006
8.642	171521	0.042	0.970	14.626	15:0 ISO FAME	32.79	ECL deviates 0.003	Reference 0.000
8.789	271077	0.042	0.967	14.719	15:0 ANTEISO FAME	51.65	ECL deviates 0.005	Reference 0.002
9.227	1238	0.041	0.957	14.998	15:0 FAME	0.23	ECL deviates -0.002	Reference -0.005
10.289	11583	0.046	0.938	15.625	16:0 ISO FAME	2.14	ECL deviates -0.002	Reference -0.005
10.919	1645	0.044	0.928	15.997	16:0 FAME	0.30	ECL deviates -0.003	Reference -0.007
11.164	3803	0.067	0.925	16.137	15:0 ISO 3OH FAME	0.69	ECL deviates 0.002	
11.324	4361	0.077	0.922	16.229	Sum In Feature 6	0.79	ECL deviates 0.003	15:0 ANTEI 3OH FAME
12.030	792	0.048	0.914	16.632	17:0 ISO FAME	0.14	ECL deviates 0.002	Reference -0.001
12.185	3782	0.049	0.912	16.721	17:0 ANTEISO FAME	0.68	ECL deviates -0.002	
15.814	5015	0.062	----	18.777		----		
15.885	1721	0.028	----	18.818		----		
16.294	20360	0.470	----	19.051		----	> max ar/ht	
16.726	13690	0.323	----	19.300		----	> max ar/ht	
17.224	12961	0.128	----	19.586		----		
17.435	1919	0.100	----	19.707		----		
17.720	27837	0.122	----	19.871		----		
17.922	1805	0.089	0.891	19.988	20:0 FAME	0.32	ECL deviates -0.012	
18.340	605	0.046	----	20.228		----	> max rt	
18.943	2634	0.038	----	20.575		----	> max rt	
19.048	1443	0.042	----	20.635		----	> max rt	
----	4361	---	----	----	Summed Feature 6	0.79	15:0 ANTEI 3OH FAME	16:1 CIS 7 DMA

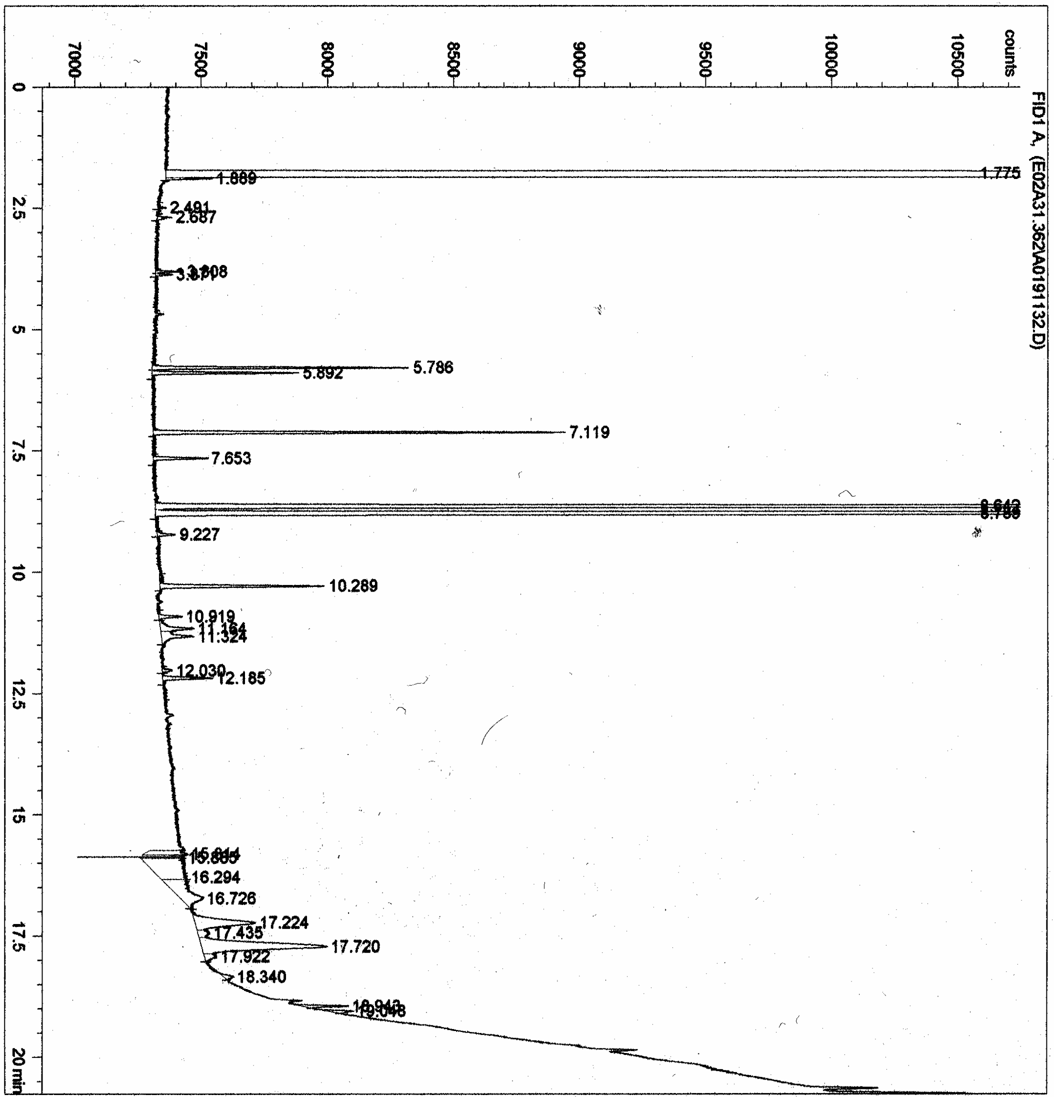
ECL Deviation: 0.004 Reference ECL Shift: 0.004 Number Reference Peaks: 12
Total Response: 605428 Total Named: 521924
Percent Named: 86.21% Total Amount: 507373

Matches:

Library	Sim Index	Entry Name
BHIBLA 3.80	0.010	Bacteroides-melaninogenicus
	0.006	Prevotella-loescheii (Bacteroides loescheii)

```
Data File C:\SHERLOCK\RAW\E02A31.362\A0191132.D                        Sample Name:
    Sherlock Id: PAM TANK 1:1000 BHI

    =====================================================================
    Injection Date  : 10/31/02 4:25:14 PM
    Sample Name     :  1132                          Location : Vial 41
    Acq. Operator   :
                                                   Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 10/31/02 4:21:07 PM
    =====================================================================
```



*** End of Report ***

E02A313.62A [1131] PAM A/C 1:1000 BHI

Volume: DATA File: E02A313.62A Seq Counter: 18 ID Number: 1131
Type: Samp Bottle: 40 Method: ANAEROBE
Created: 10/31/02 3:55:26 PM
Sample ID: PAM A/C 1:1000 BHI

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.776	2.197E+18	0.029	----	7.012	SOLVENT PEAK	----	< min rt	
1.889	2347	0.034	----	7.224		----	< min rt	
2.493	360	0.027	----	8.361		----	< min rt	
3.808	591	0.028	1.177	10.607	11:0 ISO FAME	0.17	ECL deviates 0.000	Reference -0.003
3.874	653	0.029	1.171	10.697	11:0 ANTEISO FAME	0.18	ECL deviates 0.001	Reference -0.002
5.788	5180	0.035	1.058	12.613	13:0 ISO FAME	1.31	ECL deviates -0.001	Reference -0.003
5.894	4576	0.036	1.053	12.702	13:0 ANTEISO FAME	1.16	ECL deviates -0.001	Reference -0.003
7.121	14868	0.038	1.010	13.618	14:0 ISO FAME	3.60	ECL deviates 0.000	Reference -0.002
7.655	2901	0.042	0.994	13.997	14:0 FAME	0.69	ECL deviates -0.003	Reference -0.005
8.642	96283	0.042	0.970	14.625	15:0 ISO FAME	22.40	ECL deviates 0.002	Reference 0.000
8.791	283856	0.042	0.967	14.719	15:0 ANTEISO FAME	65.81	ECL deviates 0.005	Reference 0.003
9.232	888	0.042	0.957	15.000	15:0 FAME	0.20	ECL deviates 0.000	Reference -0.002
10.291	10365	0.045	0.938	15.626	16:0 ISO FAME	2.33	ECL deviates -0.001	Reference -0.004
10.919	1664	0.047	0.928	15.997	16:0 FAME	0.37	ECL deviates -0.003	Reference -0.007
11.164	851	0.044	0.925	16.137	15:0 ISO 3OH FAME	0.19	ECL deviates 0.002	
11.326	1417	0.048	0.922	16.230	Sum In Feature 6	0.31	ECL deviates 0.004	15:0 ANTEI 3OH FAME
12.027	736	0.050	0.914	16.631	17:0 ISO FAME	0.16	ECL deviates 0.001	Reference -0.003
12.187	5049	0.047	0.912	16.723	17:0 ANTEISO FAME	1.10	ECL deviates 0.000	
15.822	584	0.052	----	18.782		----		
18.262	59261	0.147	----	20.184		----	> max rt	
18.833	532289	0.292	----	20.512		----	> max rt	
18.943	172822	0.088	----	20.575		----	> max rt	
19.047	170753	0.077	----	20.635		----	> max rt	
19.857	1.948E+6	0.435	----	21.101		----	> max rt	
19.910	108701	0.028	----	21.132		----	> max rt	
----	1417	---	----	----	Summed Feature 6	0.31	15:0 ANTEI 3OH FAME	16:1 CIS 7 DMA

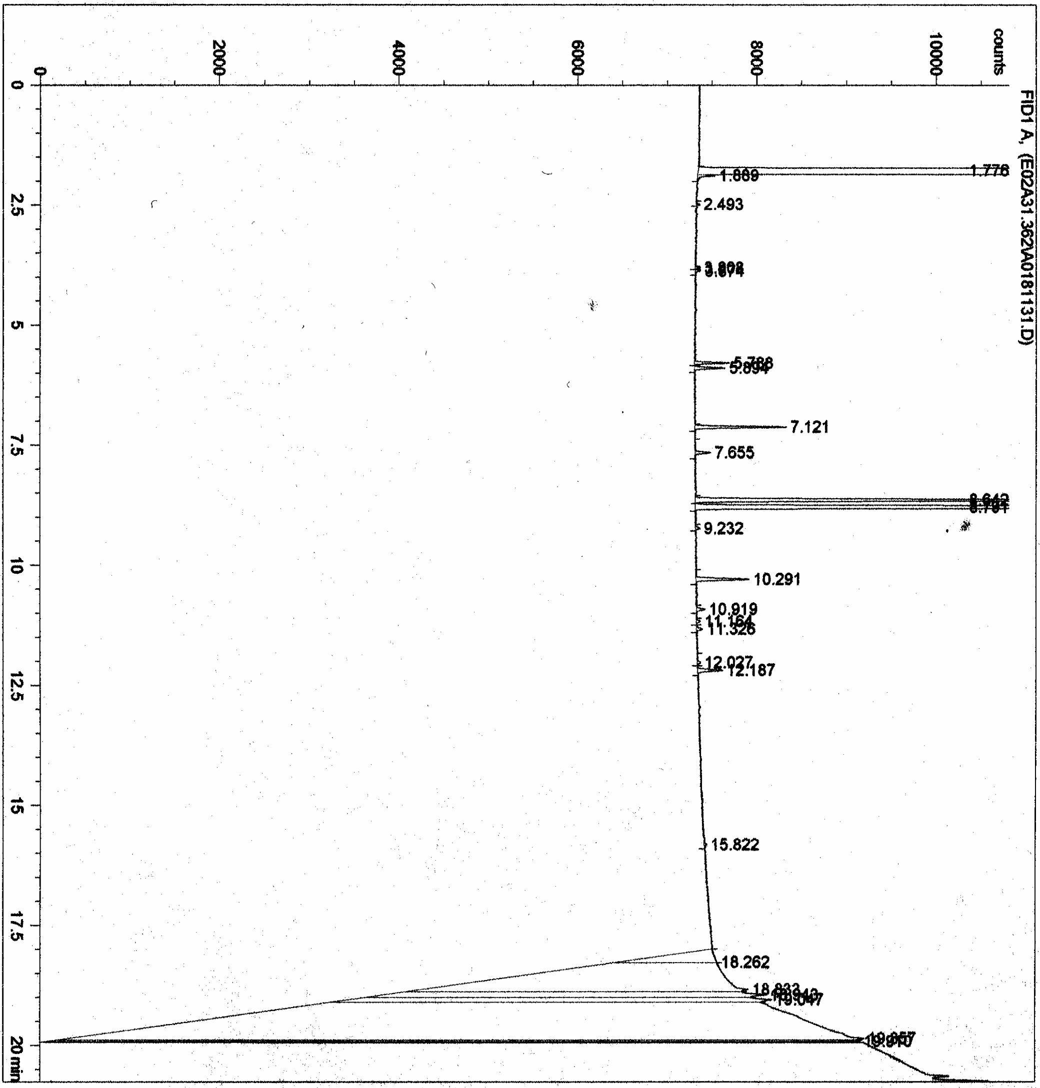
ECL Deviation: 0.002 Reference ECL Shift: 0.003 Number Reference Peaks: 12
Total Response: 430459 Total Named: 429875
Percent Named: 99.86% Total Amount: 416931

Matches:

Library	Sim Index	Entry Name
BHIBLA 3.80	0.027	Propionibacterium-propionicus (Arachnia propionica)

```
Data File C:\SHERLOCK\RAW\E02A31.362\A0181131.D                        Sample Name:
    Sherlock Id: PAM A/C 1:1000 BHI

    =====================================================================
    Injection Date  : 10/31/02 3:59:38 PM
    Sample Name     :  1131                         Location : Vial 40
    Acq. Operator   :
                                                  Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 10/31/02 3:55:26 PM
    =====================================================================
```



*** End of Report ***

Appendix F: Worksheet / Sample Data – Ellsworth AFB (KRCA)

	Site Information								Fuel	Sample
#	Sample	Type / Number	Date Sampled	Dilution	Medium	Temp °C	% Relative Humidity	Inches Rainfall (last 30 days)	Fuel Type	Sample Method
1	Neg Control	—	—	—	—	—	—	—	—	—
2	Pos Control	—	—	—	—	—	—	—	—	—
3	Tank	15	10/10/2002	Neat	TSBA	2	70	2.38	JP-8	gravity
4	Tank	15	10/10/2002	1:10	TSBA	2	70	2.38	JP-8	gravity
5	Tank	15	10/10/2002	1:100	TSBA	2	70	2.38	JP-8	gravity
6	Tank	15	10/10/2002	1:1000	TSBA	2	70	2.38	JP-8	gravity
7	Tank	15	10/10/2002	Neat	SDA	2	70	2.38	JP-8	gravity
8	Tank	15	10/10/2002	1:10	SDA	2	70	2.38	JP-8	gravity
9	Tank	15	10/10/2002	1:100	SDA	2	70	2.38	JP-8	gravity
10	Tank	15	10/10/2002	1:1000	SDA	2	70	2.38	JP-8	gravity
11	Tank	15	10/10/2002	Neat	BHIBLA	2	70	2.38	JP-8	gravity
12	Tank	15	10/10/2002	1:10	BHIBLA	2	70	2.38	JP-8	gravity
13	Tank	15	10/10/2002	1:100	BHIBLA	2	70	2.38	JP-8	gravity
14	Tank	15	10/10/2002	1:1000	BHIBLA	2	70	2.38	JP-8	gravity
15	Truck	97L359	10/10/2002	Neat	TSBA	2	70	2.38	JP-8	gravity
16	Truck	97L359	10/10/2002	1:10	TSBA	2	70	2.38	JP-8	gravity
17	Truck	97L359	10/10/2002	1:100	TSBA	2	70	2.38	JP-8	gravity
18	Truck	97L359	10/10/2002	1:1000	TSBA	2	70	2.38	JP-8	gravity
19	Truck	97L359	10/10/2002	Neat	SDA	2	70	2.38	JP-8	gravity
20	Truck	97L359	10/10/2002	1:10	SDA	2	70	2.38	JP-8	gravity
21	Truck	97L359	10/10/2002	1:100	SDA	2	70	2.38	JP-8	gravity
22	Truck	97L359	10/10/2002	1:1000	SDA	2	70	2.38	JP-8	gravity
23	Truck	97L359	10/10/2002	Neat	BHIBLA	2	70	2.38	JP-8	gravity
24	Truck	97L359	10/10/2002	1:10	BHIBLA	2	70	2.38	JP-8	gravity
25	Truck	97L359	10/10/2002	1:100	BHIBLA	2	70	2.38	JP-8	gravity
26	Truck	97L359	10/10/2002	1:1000	BHIBLA	2	70	2.38	JP-8	gravity
27	Aircraft	B-1 #83	10/10/2002	Neat	TSBA	2	70	2.38	JP-8	gravity
28	Aircraft	B-1 #83	10/10/2002	1:10	TSBA	2	70	2.38	JP-8	gravity
29	Aircraft	B-1 #83	10/10/2002	1:100	TSBA	2	70	2.38	JP-8	gravity
30	Aircraft	B-1 #83	10/10/2002	1:1000	TSBA	2	70	2.38	JP-8	gravity
31	Aircraft	B-1 #83	10/10/2002	Neat	SDA	2	70	2.38	JP-8	gravity
32	Aircraft	B-1 #83	10/10/2002	1:10	SDA	2	70	2.38	JP-8	gravity
33	Aircraft	B-1 #83	10/10/2002	1:100	SDA	2	70	2.38	JP-8	gravity
34	Aircraft	B-1 #83	10/10/2002	1:1000	SDA	2	70	2.38	JP-8	gravity
35	Aircraft	B-1 #83	10/10/2002	Neat	BHIBLA	2	70	2.38	JP-8	gravity
36	Aircraft	B-1 #83	10/10/2002	1:10	BHIBLA	2	70	2.38	JP-8	gravity
37	Aircraft	B-1 #83	10/10/2002	1:100	BHIBLA	2	70	2.38	JP-8	gravity
38	Aircraft	B-1 #83	10/10/2002	1:1000	BHIBLA	2	70	2.38	JP-8	gravity

— Indicates analysis omitted

		Sample Description					
#	Tank Composition	pH	Sample Color	General Description	Obvious Solids	TDS (mg/L)	Free Phase Water
1	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—
3	steel w/ epoxy lining	6.4	light yellow	clear	yes	20	yes
4	steel w/ epoxy lining	6.4	light yellow	clear	yes	20	yes
5	steel w/ epoxy lining	6.4	light yellow	clear	yes	20	yes
6	steel w/ epoxy lining	6.4	light yellow	clear	yes	20	yes
7	steel w/ epoxy lining	6.4	light yellow	clear	yes	20	yes
8	steel w/ epoxy lining	6.4	light yellow	clear	yes	20	yes
9	steel w/ epoxy lining	6.4	light yellow	clear	yes	20	yes
10	steel w/ epoxy lining	6.4	light yellow	clear	yes	20	yes
11	steel w/ epoxy lining	6.4	light yellow	clear	yes	20	yes
12	steel w/ epoxy lining	6.4	light yellow	clear	yes	20	yes
13	steel w/ epoxy lining	6.4	light yellow	clear	yes	20	yes
14	steel w/ epoxy lining	6.4	light yellow	clear	yes	20	yes
15	aluminum	—	light yellow	clear	no	—	no
16	aluminum	—	light yellow	clear	no	—	no
17	aluminum	—	light yellow	clear	no	—	no
18	aluminum	—	light yellow	clear	no	—	no
19	aluminum	—	light yellow	clear	no	—	no
20	aluminum	—	light yellow	clear	no	—	no
21	aluminum	—	light yellow	clear	no	—	no
22	aluminum	—	light yellow	clear	no	—	no
23	aluminum	—	light yellow	clear	no	—	no
24	aluminum	—	light yellow	clear	no	—	no
25	aluminum	—	light yellow	clear	no	—	no
26	aluminum	—	light yellow	clear	no	—	no
27	aluminum	—	light yellow	clear	no	—	no
28	aluminum	—	light yellow	clear	no	—	no
29	aluminum	—	light yellow	clear	no	—	no
30	aluminum	—	light yellow	clear	no	—	no
31	aluminum	—	light yellow	clear	no	—	no
32	aluminum	—	light yellow	clear	no	—	no
33	aluminum	—	light yellow	clear	no	—	no
34	aluminum	—	light yellow	clear	no	—	no
35	aluminum	—	light yellow	clear	no	—	no
36	aluminum	—	light yellow	clear	no	—	no
37	aluminum	—	light yellow	clear	no	—	no
38	aluminum	—	light yellow	clear	no	—	no

	Light Microscopy				Colony Description		Quantity Data		
#	Gram +/-	Organism Shape	Length of Cells (μm)	Width of Cells (μm)	Colony Type	Colony Color	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours
1	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—
3	neg	bacillus	2	0.5	round	white	0	1	0
4	—	—	—	—	—	—	0	0	0
5	neg	bacillus	1	0.5	irregular	white	0	0	4 days to grow
6	neg	coccus	1 diameter	—	round	white	0	1	0
7	—	—	—	—	—	—	0	0	0
8	—	—	—	—	—	—	0	0	0
9	—	—	—	—	—	—	0	0	0
10	—	—	—	—	—	—	0	0	0
11	—	—	—	—	—	—	0	0	0
12	—	—	—	—	—	—	0	0	0
13	neg	coccus	1 diameter	—	round	white	0	2,980	0
14	—	—	—	—	—	—	0	0	0
15	—	—	—	—	—	—	0	0	0
16	—	—	—	—	—	—	0	0	0
17	—	—	—	—	—	—	0	0	0
18	—	—	—	—	—	—	0	0	0
19	—	—	—	—	—	—	0	0	0
20	—	—	—	—	—	—	0	0	0
21	—	—	—	—	—	—	0	0	0
22	—	—	—	—	—	—	0	0	0
23	—	—	—	—	—	—	0	0	0
24	neg	bacillus	1	0.5	round	white	0	1,750	0
25	—	—	—	—	—	—	0	0	0
26	—	—	—	—	—	—	0	0	0
27	—	—	—	—	—	—	0	0	0
28	—	—	—	—	—	—	0	0	0
29	—	—	—	—	—	—	0	0	0
30	—	—	—	—	—	—	0	0	0
31	—	—	—	—	—	—	0	0	0
32	—	—	—	—	—	—	0	0	0
33	—	—	—	—	—	—	0	0	0
34	—	—	—	—	—	—	0	0	0
35	—	—	—	—	—	—	0	0	0
36	neg	bacillus	1	0.5	round	white	0	0	4 days to grow
37	neg	bacillus	1	0.5	irregular	white	0	1	0
38	neg	bacillus	1	0.5	round	white	0	0	4 days to grow

				Identification Data				
				FAME analysis			DNA gene sequence	
#	Bacteria vs. Fungi	Spores	Digital Photo	Sim Index	> .1 S. I. Separation	GC Best Match	DNA % ID	DNA Best Match
1	—	—	—	—	—	Library match not attempted	—	—
2	—	—	—	—	—	No match found *	—	—
3	bacteria	no	RCATANEATTSBA	0.530	yes	*Bacillus-megaterium-GC subgroup B*	—	—
4	—	—	—	—	—	—	—	—
5	bacteria	no	RCATA100TSBA	0.612	yes	*Bacillus-licheniformis (Bacillus subtitis group)*	—	—
6	bacteria	no	RCATA1000TSBA	0.426	no	*Micrococcus-leteus-GC subgroup A*	—	—
7	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—
13	bacteria	no	—	—	—	No match found	—	—
14	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—	—
18	—	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—
20	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—
22	—	—	—	—	—	—	—	—
23	—	—	—	—	—	—	—	—
24	bacteria	no	RCATR10BHI	—	—	No match found	—	—
25	—	—	—	—	—	—	—	—
26	—	—	—	—	—	—	—	—
27	—	—	—	—	—	—	—	—
28	—	—	—	—	—	—	—	—
29	—	—	—	—	—	—	—	—
30	—	—	—	—	—	—	—	—
31	—	—	—	—	—	—	—	—
32	—	—	—	—	—	—	—	—
33	—	—	—	—	—	—	—	—
34	—	—	—	—	—	—	—	—
35	—	—	—	—	—	—	—	—
36	bacteria	no	RCAAC10BHI	—	—	No match found	—	—
37	bacteria	no	RCAAC100BHI	—	—	Library match not attempted	—	—
38	bacteria	no	—	—	—	No match found	—	—

* GC vial cap separated; contents evaporated

Volume: DATA File: E02A313.62A Seq Counter: 30 ID Number: 1140
Type: Samp Bottle: 50 Method: TSBA40
Created: 11/1/02 10:39:56 AM
Sample ID: ELL TANK NEAT TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.773	2.334E+8	0.029	----	7.007	SOLVENT PEAK	----	< min rt	
1.886	6264	0.025	----	7.221		----	< min rt	
5.885	1016	0.040	1.049	12.702	13:0 ANTEISO	0.92	ECL deviates 0.000	Reference 0.002
7.109	11086	0.039	1.003	13.618	14:0 ISO	9.64	ECL deviates -0.001	Reference -0.001
7.644	1757	0.040	0.987	13.999	14:0	1.50	ECL deviates -0.001	Reference -0.001
8.625	20315	0.043	0.963	14.623	15:0 ISO	16.97	ECL deviates 0.000	Reference 0.000
8.770	68346	0.041	0.960	14.715	15:0 ANTEISO	56.90	ECL deviates 0.002	Reference 0.002
9.218	889	0.040	0.951	15.000	15:0	0.73	ECL deviates 0.000	Reference -0.001
9.876	3594	0.045	0.939	15.390	16:1 w7c alcohol	2.93	ECL deviates 0.003	
10.277	3465	0.049	0.933	15.627	16:0 ISO	2.80	ECL deviates 0.000	Reference -0.001
10.504	3422	0.045	0.930	15.761	16:1 w11c	2.76	ECL deviates 0.004	
10.905	2122	0.046	0.924	15.998	16:0	1.70	ECL deviates -0.002	Reference -0.003
11.589	676	0.046	0.916	16.390	ISO 17:1 w10c	0.54	ECL deviates 0.002	
11.756	974	0.051	0.914	16.486	Sum In Feature 4	0.77	ECL deviates 0.000	17:1 ANTEISO B/i I
12.011	917	0.046	0.911	16.632	17:0 ISO	0.72	ECL deviates 0.002	Reference 0.000
12.170	1405	0.044	0.909	16.722	17:0 ANTEISO	1.11	ECL deviates -0.001	Reference -0.002
----	974	---	----	----	Summed Feature 4	0.77	17:1 ISO I/ANTEI B	17:1 ANTEISO B/i I

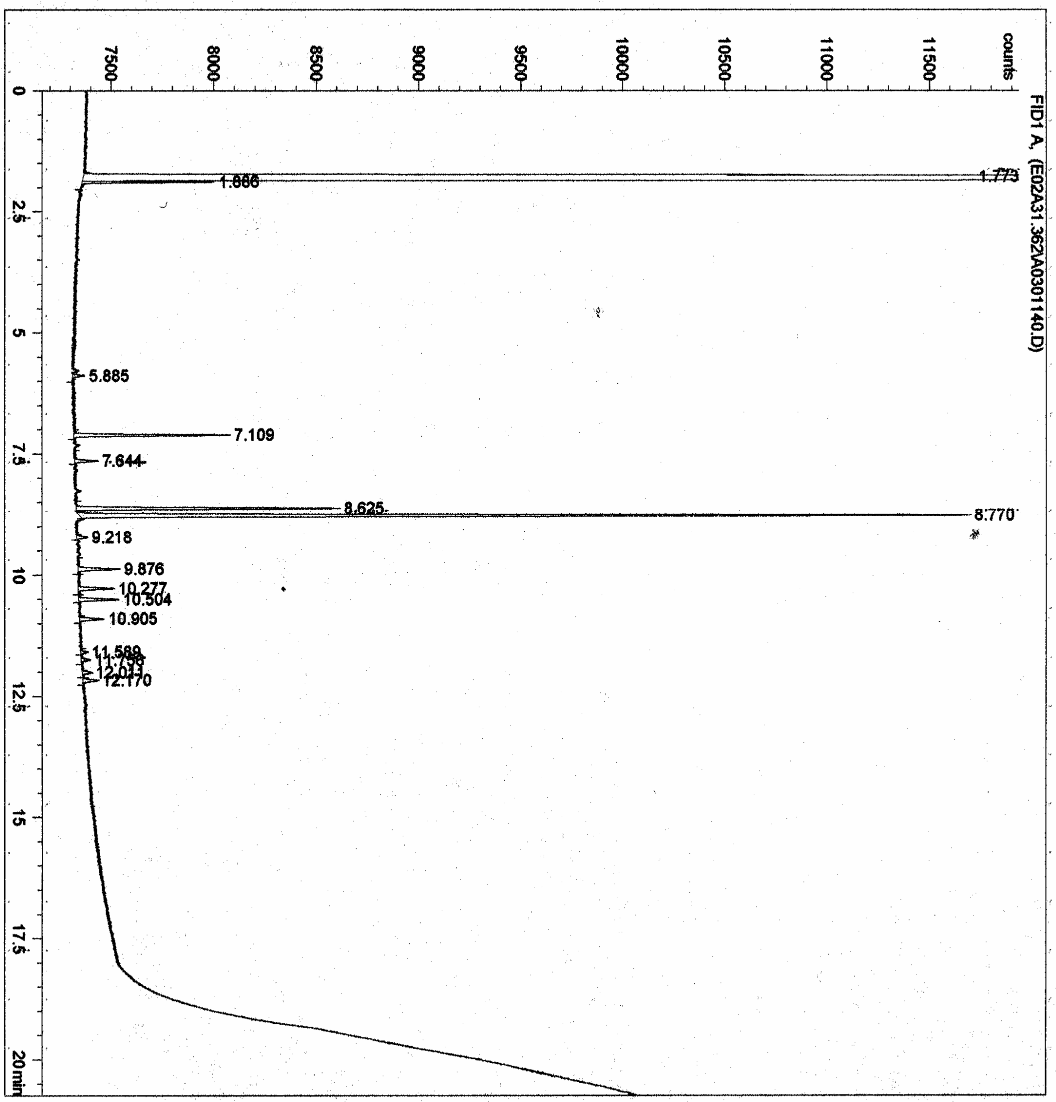
ECL Deviation: 0.002 Reference ECL Shift: 0.002 Number Reference Peaks: 10
Total Response: 119985 Total Named: 119985
Percent Named: 100.00% Total Amount: 115333

Matches:

Library	Sim Index	Entry Name
TSBA40 4.10	0.530	Bacillus-megaterium-GC subgroup B
	0.425	Bacillus-megaterium-GC subgroup A

```
Data File C:\SHERLOCK\RAW\E02A31.362\A0301140.D                     Sample Name:
    Sherlock Id: ELL TANK NEAT TSBA

    =====================================================================
    Injection Date  : 11/1/02 10:44:09 AM
    Sample Name     :  1140                         Location : Vial 50
    Acq. Operator   :
                                                  Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 11/1/02 10:39:57 AM
    =====================================================================
```



```
*** End of Report ***
```

E02A313.62A [1139] ELL TANK 1:100 TSBA

Volume: DATA File: E02A313.62A Seq Counter: 29 ID Number: 1139
Type: Samp Bottle: 49 Method: TSBA40
Created: 11/1/02 10:14:26 AM
Sample ID: ELL TANK 1:100 TSBA

Profile: ONE OR MORE NAMED PEAKS REJECTED. SEE COMMENTS IN THE REPORT.

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.773	2.342E+8	0.029	----	7.016	SOLVENT PEAK	----	< min rt	
1.887	2523	0.028	----	7.230		----	< min rt	
7.107	1128	0.044	1.003	13.618	14:0 ISO	1.01	ECL deviates -0.001	Reference -0.001
8.626	46237	0.041	0.963	14.624	15:0 ISO	39.61	ECL deviates 0.001	Reference 0.000
8.769	38600	0.041	0.960	14.715	15:0 ANTEISO	32.96	ECL deviates 0.002	Reference 0.001
9.877	1984	0.046	0.939	15.390	16:1 w7c alcohol	1.66	ECL deviates 0.003	
10.277	3009	0.044	0.933	15.627	16:0 ISO	2.50	ECL deviates 0.000	Reference -0.001
10.501	1771	0.049	0.930	15.759	16:1 w11c	1.46	ECL deviates 0.002	
10.905	1860	0.046	0.924	15.998	16:0	1.53	ECL deviates -0.002	Reference -0.003
11.590	3780	0.050	0.916	16.390	ISO 17:1 w10c	3.08	ECL deviates 0.002	
11.748	2482	0.048	0.914	16.480	Sum In Feature 4	2.02	ECL deviates 0.004	17:1 ISO I/ANTEI B
12.010	7438	0.047	0.911	16.630	17:0 ISO	6.03	ECL deviates 0.000	Reference -0.001
12.173	9037	0.046	0.909	16.723	17:0 ANTEISO	7.31	ECL deviates 0.000	Reference -0.001
17.478	1275	0.102	0.876	19.742	20:2 w6,9c	----	> max ar/ht	
17.732	4163	0.109	----	19.888		----	> max ar/ht	
17.927	1091	0.081	0.874	20.000	20:0	0.85	ECL deviates 0.000	Reference 0.001
----	2482	---	----	----	Summed Feature 4	2.02	17:1 ISO I/ANTEI B	17:1 ANTEISO B/i I

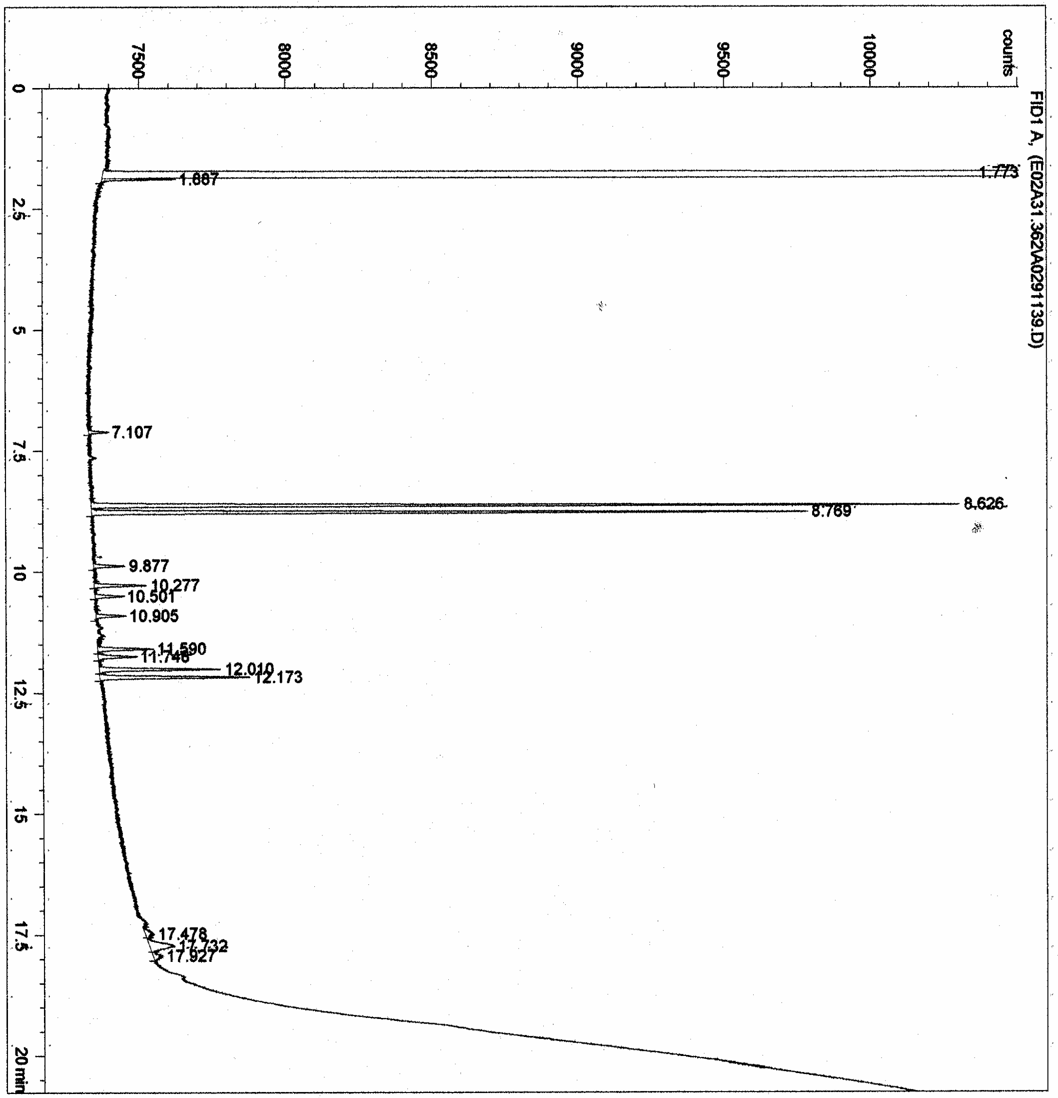
ECL Deviation: 0.003 Reference ECL Shift: 0.001 Number Reference Peaks: 8
Total Response: 123854 Total Named: 118417
Percent Named: 95.61% Total Amount: 113559
Profile: ONE OR MORE NAMED PEAKS REJECTED. SEE COMMENTS IN THE REPORT.

Matches:

Library	Sim Index	Entry Name
TSBA40 4.10	0.612	Bacillus-licheniformis* (Bacillus subtilis group)
	0.408	Bacillus-subtilis*

```
Data File C:\SHERLOCK\RAW\E02A31.362\A0291139.D                        Sample Name:
    Sherlock Id: ELL TANK 1:100 TSBA

    =====================================================================
    Injection Date  : 11/1/02 10:18:32 AM
    Sample Name     :  1139                        Location : Vial 49
    Acq. Operator   :
                                                 Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 11/1/02 10:14:27 AM
    =====================================================================
```



*** End of Report ***

E02A313.62A [1138] ELL TANK 1:1000 TSBA

Volume: DATA File: E02A313.62A Seq Counter: 28 ID Number: 1138
Type: Samp Bottle: 48 Method: TSBA40
Created: 11/1/02 9:48:49 AM
Sample ID: ELL TANK 1:1000 TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.773	2.34E+8	0.029	----	7.009	SOLVENT PEAK	----	< min rt	
1.884	777	0.027	----	7.218		----	< min rt	
3.801	1068	0.029	1.197	10.607	11:0 ISO	0.57	ECL deviates 0.001	Reference 0.002
3.865	1794	0.030	1.190	10.695	11:0 ANTEISO	0.96	ECL deviates 0.000	Reference 0.001
5.777	6130	0.033	1.054	12.613	13:0 ISO	2.90	ECL deviates -0.001	Reference -0.001
5.883	9558	0.036	1.049	12.702	13:0 ANTEISO	4.50	ECL deviates 0.000	Reference 0.000
7.108	7063	0.039	1.003	13.618	14:0 ISO	3.18	ECL deviates -0.001	Reference -0.001
7.643	2551	0.045	0.987	13.998	14:0	1.13	ECL deviates -0.002	Reference -0.002
8.627	50753	0.041	0.963	14.624	15:0 ISO	21.95	ECL deviates 0.001	Reference 0.001
8.773	146078	0.042	0.960	14.717	15:0 ANTEISO	62.98	ECL deviates 0.004	Reference 0.004
10.275	1514	0.046	0.933	15.626	16:0 ISO	0.63	ECL deviates -0.001	Reference -0.002
10.906	1762	0.049	0.924	15.999	16:0	0.73	ECL deviates -0.001	Reference -0.002
11.313	1174	0.045	----	16.232		----		
11.521	611	0.043	----	16.351		----		
12.171	972	0.043	0.909	16.723	17:0 ANTEISO	0.40	ECL deviates 0.000	Reference -0.002
17.054	189	0.055	----	19.501		----		
17.400	501	0.191	----	19.700		----	> max ar/ht	
17.517	129	0.039	0.875	19.768	20:1 w9c	0.05	ECL deviates -0.002	
17.595	186	0.045	----	19.813		----		
19.039	811	0.029	----	20.646		----	> max rt	

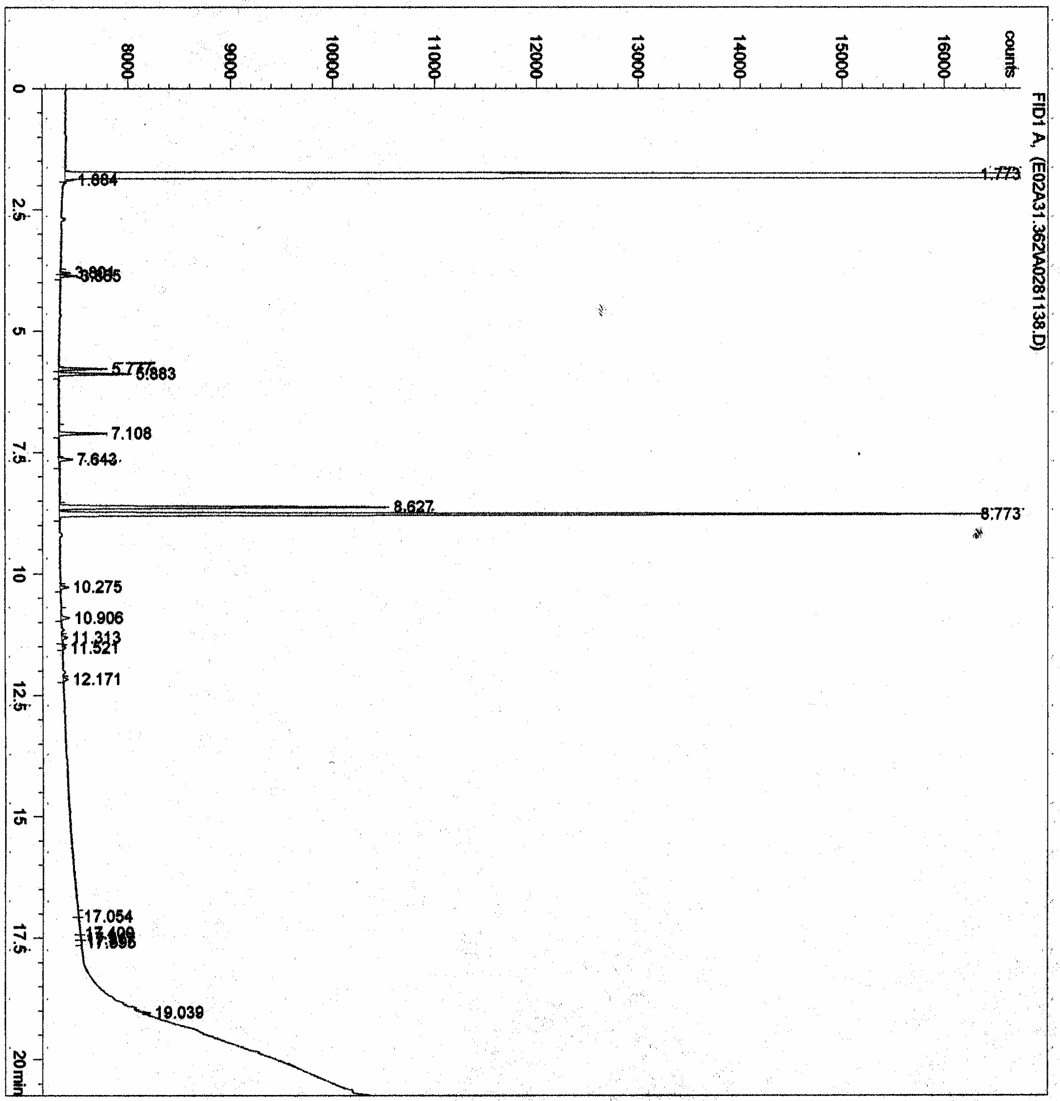
ECL Deviation: 0.002 Reference ECL Shift: 0.002 Number Reference Peaks: 11
Total Response: 232033 Total Named: 229372
Percent Named: 98.85% Total Amount: 222697

Matches:

Library	Sim Index	Entry Name
TSBA40 4.10	0.426	Micrococcus-lylae-GC subgroup A
	0.420	Micrococcus-luteus-GC subgroup C*
	0.386	Micrococcus-luteus-GC subgroup B*

```
Data File C:\SHERLOCK\RAW\E02A31.362\A0281138.D                          Sample Name:
    Sherlock Id: ELL TANK 1:1000 TSBA

    =====================================================================
    Injection Date  : 11/1/02 9:52:58 AM
    Sample Name     :  1138                          Location : Vial 48
    Acq. Operator   :
                                                   Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 11/1/02 9:48:50 AM
    =====================================================================
```



*** End of Report ***

Appendix G: Worksheet / Sample Data – Edwards AFB (KEDW)

	Site Information									
#	Sample	Type / Number	Date Sampled	Dilution	Medium	Temp °C	% Relative Humidity	Inches Rainfall (last 30 days)	Fuel Type	Sample Method
1	Neg Control	—	—	—	—	—	—	—	—	—
2	Pos Control	—	—	—	—	—	—	—	—	—
3	Tank	28	10/24/2002	Neat	TSBA	17	41	trace	JP-8	gravity
4	Tank	28	10/24/2002	1:10	TSBA	17	41	trace	JP-8	gravity
5	Tank	28	10/24/2002	1:100	TSBA	17	41	trace	JP-8	gravity
6	Tank	28	10/24/2002	1:1000	TSBA	17	41	trace	JP-8	gravity
7	Tank	28	10/24/2002	Neat	SDA	17	41	trace	JP-8	gravity
8	Tank	28	10/24/2002	1:10	SDA	17	41	trace	JP-8	gravity
9	Tank	28	10/24/2002	1:100	SDA	17	41	trace	JP-8	gravity
10	Tank	28	10/24/2002	1:1000	SDA	17	41	trace	JP-8	gravity
11	Tank	28	10/24/2002	Neat	BHIBLA	17	41	trace	JP-8	gravity
12	Tank	28	10/24/2002	1:10	BHIBLA	17	41	trace	JP-8	gravity
13	Tank	28	10/24/2002	1:100	BHIBLA	17	41	trace	JP-8	gravity
14	Tank	28	10/24/2002	1:1000	BHIBLA	17	41	trace	JP-8	gravity
15	Truck 1	494	10/23/2002	Neat	TSBA	17	41	trace	JP-8 +100	gravity
16	Truck 1	494	10/23/2002	1:10	TSBA	17	41	trace	JP-8 +100	gravity
17	Truck 1	494	10/23/2002	1:100	TSBA	17	41	trace	JP-8 +100	gravity
18	Truck 1	494	10/23/2002	1:1000	TSBA	17	41	trace	JP-8 +100	gravity
19	Truck 1	494	10/23/2002	Neat	SDA	17	41	trace	JP-8 +100	gravity
20	Truck 1	494	10/23/2002	1:10	SDA	17	41	trace	JP-8 +100	gravity
21	Truck 1	494	10/23/2002	1:100	SDA	17	41	trace	JP-8 +100	gravity
22	Truck 1	494	10/23/2002	1:1000	SDA	17	41	trace	JP-8 +100	gravity
23	Truck 1	494	10/23/2002	Neat	BHIBLA	17	41	trace	JP-8 +100	gravity
24	Truck 1	494	10/23/2002	1:10	BHIBLA	17	41	trace	JP-8 +100	gravity
25	Truck 1	494	10/23/2002	1:100	BHIBLA	17	41	trace	JP-8 +100	gravity
26	Truck 1	494	10/23/2002	1:1000	BHIBLA	17	41	trace	JP-8 +100	gravity
27	Truck 2	639	10/24/2002	Neat	TSBA	17	41	trace	JP-8	gravity
28	Truck 2	639	10/24/2002	1:10	TSBA	17	41	trace	JP-8	gravity
29	Truck 2	639	10/24/2002	1:100	TSBA	17	41	trace	JP-8	gravity
30	Truck 2	639	10/24/2002	1:1000	TSBA	17	41	trace	JP-8	gravity
31	Truck 2	639	10/24/2002	Neat	SDA	17	41	trace	JP-8	gravity
32	Truck 2	639	10/24/2002	1:10	SDA	17	41	trace	JP-8	gravity
33	Truck 2	639	10/24/2002	1:100	SDA	17	41	trace	JP-8	gravity
34	Truck 2	639	10/24/2002	1:1000	SDA	17	41	trace	JP-8	gravity
35	Truck 2	639	10/24/2002	Neat	BHIBLA	17	41	trace	JP-8	gravity
36	Truck 2	639	10/24/2002	1:10	BHIBLA	17	41	trace	JP-8	gravity
37	Truck 2	639	10/24/2002	1:100	BHIBLA	17	41	trace	JP-8	gravity
38	Truck 2	639	10/24/2002	1:1000	BHIBLA	17	41	trace	JP-8	gravity

— Indicates analysis omitted

		Sample Description					
#	Tank Composition	pH	Sample Color	General Description	Obvious Solids	TDS (mg/L)	Free Phase Water
1	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—
3	steel w/ epoxy lining	7.76	orange	clear	yes	50	yes
4	steel w/ epoxy lining	7.76	orange	clear	yes	50	yes
5	steel w/ epoxy lining	7.76	orange	clear	yes	50	yes
6	steel w/ epoxy lining	7.76	orange	clear	yes	50	yes
7	steel w/ epoxy lining	7.76	orange	clear	yes	50	yes
8	steel w/ epoxy lining	7.76	orange	clear	yes	50	yes
9	steel w/ epoxy lining	7.76	orange	clear	yes	50	yes
10	steel w/ epoxy lining	7.76	orange	clear	yes	50	yes
11	steel w/ epoxy lining	7.76	orange	clear	yes	50	yes
12	steel w/ epoxy lining	7.76	orange	clear	yes	50	yes
13	steel w/ epoxy lining	7.76	orange	clear	yes	50	yes
14	steel w/ epoxy lining	7.76	orange	clear	yes	50	yes
15	aluminum	—	light yellow	clear	yes	—	yes
16	aluminum	—	light yellow	clear	yes	—	yes
17	aluminum	—	light yellow	clear	yes	—	yes
18	aluminum	—	light yellow	clear	yes	—	yes
19	aluminum	—	light yellow	clear	yes	—	yes
20	aluminum	—	light yellow	clear	yes	—	yes
21	aluminum	—	light yellow	clear	yes	—	yes
22	aluminum	—	light yellow	clear	yes	—	yes
23	aluminum	—	light yellow	clear	yes	—	yes
24	aluminum	—	light yellow	clear	yes	—	yes
25	aluminum	—	light yellow	clear	yes	—	yes
26	aluminum	—	light yellow	clear	yes	—	yes
27	aluminum	—	light brown	clear	yes	—	yes
28	aluminum	—	light brown	clear	yes	—	yes
29	aluminum	—	light brown	clear	yes	—	yes
30	aluminum	—	light brown	clear	yes	—	yes
31	aluminum	—	light brown	clear	yes	—	yes
32	aluminum	—	light brown	clear	yes	—	yes
33	aluminum	—	light brown	clear	yes	—	yes
34	aluminum	—	light brown	clear	yes	—	yes
35	aluminum	—	light brown	clear	yes	—	yes
36	aluminum	—	light brown	clear	yes	—	yes
37	aluminum	—	light brown	clear	yes	—	yes
38	aluminum	—	light brown	clear	yes	—	yes

	Light Microscopy				Colony Description		Quantity Data		
#	Gram +/-	Organism Shape	Length of Cells (μm)	Width of Cells (μm)	Colony Type	Colony Color	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours
1	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	0	0	0
4	—	—	—	—	—	—	0	0	0
5	neg	coccus	1 diameter	—	round	white	112	422	112,000
6	—	varied	varied	varied	irregular	white	5	11	50,000
7	—	—	—	—	—	—	0	0	0
8	—	varied	varied	varied	round	white	1	1	100
9	—	varied	varied	varied	round	white	149	518	149,000
10	—	varied	varied	varied	round	white	1	3	10,000
11	—	—	—	—	—	—	0	0	0
12	—	—	—	—	—	—	0	0	0
13	—	varied	varied	varied	round	white	398	574	398,000
14	—	—	—	—	—	—	0	0	0
15	—	varied	varied	varied	round	white	1	1	10
16	—	—	—	—	—	—	0	0	0
17	—	—	—	—	—	—	0	0	0
18	—	—	—	—	—	—	0	0	0
19	—	—	—	—	—	—	0	0	0
20	—	—	—	—	—	—	0	0	0
21	—	—	—	—	—	—	0	0	0
22	—	—	—	—	—	—	0	0	0
23	—	—	—	—	—	—	0	0	0
24	—	varied	varied	varied	irregular	white	520	880	52,000
25	—	—	—	—	—	—	0	0	0
26	—	—	—	—	—	—	0	0	0
27	—	—	—	—	—	—	0	0	0
28	—	—	—	—	—	—	0	0	0
29	—	—	—	—	—	—	0	0	0
30	—	varied	varied	varied	round	white	89	210	890,000
31	—	—	—	—	—	—	0	0	0
32	—	—	—	—	—	—	0	0	0
33	—	—	—	—	—	—	0	0	0
34	neg	coccus	1 diameter	—	round	white	21	51	210,000
35	—	—	—	—	—	—	0	0	0
36	—	—	—	—	—	—	0	0	0
37	—	—	—	—	—	—	0	0	0
38	—	—	—	—	—	—	0	0	0

				Identification Data				
				FAME analysis			DNA gene sequence	
#	Bacteria vs. Fungi	Spores	Digital Photo	Sim Index	> .1 S. I. Separation	GC Best Match	DNA % ID	DNA Best Match
1	—	—	—	—	—	Library match not attempted	—	—
2	—	—	—	0.271	yes	*Stenotrophomonas-maltophilia*	—	—
3	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—
5	bacteria	no	EDWTA100TSBA	0.588	no	*Xenorhabdus-nematophilus (48h)*	—	—
6	fungi	no	EDWTA1000TSBA	—	—	No match found	—	—
7	—	—	—	—	—	—	—	—
8	fungi	no	EDWTA10SDA	—	—	No match found	—	—
9	fungi	no	EDWTA100SDA	—	—	No match found	—	—
10	fungi	no	EDWTA1000SDA	—	—	No match found	—	—
11	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—
13	fungi	no	EDWTA100BHI	—	—	No match found	—	—
14	—	—	—	—	—	—	—	—
15	fungi	no	EDWTR1NEATTSBA	0.583	yes	*Xenorhabdus-nematophilus (48h)*	—	—
16	—	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—	—
18	—	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—
20	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—
22	—	—	—	—	—	—	—	—
23	—	—	—	—	—	—	—	—
24	fungi	no	EDWTR110BHI	—	—	No match found	—	—
25	—	—	—	—	—	—	—	—
26	—	—	—	—	—	—	—	—
27	—	—	—	—	—	—	—	—
28	—	—	—	—	—	—	—	—
29	—	—	—	—	—	—	—	—
30	fungi	no	EDWTR21000TSBA	0.675	yes	*Xenorhabdus-nematophilus (48h)*	—	—
31	—	—	—	—	—	—	—	—
32	—	—	—	—	—	—	—	—
33	—	—	—	—	—	—	—	—
34	bacteria	no	EDWTR21000SDA	—	—	No match found	—	—
35	—	—	—	—	—	—	—	—
36	—	—	—	—	—	—	—	—
37	—	—	—	—	—	—	—	—
38	—	—	—	—	—	—	—	—

E02B046.67A [1150] EDW TRUCK2 1:1000 TSBA

Volume: DATA File: E02B046.67A Seq Counter: 9 ID Number: 1150
Type: Samp Bottle: 7 Method: TSBA40
Created: 11/4/02 7:24:41 PM
Sample ID: EDW TRUCK2 1:1000 TSBA

Profile: TOTAL RESPONSE LESS THAN 50000.0. CONCENTRATE AND RE-RUN.

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.437	3.568E+8	0.028	----	7.066	SOLVENT PEAK	----	< min rt	
4.228	1772	0.028	1.087	12.000	12:0	4.49	ECL deviates 0.000	Reference -0.003
6.597	2672	0.037	0.991	13.999	14:0	6.17	ECL deviates -0.001	Reference -0.004
8.075	929	0.037	0.956	15.000	15:0	2.07	ECL deviates 0.000	Reference -0.003
8.874	3285	0.042	0.942	15.496	Sum In Feature 2	7.21	ECL deviates 0.008	14:0 3OH/16:1 ISO I
9.390	3460	0.044	0.934	15.815	Sum In Feature 3	7.53	ECL deviates -0.007	16:1 w7c/15 iso 2OH
9.686	14430	0.039	0.930	15.999	16:0	31.25	ECL deviates -0.001	Reference -0.005
10.653	1206	0.320	0.918	16.573	unknown 16.582	----	> max ar/ht	
10.995	469	0.137	0.914	16.776	17:1 w9c	----	> max ar/ht	
11.183	11623	0.041	0.912	16.888	17:0 CYCLO	24.69	ECL deviates 0.000	Reference -0.004
11.377	502	0.045	0.910	17.003	17:0	1.06	ECL deviates 0.003	Reference -0.001
12.697	510	0.080	0.899	17.767	18:1 w9c	1.07	ECL deviates -0.002	
12.792	3651	0.042	0.898	17.821	18:1 w7c	7.64	ECL deviates -0.002	
13.426	273	0.085	----	18.190		----		
13.797	736	0.190	----	18.406		----	> max ar/ht	
13.905	466	0.055	0.892	18.469	19:1 ISO I	0.97	ECL deviates -0.004	
14.308	987	0.206	----	18.704		----	> max ar/ht	
14.646	2829	0.052	0.888	18.901	19:0 CYCLO w8c	5.85	ECL deviates -0.001	Reference -0.007
----	3285	---	----	----	Summed Feature 2	7.21	12:0 ALDE ?	unknown 10.928
----	-----	---	----	----		----	16:1 ISO I/14:0 3OH	14:0 3OH/16:1 ISO I
----	3460	---	----	----	Summed Feature 3	7.53	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c

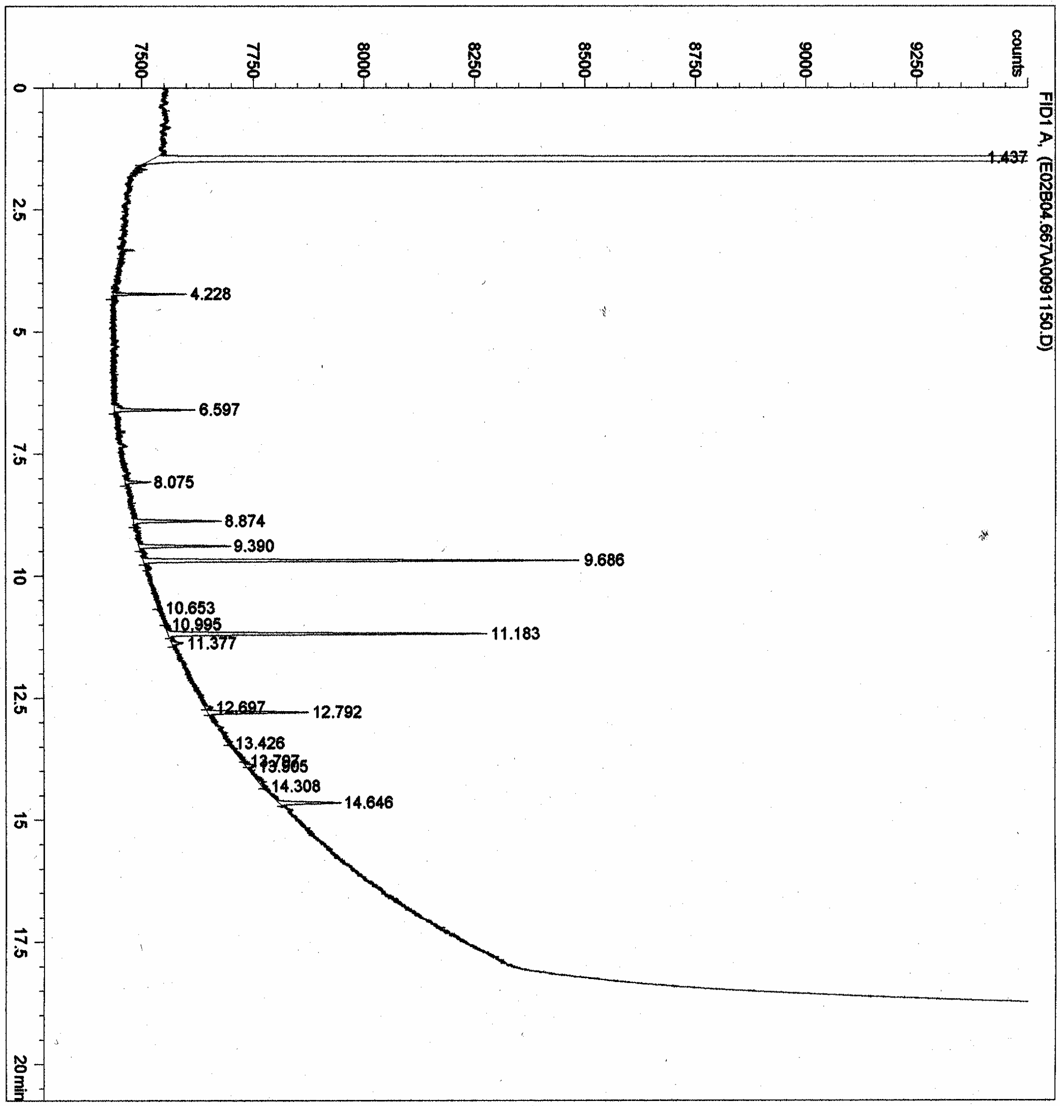
ECL Deviation: 0.004 Reference ECL Shift: 0.004 Number Reference Peaks: 7
Total Response: 49799 Total Named: 46129
Percent Named: 92.63% Total Amount: 44463
Profile: TOTAL RESPONSE LESS THAN 50000.0. CONCENTRATE AND RE-RUN.

Matches:

Library	**Sim Index**	**Entry Name**
TSBA40 4.10	0.675	Xenorhabdus-nematophilus* (48h)
	0.559	Morganella-morganii
	0.396	Rahnella-aquatilis*

```
Data File C:\SHERLOCK\RAW\E02B04.667\A0091150.D                    Sample Name:
    Sherlock Id: EDW TRUCK2 1:1000 TSBA

    ===================================================================
    Injection Date  : 11/4/02 7:28:54 PM
    Sample Name     :  1150                           Location : Vial 7
    Acq. Operator   :
                                                    Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 11/4/02 7:24:41 PM
    ===================================================================
```



*** End of Report ***

E02B046.67A [1149] EDW TRUCK1 NEAT TSBA

Volume: DATA File: E02B046.67A Seq Counter: 8 ID Number: 1149
Type: Samp Bottle: 6 Method: TSBA40
Created: 11/4/02 6:59:00 PM
Sample ID: EDW TRUCK1 NEAT TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.436	3.644E+8	0.024	----	7.070	SOLVENT PEAK	----	< min rt	
4.228	3872	0.026	1.087	11.999	12:0	4.46	ECL deviates -0.001	Reference -0.003
6.598	6310	0.036	0.991	14.000	14:0	6.63	ECL deviates 0.000	Reference -0.004
7.341	780	0.050	0.972	14.504	unknown 14.502	0.80	ECL deviates 0.002	
8.077	1735	0.036	0.956	15.002	15:0	1.76	ECL deviates 0.002	Reference -0.002
8.875	8102	0.037	0.942	15.497	Sum In Feature 2	8.10	ECL deviates 0.009	14:0 3OH/16:1 ISO I
9.391	8250	0.040	0.934	15.816	Sum In Feature 3	8.17	ECL deviates -0.006	16:1 w7c/15 iso 2OH
9.687	29551	0.038	0.930	15.999	16:0	29.14	ECL deviates -0.001	Reference -0.004
11.184	27823	0.041	0.912	16.887	17:0 CYCLO	26.91	ECL deviates -0.001	Reference -0.004
11.375	810	0.040	0.910	17.000	17:0	0.78	ECL deviates 0.000	Reference -0.002
12.290	312	0.071	0.902	17.528	16:0 3OH	0.30	ECL deviates 0.009	
12.682	602	0.050	----	17.754		----		
12.792	8425	0.043	0.898	17.818	18:1 w7c	8.03	ECL deviates -0.005	
13.098	485	0.071	0.896	17.994	18:0	0.46	ECL deviates -0.006	Reference -0.006
13.222	194	0.047	0.895	18.066	11 methyl 18:1 w7c	0.18	ECL deviates -0.015	
13.431	193	0.065	----	18.188		----		
13.629	101	0.036	----	18.303		----		
13.779	364	0.086	0.892	18.390	TBSA 10Me18:0	0.34	ECL deviates -0.002	
13.882	800	0.056	----	18.450		----		
14.135	243	0.108	----	18.597		----	> max ar/ht	
14.222	290	0.047	0.890	18.647	19:0 ISO	0.27	ECL deviates 0.013	Reference 0.014
14.648	3890	0.046	0.888	18.895	19:0 CYCLO w8c	3.66	ECL deviates -0.007	Reference -0.006
14.792	296	0.036	----	18.978		----		
15.181	137	0.042	----	19.205		----		
15.729	773	0.182	----	19.525		----	> max ar/ht	
16.232	754	0.229	----	19.819		----	> max ar/ht	
16.682	553	0.174	----	20.081		----	> max rt	
----	8102	---	----	----	Summed Feature 2	8.10	12:0 ALDE ?	unknown 10.928
----	-----	---	----	----		----	16:1 ISO I/14:0 3OH	14:0 3OH/16:1 ISO I
----	8250	---	----	----	Summed Feature 3	8.17	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c

ECL Deviation: 0.007
Reference ECL Shift: 0.006
Number Reference Peaks: 9
Total Response: 105091
Total Named: 101191
Percent Named: 96.29%
Total Amount: 94298

Matches:

Library	Sim Index	Entry Name
TSBA40 4.10	0.583	Xenorhabdus-nematophilus* (48h)
	0.455	Yersinia-enterocolitica
	0.413	Serratia-plymuthica*
	0.406	Rahnella-aquatilis*
	0.398	Morganella-morganii
	0.396	Yersinia-pseudotuberculosis*
	0.360	Salmonella-choleraesuis-houtenae
	0.339	Hafnia-alvei*
	0.294	Klebsiella-pneumoniae-ozaenae*

E02B046.67A [1147] EDW TANK 1:100 TSBA

Volume: DATA File: E02B046.67A Seq Counter: 5 ID Number: 1147
Type: Samp Bottle: 4 Method: TSBA40
Created: 11/4/02 5:42:07 PM
Sample ID: EDW TANK 1:100 TSBA

Profile: ONE OR MORE NAMED PEAKS REJECTED. SEE COMMENTS IN THE REPORT.

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.442	3.482E+8	0.024	----	7.075	SOLVENT PEAK	----	< min rt	
4.238	5339	0.028	1.080	12.000	12:0	4.55	ECL deviates 0.000	Reference -0.008
6.611	8176	0.036	0.988	14.000	14:0	6.37	ECL deviates 0.000	Reference -0.009
7.356	911	0.042	0.970	14.505	unknown 14.502	0.70	ECL deviates 0.003	
8.090	2815	0.040	0.954	15.001	15:0	2.12	ECL deviates 0.001	Reference -0.008
8.891	11137	0.038	0.940	15.497	Sum In Feature 2	8.26	ECL deviates 0.009	14:0 3OH/16:1 ISO I
9.407	11040	0.040	0.932	15.816	Sum In Feature 3	8.12	ECL deviates -0.006	16:1 w7c/15 iso 2OH
9.703	41512	0.039	0.928	15.999	16:0	30.39	ECL deviates -0.001	Reference -0.009
9.832	872	0.069	----	16.076		----		
11.108	438	0.065	----	16.832		----		
11.201	36740	0.042	0.912	16.887	17:0 CYCLO	26.44	ECL deviates -0.001	Reference -0.008
11.392	1114	0.040	0.911	17.000	17:0	0.80	ECL deviates 0.000	Reference -0.007
11.835	536	0.141	----	17.256		----	> max ar/ht	
12.360	594	0.128	----	17.560		----	> max ar/ht	
12.705	447	0.036	----	17.758		----		
12.807	10387	0.041	0.903	17.818	18:1 w7c	7.40	ECL deviates -0.005	
13.116	917	0.071	0.902	17.996	18:0	0.65	ECL deviates -0.004	Reference -0.010
13.897	2122	0.086	----	18.450		----		
14.234	671	0.063	0.902	18.645	19:0 ISO	0.48	ECL deviates 0.011	Reference 0.006
14.663	5241	0.042	0.903	18.895	19:0 CYCLO w8c	3.73	ECL deviates -0.007	Reference -0.012
14.810	390	0.040	----	18.980		----		
15.055	292	0.067	----	19.123		----		
15.290	275	0.132	0.905	19.260	18:0 2OH	----	> max ar/ht	
15.396	98	0.042	----	19.322		----		
16.172	548	0.236	0.910	19.775	20:1 w9c	----	> max ar/ht	
16.647	13313	0.113	----	20.053		----	> max rt	
16.938	3294	0.139	----	20.222		----	> max rt	
17.317	4470	0.106	----	20.444		----	> max rt	
17.560	1416	0.141	----	20.586		----	> max rt	
----	11137	---	----	----	Summed Feature 2	8.26	12:0 ALDE ?	unknown 10.928
----	-----	---	----	----		----	16:1 ISO I/14:0 3OH	14:0 3OH/16:1 ISO I
----	11040	---	----	----	Summed Feature 3	8.12	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c

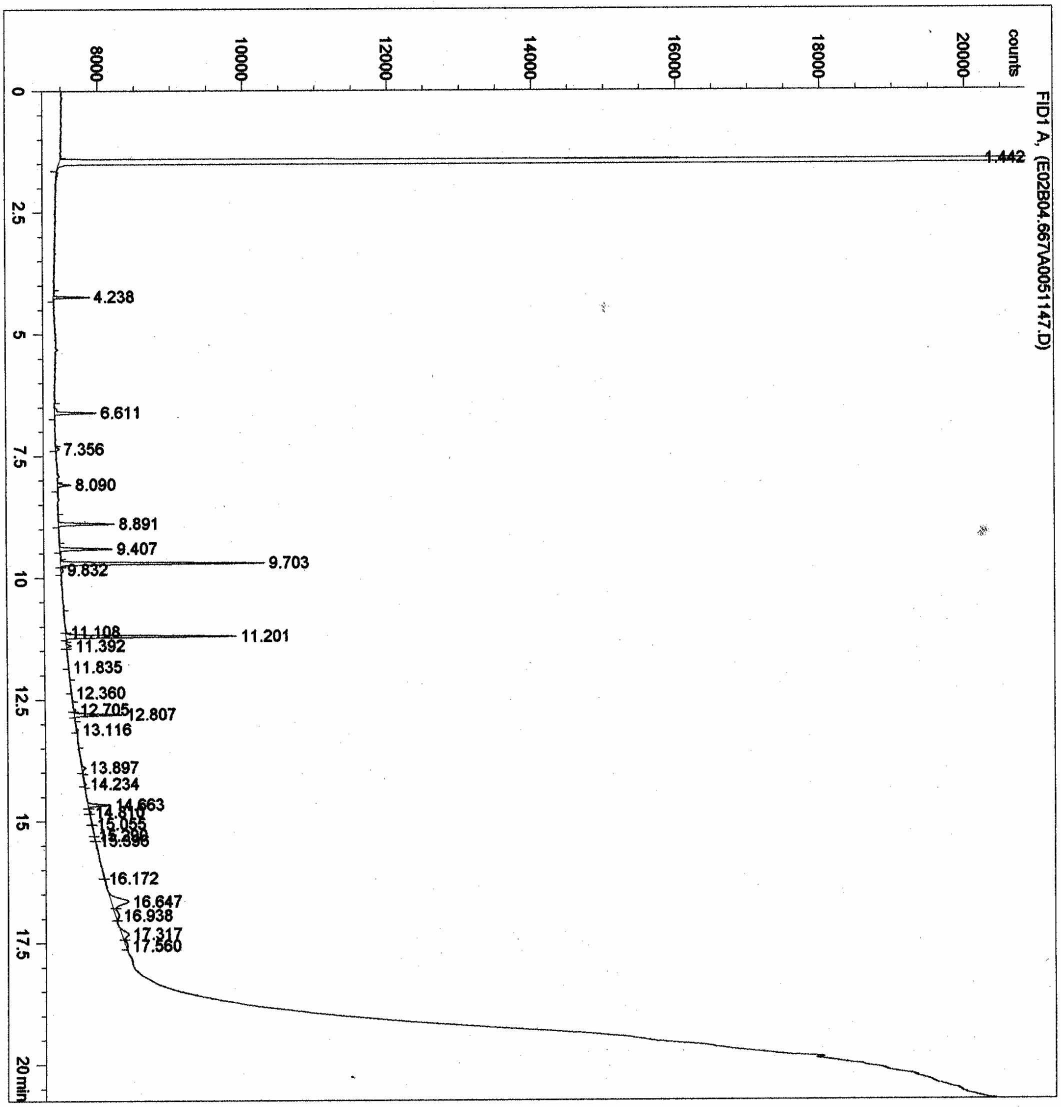
ECL Deviation: 0.005 Reference ECL Shift: 0.009 Number Reference Peaks: 9
Total Response: 142614 Total Named: 136002
Percent Named: 95.36% Total Amount: 127536
Profile: ONE OR MORE NAMED PEAKS REJECTED. SEE COMMENTS IN THE REPORT.

Matches:

Library	Sim Index	Entry Name
TSBA40 4.10	0.588	Xenorhabdus-nematophilus* (48h)
	0.509	Rahnella-aquatilis*
	0.494	Yersinia-enterocolitica
	0.464	Serratia-plymuthica*
	0.442	Morganella-morganii
	0.419	Yersinia-pseudotuberculosis*
	0.406	Hafnia-alvei*
	0.317	Salmonella-choleraesuis-houtenae
	0.316	Klebsiella-pneumoniae-ozaenae*

```
Data File C:\SHERLOCK\RAW\E02B04.667\A0051147.D                    Sample Name:
    Sherlock Id: EDW TANK 1:100 TSBA

    =================================================================
    Injection Date  : 11/4/02 5:46:16 PM
    Sample Name     :  1147                        Location : Vial 4
    Acq. Operator   :
                                                 Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 11/4/02 5:42:07 PM
    =================================================================
```



*** End of Report ***

Appendix H: Worksheet / Sample Data – Undisclosed Overseas Air Base

	Site Information									
#	Sample	Type / Number	Date Sampled	Dilution	Medium	Temp °C	% Relative Humidity	Inches Rainfall (last 30 days)	Fuel Type	Sample Method
1	Neg Control	—	—	—	—	—	—	—	—	—
2	Pos Control	—	—	—	—	—	—	—	—	—
3	Bladder	N/A	10/25/2002	Neat	TSBA	35	74	trace	JPTS	gravity
4	Bladder	N/A	10/25/2002	1:10	TSBA	35	74	trace	JPTS	gravity
5	Bladder	N/A	10/25/2002	1:100	TSBA	35	74	trace	JPTS	gravity
6	Bladder	N/A	10/25/2002	1:1000	TSBA	35	74	trace	JPTS	gravity
7	Bladder	N/A	10/25/2002	Neat	SDA	35	74	trace	JPTS	gravity
8	Bladder	N/A	10/25/2002	1:10	SDA	35	74	trace	JPTS	gravity
9	Bladder	N/A	10/25/2002	1:100	SDA	35	74	trace	JPTS	gravity
10	Bladder	N/A	10/25/2002	1:1000	SDA	35	74	trace	JPTS	gravity
11	Bladder	N/A	10/25/2002	Neat	BHIBLA	35	74	trace	JPTS	gravity
12	Bladder	N/A	10/25/2002	1:10	BHIBLA	35	74	trace	JPTS	gravity
13	Bladder	N/A	10/25/2002	1:100	BHIBLA	35	74	trace	JPTS	gravity
14	Bladder	N/A	10/25/2002	1:1000	BHIBLA	35	74	trace	JPTS	gravity
15	Liner	N/A	10/25/2002	Neat	TSBA	35	74	trace	JPTS	gravity
16	Liner	N/A	10/25/2002	1:10	TSBA	35	74	trace	JPTS	gravity
17	Liner	N/A	10/25/2002	1:100	TSBA	35	74	trace	JPTS	gravity
18	Liner	N/A	10/25/2002	1:1000	TSBA	35	74	trace	JPTS	gravity
19	Liner	N/A	10/25/2002	Neat	SDA	35	74	trace	JPTS	gravity
20	Liner	N/A	10/25/2002	1:10	SDA	35	74	trace	JPTS	gravity
21	Liner	N/A	10/25/2002	1:100	SDA	35	74	trace	JPTS	gravity
22	Liner	N/A	10/25/2002	1:1000	SDA	35	74	trace	JPTS	gravity
23	Liner	N/A	10/25/2002	Neat	BHIBLA	35	74	trace	JPTS	gravity
24	Liner	N/A	10/25/2002	1:10	BHIBLA	35	74	trace	JPTS	gravity
25	Liner	N/A	10/25/2002	1:100	BHIBLA	35	74	trace	JPTS	gravity
26	Liner	N/A	10/25/2002	1:1000	BHIBLA	35	74	trace	JPTS	gravity
27	Drain	N/A	10/25/2002	Neat	TSBA	35	74	trace	JPTS	gravity
28	Drain	N/A	10/25/2002	1:10	TSBA	35	74	trace	JPTS	gravity
29	Drain	N/A	10/25/2002	1:100	TSBA	35	74	trace	JPTS	gravity
30	Drain	N/A	10/25/2002	1:1000	TSBA	35	74	trace	JPTS	gravity
31	Drain	N/A	10/25/2002	Neat	SDA	35	74	trace	JPTS	gravity
32	Drain	N/A	10/25/2002	1:10	SDA	35	74	trace	JPTS	gravity
33	Drain	N/A	10/25/2002	1:100	SDA	35	74	trace	JPTS	gravity
34	Drain	N/A	10/25/2002	1:1000	SDA	35	74	trace	JPTS	gravity
35	Drain	N/A	10/25/2002	Neat	BHIBLA	35	74	trace	JPTS	gravity
36	Drain	N/A	10/25/2002	1:10	BHIBLA	35	74	trace	JPTS	gravity
37	Drain	N/A	10/25/2002	1:100	BHIBLA	35	74	trace	JPTS	gravity
38	Drain	N/A	10/25/2002	1:1000	BHIBLA	35	74	trace	JPTS	gravity

— Indicates analysis omitted

	Site Information								Fuel Type	Sample Method
#	Sample	Type / Number	Date Sampled	Dilution	Medium	Temp °C	% Relative Humidity	Inches Rainfall (last 30 days)		
39	Vent	N/A	10/25/2002	Neat	TSBA	35	74	trace	JPTS	gravity
40	Vent	N/A	10/25/2002	1:10	TSBA	35	74	trace	JPTS	gravity
41	Vent	N/A	10/25/2002	1:100	TSBA	35	74	trace	JPTS	gravity
42	Vent	N/A	10/25/2002	1:1000	TSBA	35	74	trace	JPTS	gravity
43	Vent	N/A	10/25/2002	Neat	SDA	35	74	trace	JPTS	gravity
44	Vent	N/A	10/25/2002	1:10	SDA	35	74	trace	JPTS	gravity
45	Vent	N/A	10/25/2002	1:100	SDA	35	74	trace	JPTS	gravity
46	Vent	N/A	10/25/2002	1:1000	SDA	35	74	trace	JPTS	gravity
47	Vent	N/A	10/25/2002	Neat	BHIBLA	35	74	trace	JPTS	gravity
48	Vent	N/A	10/25/2002	1:10	BHIBLA	35	74	trace	JPTS	gravity
49	Vent	N/A	10/25/2002	1:100	BHIBLA	35	74	trace	JPTS	gravity
50	Vent	N/A	10/25/2002	1:1000	BHIBLA	35	74	trace	JPTS	gravity

— Indicates analysis omitted

#	Tank Composition	Sample Description pH	Sample Color	General Description	Obvious Solids	TDS (mg/L)	Free Phase Water
1	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—
3	Nylon w/ polyester lining	—	colorless	clear	yes	—	no
4	Nylon w/ polyester lining	—	colorless	clear	yes	—	no
5	Nylon w/ polyester lining	—	colorless	clear	yes	—	no
6	Nylon w/ polyester lining	—	colorless	clear	yes	—	no
7	Nylon w/ polyester lining	—	colorless	clear	yes	—	no
8	Nylon w/ polyester lining	—	colorless	clear	yes	—	no
9	Nylon w/ polyester lining	—	colorless	clear	yes	—	no
10	Nylon w/ polyester lining	—	colorless	clear	yes	—	no
11	Nylon w/ polyester lining	—	colorless	clear	yes	—	no
12	Nylon w/ polyester lining	—	colorless	clear	yes	—	no
13	Nylon w/ polyester lining	—	colorless	clear	yes	—	no
14	Nylon w/ polyester lining	—	colorless	clear	yes	—	no
15	Nylon w/ polyester lining	—	dark yellow	clear	yes	—	no
16	Nylon w/ polyester lining	—	dark yellow	clear	yes	—	no
17	Nylon w/ polyester lining	—	dark yellow	clear	yes	—	no
18	Nylon w/ polyester lining	—	dark yellow	clear	yes	—	no
19	Nylon w/ polyester lining	—	dark yellow	clear	yes	—	no
20	Nylon w/ polyester lining	—	dark yellow	clear	yes	—	no
21	Nylon w/ polyester lining	—	dark yellow	clear	yes	—	no
22	Nylon w/ polyester lining	—	dark yellow	clear	yes	—	no
23	Nylon w/ polyester lining	—	dark yellow	clear	yes	—	no
24	Nylon w/ polyester lining	—	dark yellow	clear	yes	—	no
25	Nylon w/ polyester lining	—	dark yellow	clear	yes	—	no
26	Nylon w/ polyester lining	—	dark yellow	clear	yes	—	no
27	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
28	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
29	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
30	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
31	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
32	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
33	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
34	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
35	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
36	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
37	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
38	Nylon w/ polyester lining	—	light yellow	clear	no	—	no

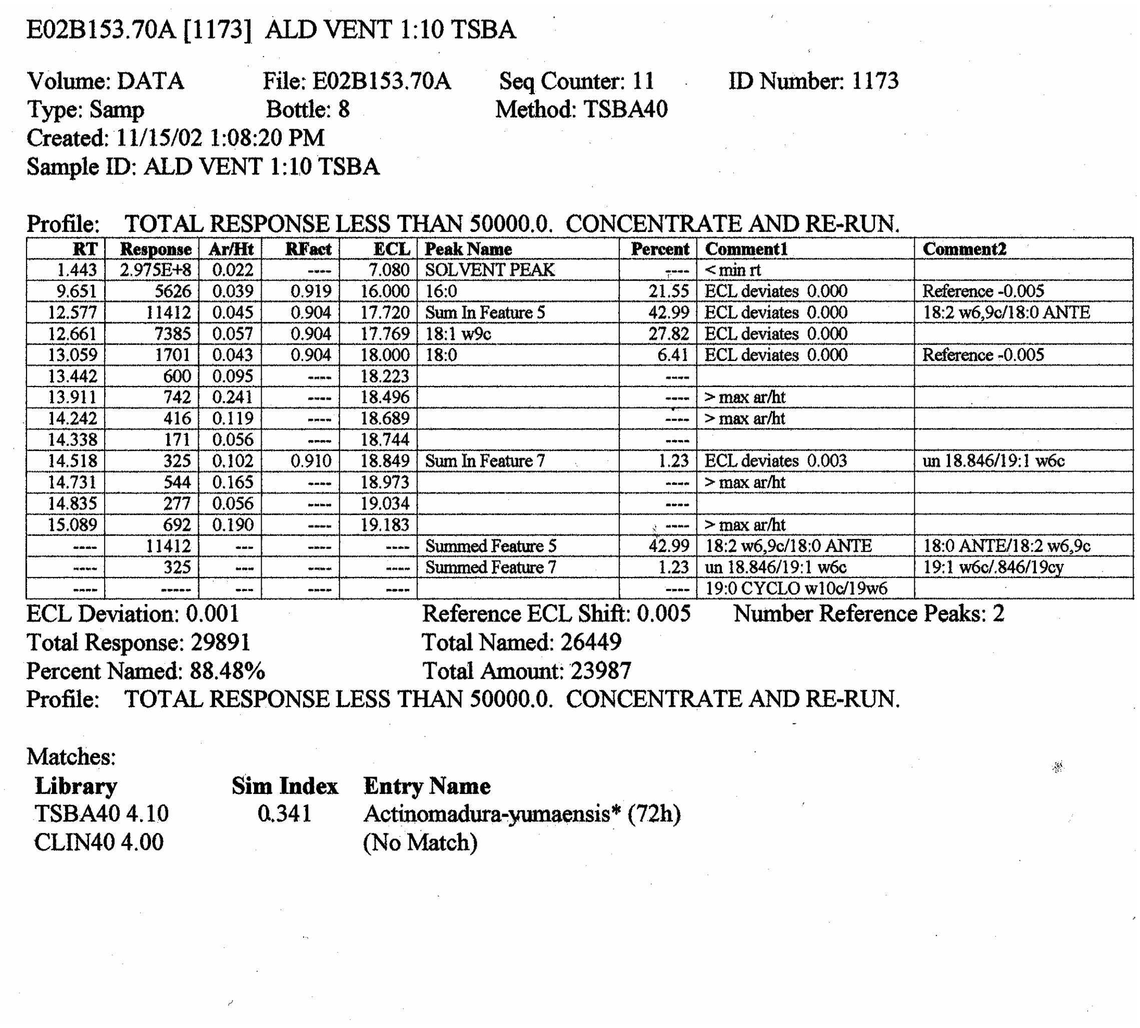
		Sample Description					
#	Tank Composition	pH	Sample Color	General Description	Obvious Solids	TDS (mg/L)	Free Phase Water
39	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
40	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
41	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
42	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
43	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
44	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
45	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
46	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
47	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
48	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
49	Nylon w/ polyester lining	—	light yellow	clear	no	—	no
50	Nylon w/ polyester lining	—	light yellow	clear	no	—	no

	Light Microscopy				Colony Description		Quantity Data		
#	Gram +/-	Organism Shape	Length of Cells (μm)	Width of Cells (μm)	Colony Type	Colony Color	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours
1	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	0	0	0
4	—	—	—	—	—	—	0	0	0
5	—	—	—	—	—	—	0	0	0
6	—	varied	varied	varied	round	white	0	0	5 days to grow
7	—	—	—	—	—	—	0	0	0
8	—	—	—	—	—	—	0	0	0
9	—	—	—	—	—	—	0	0	0
10	—	—	—	—	—	—	0	0	0
11	neg	coccus	1 diameter	—	irregular	white	0	0	5 days to grow
12	—	varied	varied	varied	round	white	0	0	5 days to grow
13	neg	bacillus	1	0.5	irregular	white	640	2,520	640,000
14	—	varied	varied	varied	round	white	710	3,450	7,100,000
15	—	—	—	—	—	—	0	0	0
16	—	—	—	—	—	—	0	0	0
17	—	—	—	—	—	—	0	0	0
18	neg	coccus	1 diameter	—	round	white	0	0	5 days to grow
19	—	—	—	—	—	—	0	0	0
20	—	—	—	—	—	—	0	0	0
21	—	—	—	—	—	—	0	0	0
22	—	—	—	—	—	—	0	0	0
23	neg	coccus	1 diameter	—	round	white	0	0	5 days to grow
24	neg	coccus	1 diameter	—	round	white	605	1,200	60,500
25	—	—	—	—	—	—	0	0	0
26	neg	coccus	1 diameter	—	round	white	0	0	5 days to grow
27	neg	coccus	1 diameter	—	round	white	0	0	5 days to grow
28	—	varied	varied	varied	round	white	0	0	5 days to grow
29	—	—	—	—	—	—	0	0	0
30	—	—	—	—	—	—	0	0	0
31	—	—	—	—	—	—	0	0	0
32	—	—	—	—	—	—	0	0	0
33	—	—	—	—	—	—	0	0	0
34	—	—	—	—	—	—	0	0	0
35	neg	bacillus	1	0.5	round	white	610	1,200	61,000
36	neg	bacillus	1	0.5	round	white	0	0	5 days to grow
37	—	—	—	—	—	—	0	0	0
38	neg	bacillus	1	0.5	round	white	690	3,450	6,900,000

	Light Microscopy				Colony Description		Quantity Data		
#	Gram +/-	Organism Shape	Length of Cells (μm)	Width of Cells (μm)	Colony Type	Colony Color	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours
39	—	—	—	—	—	—	0	0	0
40	neg	bacillus	1	0.5	round	white	0	0	5 days to grow
41	—	—	—	—	—	—	0	0	0
42	—	—	—	—	—	—	0	0	0
43	—	—	—	—	—	—	0	0	0
44	—	—	—	—	—	—	0	0	0
45	—	—	—	—	—	—	0	0	0
46	—	—	—	—	—	—	0	0	0
47	—	varied	varied	varied	round	white	210	345	2,100
48	—	—	—	—	—	—	0	0	0
49	—	varied	varied	varied	round	white	0	0	5 days to grow
50	—	varied	varied	varied	round	white	490	890	4,900,000

				Identification Data				
				FAME analysis			DNA gene sequence	
#	Bacteria vs. Fungi	Spores	Digital Photo	Sim Index	> .1 S. I. Separation	GC Best Match	DNA % ID	DNA Best Match
1	—	—	—	—	—	Library match not attempted	—	—
2	—	—	—	—	—	No match found	—	—
3	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—
6	fungi	no	ALDBL1000TSBA	0.746	yes	*Kocuria-kristinea (Micrococcus)*	—	—
7	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—
11	bacteria	no	ALDBLNEATBHI	—	—	No match found	99.91	*Bacillus licheniformis*
12	fungi	no	ALDBL10BHI	—	—	No match found	—	—
13	bacteria	no	ALDBL100BHI	—	—	No match found	—	—
14	fungi	no	ALDBL1000BHI	—	—	Library match not attempted	—	—
15	—	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—	—
18	bacteria	no	ALDLI1000TSBA	0.314	yes	*Staphylococcus-xylosus*	—	—
19	—	—	—	—	—	—	—	—
20	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—
22	—	—	—	—	—	—	—	—
23	bacteria	no	ALDLINEATBHI	0.019	no	*Prevotella-loescheii (Bacteroides loescheii)*	—	—
24	bacteria	no	ALDLI10BHI	—	—	Library match not attempted	—	—
25	—	—	—	—	—	—	—	—
26	bacteria	no	ALDLI1000BHI	—	—	No match found	—	—
27	bacteria	no	ALDDRNEATTSBA	0.378	no	*Bacillus-megaterium-GC subgroup B*	99.91	*Micrococcus luteus*
28	fungi	no	ALDDR10TSBA	—	—	No match found	—	—
29	—	—	—	—	—	—	—	—
30	—	—	—	—	—	—	—	—
31	—	—	—	—	—	—	—	—
32	—	—	—	—	—	—	—	—
33	—	—	—	—	—	—	—	—
34	—	—	—	—	—	—	—	—
35	bacteria	no	ALDDRNEATBHI	—	—	No match found	99.91	*Bacillus pumilus*
36	bacteria	no	ALDDR10BHI	—	—	No match found	—	—
37	—	—	—	—	—	—	—	—
38	bacteria	no	ALDDR1000BHI	—	—	No match found	—	—

				Identification Data				
				FAME analysis			**DNA gene sequence**	
#	**Bacteria vs. Fungi**	**Spores**	**Digital Photo**	**Sim Index**	**> .1 S. I. Separation**	**GC Best Match**	**DNA % ID**	**DNA Best Match**
39	—	—	—	—	—	—	—	—
40	bacteria	no	ALDVE10TSBA	0.341	yes	*Actinomadura-yumaensis (72h)*	—	—
41	—	—	—	—	—	—	—	—
42	—	—	—	—	—	—	—	—
43	—	—	—	—	—	—	—	—
44	—	—	—	—	—	—	—	—
45	—	—	—	—	—	—	—	—
46	—	—	—	—	—	—	—	—
47	bacteria	no	ALDVENEATBHI			No match found	99.91	*Bacillus licheniformis*
48	—	—	—	—	—	—	—	—
49	fungi	no	—	—	—	Library match not attempted	—	—
50	fungi	no	—	—	—	No match found	—	—



E02B153.70A [1173] ALD VENT 1:10 TSBA

Volume: DATA File: E02B153.70A Seq Counter: 11 ID Number: 1173
Type: Samp Bottle: 8 Method: TSBA40
Created: 11/15/02 1:08:20 PM
Sample ID: ALD VENT 1:10 TSBA

Profile: TOTAL RESPONSE LESS THAN 50000.0. CONCENTRATE AND RE-RUN.

RT	**Response**	**Ar/Ht**	**RFact**	**ECL**	**Peak Name**	**Percent**	**Comment1**	**Comment2**
1.443	2.975E+8	0.022	----	7.080	SOLVENT PEAK	----	< min rt	
9.651	5626	0.039	0.919	16.000	16:0	21.55	ECL deviates 0.000	Reference -0.005
12.577	11412	0.045	0.904	17.720	Sum In Feature 5	42.99	ECL deviates 0.000	18:2 w6,9c/18:0 ANTE
12.661	7385	0.057	0.904	17.769	18:1 w9c	27.82	ECL deviates 0.000	
13.059	1701	0.043	0.904	18.000	18:0	6.41	ECL deviates 0.000	Reference -0.005
13.442	600	0.095	----	18.223		----		
13.911	742	0.241	----	18.496		----	> max ar/ht	
14.242	416	0.119	----	18.689		----	> max ar/ht	
14.338	171	0.056	----	18.744		----		
14.518	325	0.102	0.910	18.849	Sum In Feature 7	1.23	ECL deviates 0.003	un 18.846/19:1 w6c
14.731	544	0.165	----	18.973		----	> max ar/ht	
14.835	277	0.056	----	19.034		----		
15.089	692	0.190	----	19.183		----	> max ar/ht	
----	11412	---	----	----	Summed Feature 5	42.99	18:2 w6,9c/18:0 ANTE	18:0 ANTE/18:2 w6,9c
----	325	---	----	----	Summed Feature 7	1.23	un 18.846/19:1 w6c	19:1 w6c/.846/19cy
----	-----	---	----	----		----	19:0 CYCLO w10c/19w6	

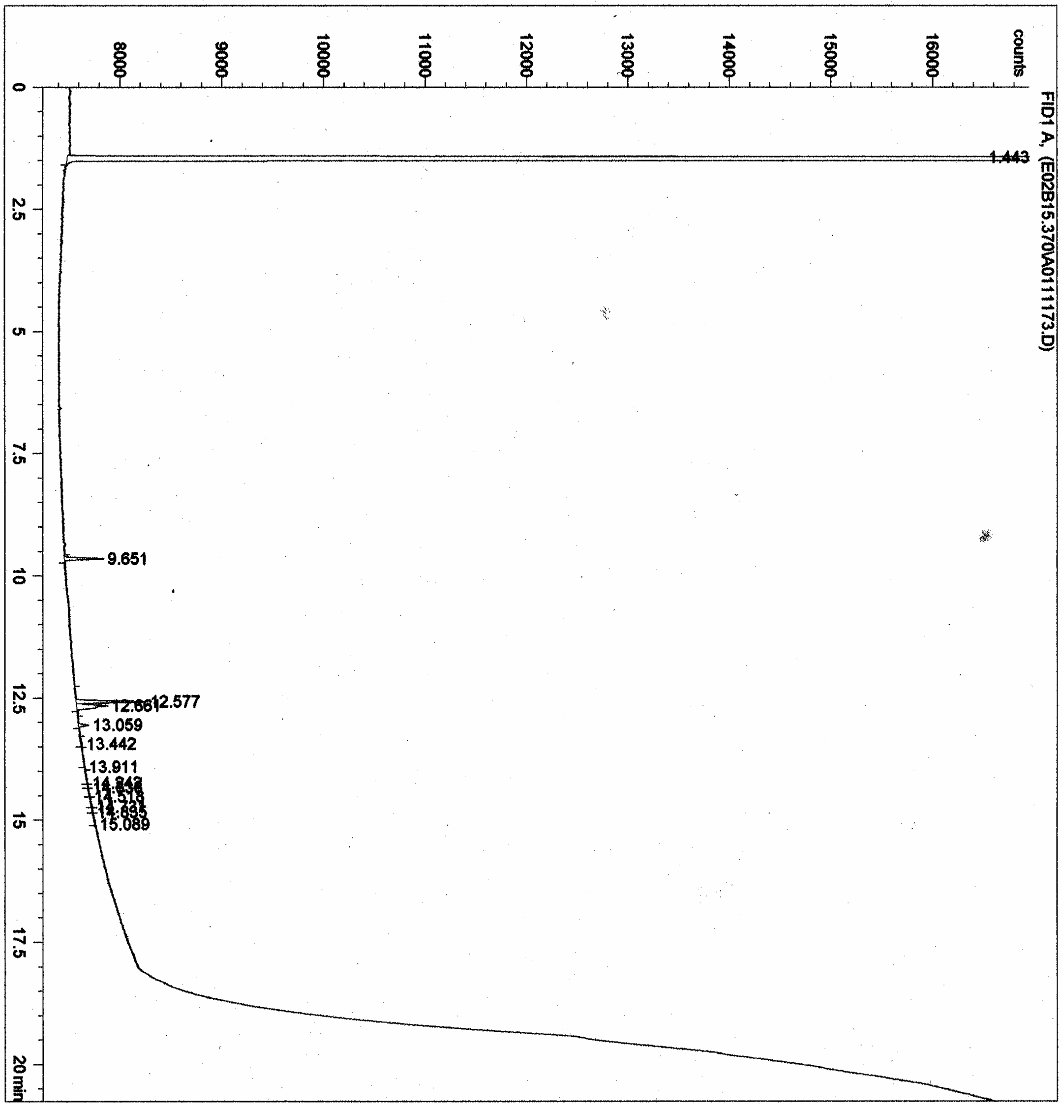
ECL Deviation: 0.001 Reference ECL Shift: 0.005 Number Reference Peaks: 2
Total Response: 29891 Total Named: 26449
Percent Named: 88.48% Total Amount: 23987
Profile: TOTAL RESPONSE LESS THAN 50000.0. CONCENTRATE AND RE-RUN.

Matches:

Library	**Sim Index**	**Entry Name**
TSBA40 4.10	0.341	Actinomadura-yumaensis* (72h)
CLIN40 4.00		(No Match)

```
Data File C:\SHERLOCK\RAW\E02B15.370\A0111173.D                        Sample Name:
    Sherlock Id: ALD VENT 1:10 TSBA

    =====================================================================
    Injection Date  : 11/15/02 1:12:29 PM
    Sample Name     :  1173                          Location : Vial 8
    Acq. Operator   :
                                                   Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 11/15/02 1:08:20 PM
    =====================================================================
```



*** End of Report ***

E02B153.70A [1170] ALD DRAIN NEAT TSBA

Volume: DATA File: E02B153.70A Seq Counter: 9 ID Number: 1170
Type: Samp Bottle: 6 Method: TSBA40
Created: 11/15/02 12:16:59 PM
Sample ID: ALD DRAIN NEAT TSBA

Profile: ONE OR MORE NAMED PEAKS REJECTED. SEE COMMENTS IN THE REPORT.

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.444	2.915E+8	0.027	----	7.071	SOLVENT PEAK	----	< min rt	
2.210	669	0.043	----	8.788		----	< min rt	
3.185	908	0.023	1.161	10.694	11:0 ANTEISO	0.23	ECL deviates -0.001	Reference 0.003
3.891	1613	0.028	1.097	11.614	12:0 ISO	0.38	ECL deviates 0.005	Reference 0.007
4.788	549	0.027	----	12.536		----		
4.868	1812	0.029	1.038	12.611	13:0 ISO	0.40	ECL deviates -0.003	Reference -0.002
4.964	7969	0.029	1.033	12.700	13:0 ANTEISO	1.77	ECL deviates -0.002	Reference 0.000
6.081	22395	0.031	0.990	13.616	14:0 ISO	4.77	ECL deviates -0.003	Reference -0.002
6.576	1511	0:033	0.974	13.999	14:0	0.32	ECL deviates -0.001	Reference 0.000
7.097	5473	0.069	----	14.354		----		
7.158	1241	0.017	0.960	14.395	ISO 15:1 AT 5	----	< min ar/ht	
7.176	4264	0.053	0.959	14.408	15:1 ISO F	0.88	ECL deviates -0.007	
7.277	7019	0.063	0.957	14.476	Sum In Feature 1	1.45	ECL deviates -0.002	15:1 ISO I/13:0 3OH
7.336	4516	0.055	----	14.516		----		
7.494	57293	0.039	0.952	14.623	15:0 ISO	11.75	ECL deviates 0.000	Reference 0.000
7.637	360916	0.036	0.950	14.720	15:0 ANTEISO	73.78	ECL deviates 0.007	Reference 0.007
7.782	1662	0.047	----	14.819		----		
7.835	3402	0.090	0.945	14.855	15:1 w6c	0.69	ECL deviates -0.001	
8.050	5127	0.147	0.941	15.001	15:0	----	> max ar/ht	
8.227	1142	0.100	0.938	15.111	14:0 ISO 3OH	0.23	ECL deviates -0.008	
8.783	1235	0.055	0.930	15.457	16:1 ISO H	0.25	ECL deviates -0.004	
9.055	9374	0.039	0.926	15.626	16:0 ISO	1.87	ECL deviates -0.001	Reference -0.002
9.360	1329	0.045	0.922	15.815	Sum In Feature 3	0.26	ECL deviates -0.007	16:1 w7c/15 iso 2OH
9.656	1030	0.064	0.919	16.000	16:0	0.20	ECL deviates 0.000	Reference -0.002
10.045	1135	0.102	0.915	16.231	15:0 2OH	----	> max ar/ht	
10.536	835	0.052	0.911	16.523	ANTEISO 17:1 w9c	0.16	ECL deviates -0.001	
10.871	3107	0.043	0.909	16.722	17:0 ANTEISO	0.61	ECL deviates -0.001	Reference -0.003
11.823	1531	0.088	----	17.281		----		
12.642	1167	0.226	----	17.757		----	> max ar/ht	
17.916	3204	0.068	----	20.842		----	> max rt	
19.740	57462	0.227	----	21.913		----	> max rt	
19.829	9771	0.048	----	21.965		----	> max rt	
19.914	16887	0.029	----	22.014		----	> max rt	
----	7019	---	----	----	Summed Feature 1	1.45	15:1 ISO H/13:0 3OH	13:0 3OH/15:1 i I/H
----	-----	---	----	----		----	15:1 ISO I/13:0 3OH	
----	1329	---	----	----	Summed Feature 3	0.26	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c

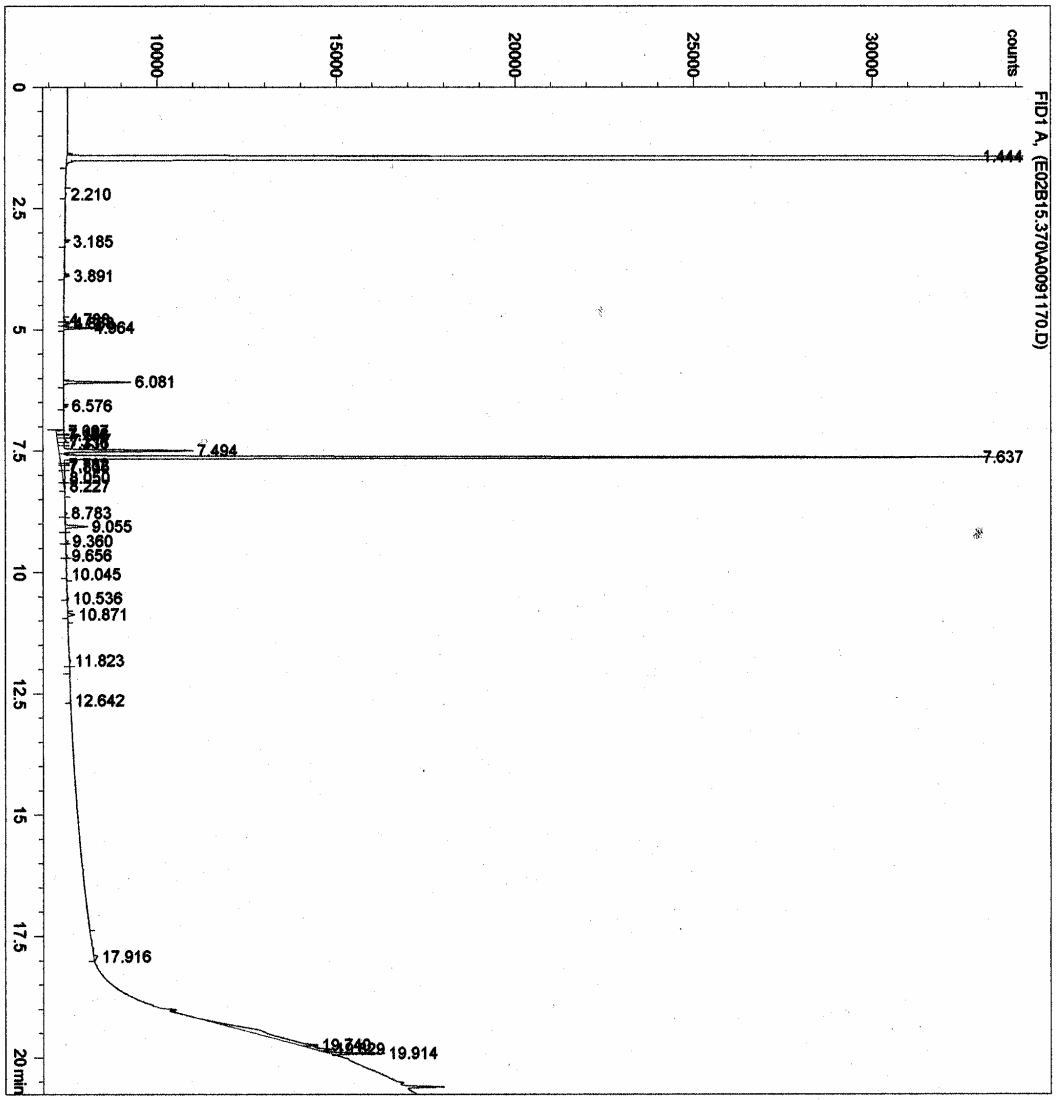
ECL Deviation: 0.005 Reference ECL Shift: 0.004 Number Reference Peaks: 11
Total Response: 509553 Total Named: 487153
Percent Named: 95.60% Total Amount: 471594
Profile: ONE OR MORE NAMED PEAKS REJECTED. SEE COMMENTS IN THE REPORT.

Matches:

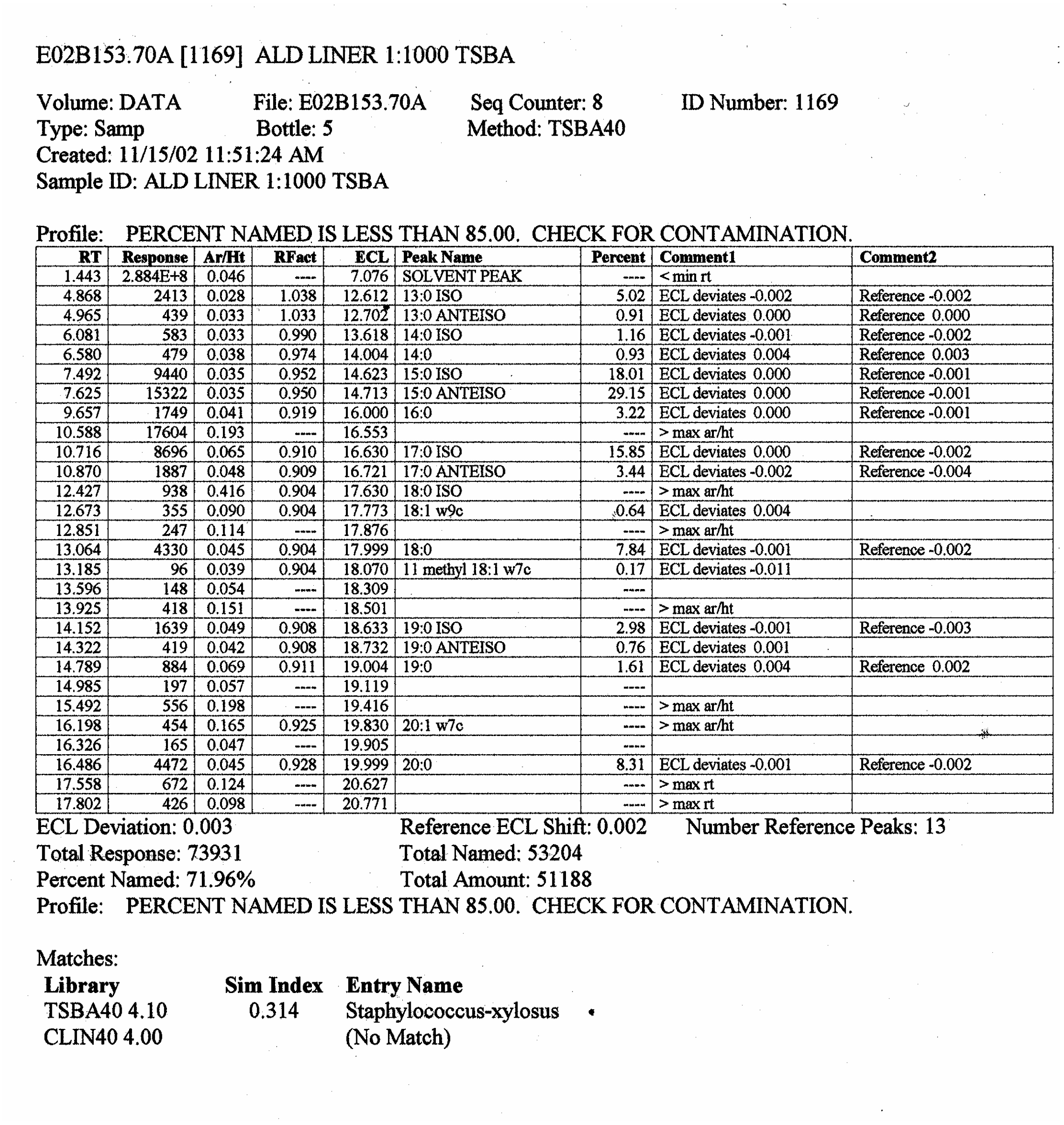
Library	**Sim Index**	**Entry Name**
TSBA40 4.10	0.378	Bacillus-megaterium-GC subgroup B
	0.373	Micrococcus-lylae-GC subgroup A
	0.314	Arthrobacter-aurescens
	0.254	Kocuria-varians* (Micrococcus)
CLIN40 4.00	0.427	Micrococcus-luteus-GC subgroup C**
	0.280	Micrococcus-luteus-GC subgroup A*

```
Data File C:\SHERLOCK\RAW\E02B15.370\A0091170.D                   Sample Name:
    Sherlock Id: ALD DRAIN NEAT TSBA

    =====================================================================
    Injection Date  : 11/15/02 12:21:14 PM
    Sample Name     :  1170.                          Location : Vial 6
    Acq. Operator   :
                                                    Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 11/15/02 12:17:00 PM
    =====================================================================
```



*** End of Report ***



E02B153.70A [1169] ALD LINER 1:1000 TSBA

Volume: DATA	File: E02B153.70A	Seq Counter: 8	ID Number: 1169
Type: Samp	Bottle: 5	Method: TSBA40	

Created: 11/15/02 11:51:24 AM
Sample ID: ALD LINER 1:1000 TSBA

Profile: PERCENT NAMED IS LESS THAN 85.00. CHECK FOR CONTAMINATION.

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.443	2.884E+8	0.046	----	7.076	SOLVENT PEAK	----	< min rt	
4.868	2413	0.028	1.038	12.612	13:0 ISO	5.02	ECL deviates -0.002	Reference -0.002
4.965	439	0.033	1.033	12.702	13:0 ANTEISO	0.91	ECL deviates 0.000	Reference 0.000
6.081	583	0.033	0.990	13.618	14:0 ISO	1.16	ECL deviates -0.001	Reference -0.002
6.580	479	0.038	0.974	14.004	14:0	0.93	ECL deviates 0.004	Reference 0.003
7.492	9440	0.035	0.952	14.623	15:0 ISO	18.01	ECL deviates 0.000	Reference -0.001
7.625	15322	0.035	0.950	14.713	15:0 ANTEISO	29.15	ECL deviates 0.000	Reference -0.001
9.657	1749	0.041	0.919	16.000	16:0	3.22	ECL deviates 0.000	Reference -0.001
10.588	17604	0.193	----	16.553		----	> max ar/ht	
10.716	8696	0.065	0.910	16.630	17:0 ISO	15.85	ECL deviates 0.000	Reference -0.002
10.870	1887	0.048	0.909	16.721	17:0 ANTEISO	3.44	ECL deviates -0.002	Reference -0.004
12.427	938	0.416	0.904	17.630	18:0 ISO	----	> max ar/ht	
12.673	355	0.090	0.904	17.773	18:1 w9c	0.64	ECL deviates 0.004	
12.851	247	0.114	----	17.876		----	> max ar/ht	
13.064	4330	0.045	0.904	17.999	18:0	7.84	ECL deviates -0.001	Reference -0.002
13.185	96	0.039	0.904	18.070	11 methyl 18:1 w7c	0.17	ECL deviates -0.011	
13.596	148	0.054	----	18.309		----		
13.925	418	0.151	----	18.501		----	> max ar/ht	
14.152	1639	0.049	0.908	18.633	19:0 ISO	2.98	ECL deviates -0.001	Reference -0.003
14.322	419	0.042	0.908	18.732	19:0 ANTEISO	0.76	ECL deviates 0.001	
14.789	884	0.069	0.911	19.004	19:0	1.61	ECL deviates 0.004	Reference 0.002
14.985	197	0.057	----	19.119		----		
15.492	556	0.198	----	19.416		----	> max ar/ht	
16.198	454	0.165	0.925	19.830	20:1 w7c	----	> max ar/ht	
16.326	165	0.047	----	19.905		----		
16.486	4472	0.045	0.928	19.999	20:0	8.31	ECL deviates -0.001	Reference -0.002
17.558	672	0.124	----	20.627		----	> max rt	
17.802	426	0.098	----	20.771		----	> max rt	

ECL Deviation: 0.003	Reference ECL Shift: 0.002	Number Reference Peaks: 13
Total Response: 73931	Total Named: 53204	
Percent Named: 71.96%	Total Amount: 51188	

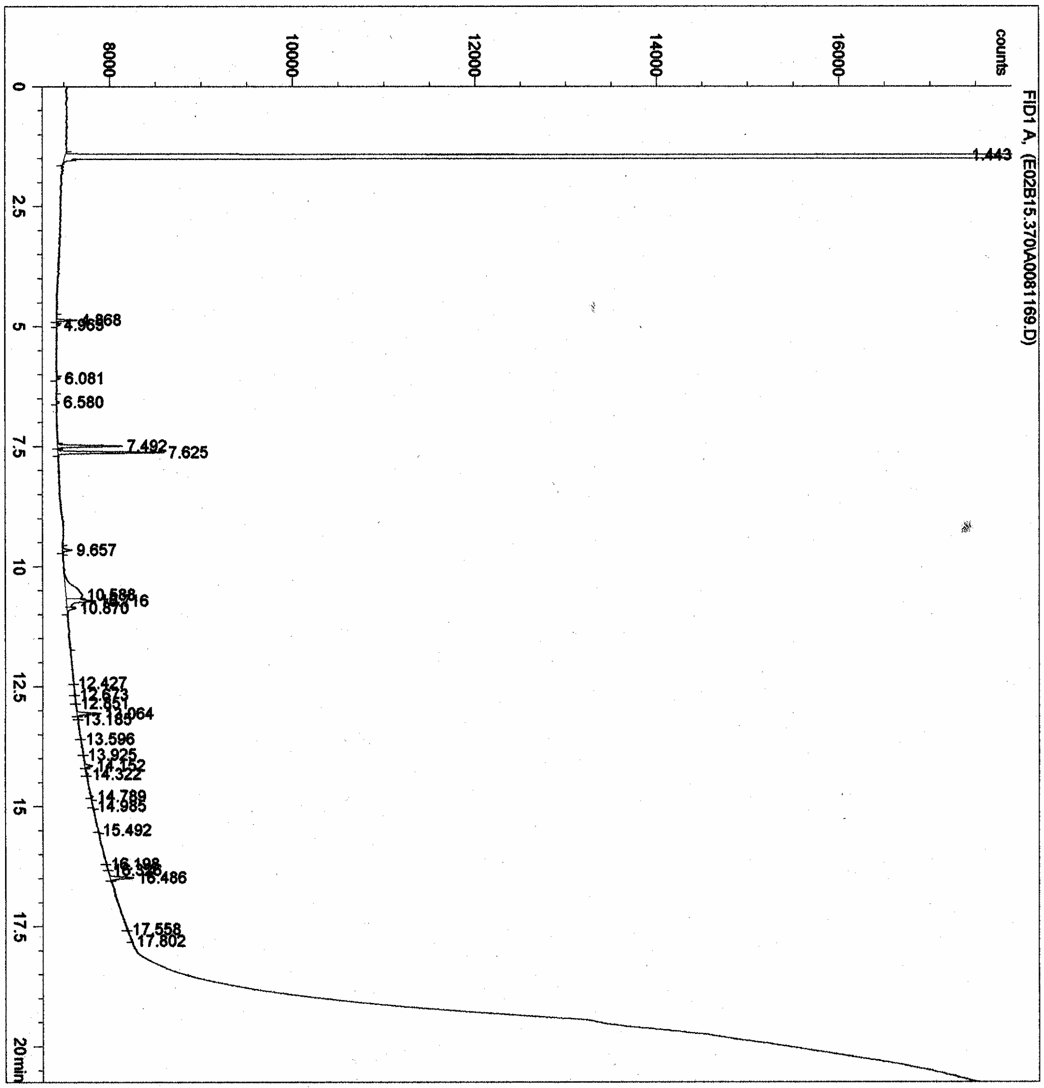
Profile: PERCENT NAMED IS LESS THAN 85.00. CHECK FOR CONTAMINATION.

Matches:

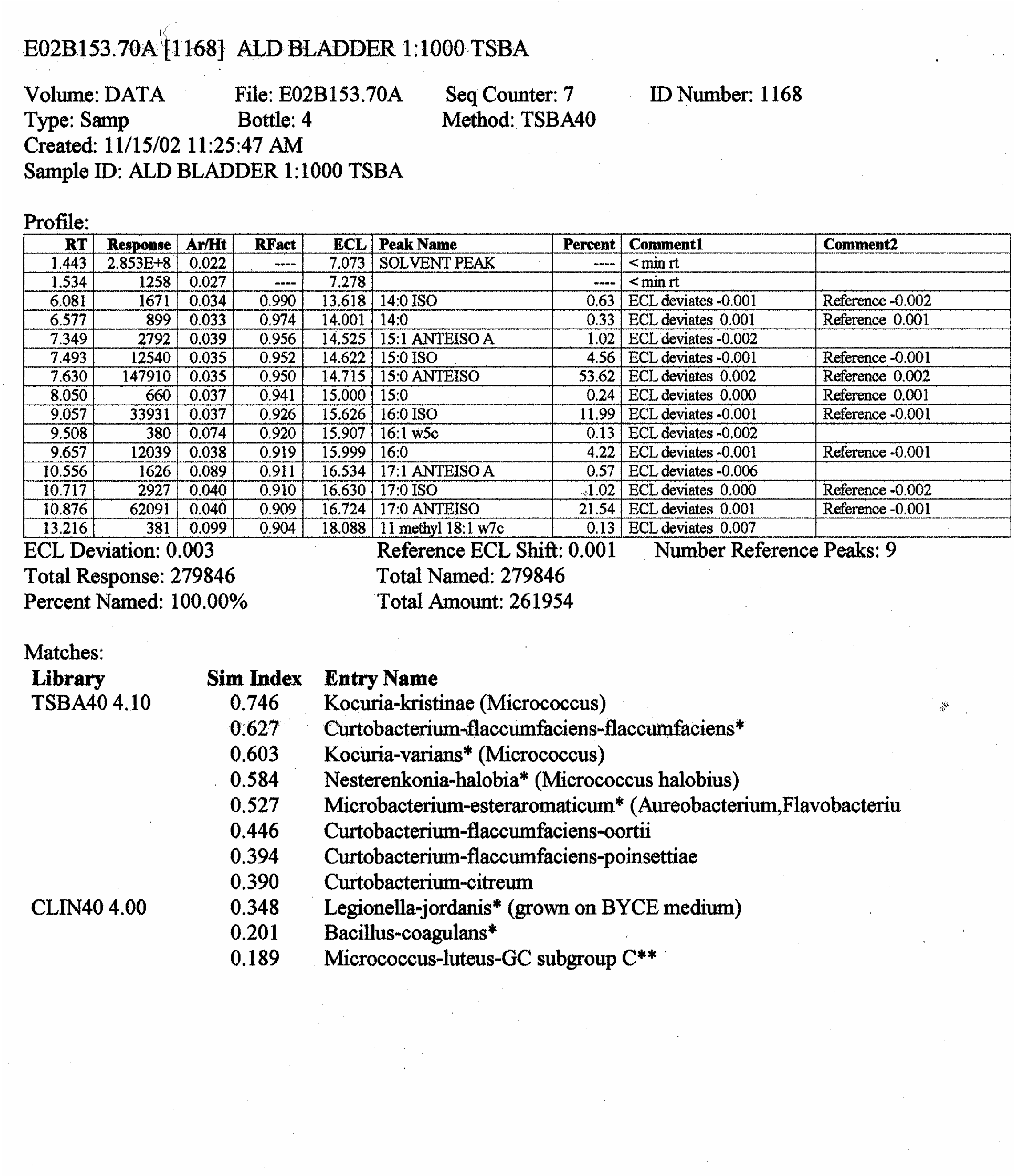
Library	Sim Index	Entry Name
TSBA40 4.10	0.314	Staphylococcus-xylosus
CLIN40 4.00		(No Match)

```
Data File C:\SHERLOCK\RAW\E02B15.370\A0081169.D                          Sample Name:
    Sherlock Id: ALD LINER 1:1000 TSBA

    =====================================================================
    Injection Date  : 11/15/02 11:55:34 AM
    Sample Name     :  1169                        Location : Vial 5
    Acq. Operator   :
                                                 Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 11/15/02 11:51:25 AM
    =====================================================================
```



*** End of Report ***



E02B153.70A [1168] ALD BLADDER 1:1000 TSBA

Volume: DATA File: E02B153.70A Seq Counter: 7 ID Number: 1168
Type: Samp Bottle: 4 Method: TSBA40
Created: 11/15/02 11:25:47 AM
Sample ID: ALD BLADDER 1:1000 TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.443	2.853E+8	0.022	----	7.073	SOLVENT PEAK	----	< min rt	
1.534	1258	0.027	----	7.278		----	< min rt	
6.081	1671	0.034	0.990	13.618	14:0 ISO	0.63	ECL deviates -0.001	Reference -0.002
6.577	899	0.033	0.974	14.001	14:0	0.33	ECL deviates 0.001	Reference 0.001
7.349	2792	0.039	0.956	14.525	15:1 ANTEISO A	1.02	ECL deviates -0.002	
7.493	12540	0.035	0.952	14.622	15:0 ISO	4.56	ECL deviates -0.001	Reference -0.001
7.630	147910	0.035	0.950	14.715	15:0 ANTEISO	53.62	ECL deviates 0.002	Reference 0.002
8.050	660	0.037	0.941	15.000	15:0	0.24	ECL deviates 0.000	Reference 0.001
9.057	33931	0.037	0.926	15.626	16:0 ISO	11.99	ECL deviates -0.001	Reference -0.001
9.508	380	0.074	0.920	15.907	16:1 w5c	0.13	ECL deviates -0.002	
9.657	12039	0.038	0.919	15.999	16:0	4.22	ECL deviates -0.001	Reference -0.001
10.556	1626	0.089	0.911	16.534	17:1 ANTEISO A	0.57	ECL deviates -0.006	
10.717	2927	0.040	0.910	16.630	17:0 ISO	1.02	ECL deviates 0.000	Reference -0.002
10.876	62091	0.040	0.909	16.724	17:0 ANTEISO	21.54	ECL deviates 0.001	Reference -0.001
13.216	381	0.099	0.904	18.088	11 methyl 18:1 w7c	0.13	ECL deviates 0.007	

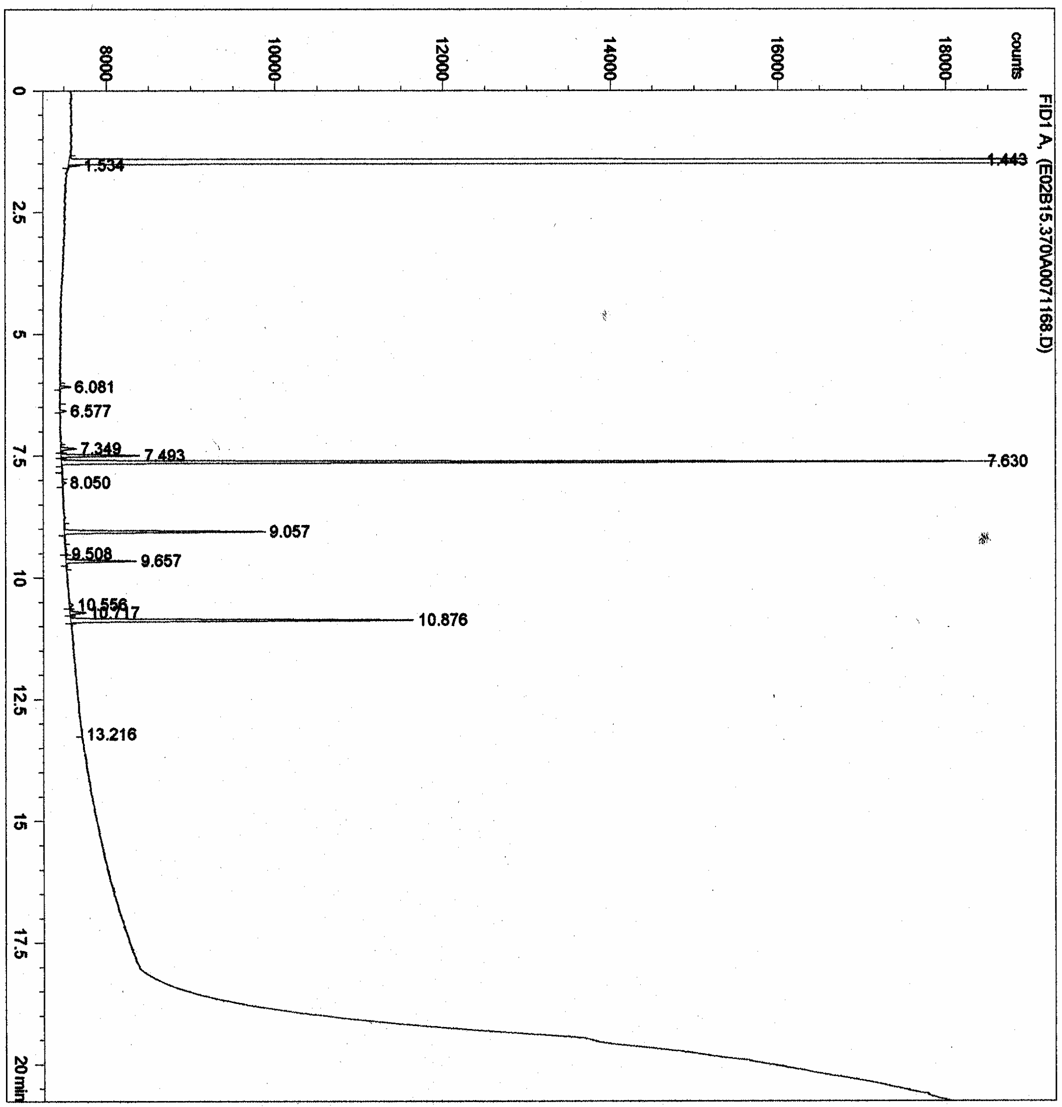
ECL Deviation: 0.003 Reference ECL Shift: 0.001 Number Reference Peaks: 9
Total Response: 279846 Total Named: 279846
Percent Named: 100.00% Total Amount: 261954

Matches:

Library	**Sim Index**	**Entry Name**
TSBA40 4.10	0.746	Kocuria-kristinae (Micrococcus)
	0.627	Curtobacterium-flaccumfaciens-flaccumfaciens*
	0.603	Kocuria-varians* (Micrococcus)
	0.584	Nesterenkonia-halobia* (Micrococcus halobius)
	0.527	Microbacterium-esteraromaticum* (Aureobacterium,Flavobacteriu
	0.446	Curtobacterium-flaccumfaciens-oortii
	0.394	Curtobacterium-flaccumfaciens-poinsettiae
	0.390	Curtobacterium-citreum
CLIN40 4.00	0.348	Legionella-jordanis* (grown on BYCE medium)
	0.201	Bacillus-coagulans*
	0.189	Micrococcus-luteus-GC subgroup C**

```
Data File C:\SHERLOCK\RAW\E02B15.370\A0071168.D                    Sample Name:
    Sherlock Id: ALD BLADDER 1:1000 TSBA

    =====================================================================
    Injection Date  : 11/15/02 11:29:56 AM
    Sample Name     :  1168                        Location : Vial 4
    Acq. Operator   :
                                                 Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 11/15/02 11:25:47 AM
    =====================================================================
```



```
*** End of Report ***
```

Appendix I: Worksheet / Sample Data – Davis-Monthan AFB (KDMA)

	Site Information									
#	Sample	Type / Number	Date Sampled	Dilution	Medium	Temp °C	% Relative Humidity	Inches Rainfall (last 30 days)	Fuel Type	Sample Method
1	Neg Control	—	—	—	—	—	—	—	—	—
2	Pos Control	—	—	—	—	—	—	—	—	—
3	Tank	25	11/8/2002	Neat	TSBA	25	48	0.31	JP-8	gravity
4	Tank	25	11/8/2002	1:10	TSBA	25	48	0.31	JP-8	gravity
5	Tank	25	11/8/2002	1:100	TSBA	25	48	0.31	JP-8	gravity
6	Tank	25	11/8/2002	1:1000	TSBA	25	48	0.31	JP-8	gravity
7	Tank	25	11/8/2002	Neat	SDA	25	48	0.31	JP-8	gravity
8	Tank	25	11/8/2002	1:10	SDA	25	48	0.31	JP-8	gravity
9	Tank	25	11/8/2002	1:100	SDA	25	48	0.31	JP-8	gravity
10	Tank	25	11/8/2002	1:1000	SDA	25	48	0.31	JP-8	gravity
11	Tank	25	11/8/2002	Neat	BHIBLA	25	48	0.31	JP-8	gravity
12	Tank	25	11/8/2002	1:10	BHIBLA	25	48	0.31	JP-8	gravity
13	Tank	25	11/8/2002	1:100	BHIBLA	25	48	0.31	JP-8	gravity
14	Tank	25	11/8/2002	1:1000	BHIBLA	25	48	0.31	JP-8	gravity
15	Truck	570	11/8/2002	Neat	TSBA	25	48	0.31	JP-8	gravity
16	Truck	570	11/8/2002	1:10	TSBA	25	48	0.31	JP-8	gravity
17	Truck	570	11/8/2002	1:100	TSBA	25	48	0.31	JP-8	gravity
18	Truck	570	11/8/2002	1:1000	TSBA	25	48	0.31	JP-8	gravity
19	Truck	570	11/8/2002	Neat	SDA	25	48	0.31	JP-8	gravity
20	Truck	570	11/8/2002	1:10	SDA	25	48	0.31	JP-8	gravity
21	Truck	570	11/8/2002	1:100	SDA	25	48	0.31	JP-8	gravity
22	Truck	570	11/8/2002	1:1000	SDA	25	48	0.31	JP-8	gravity
23	Truck	570	11/8/2002	Neat	BHIBLA	25	48	0.31	JP-8	gravity
24	Truck	570	11/8/2002	1:10	BHIBLA	25	48	0.31	JP-8	gravity
25	Truck	570	11/8/2002	1:100	BHIBLA	25	48	0.31	JP-8	gravity
26	Truck	570	11/8/2002	1:1000	BHIBLA	25	48	0.31	JP-8	gravity
27	Aircraft	A-10	11/8/2002	Neat	TSBA	25	48	0.31	JP-8	gravity
28	Aircraft	A-10	11/8/2002	1:10	TSBA	25	48	0.31	JP-8	gravity
29	Aircraft	A-10	11/8/2002	1:100	TSBA	25	48	0.31	JP-8	gravity
30	Aircraft	A-10	11/8/2002	1:1000	TSBA	25	48	0.31	JP-8	gravity
31	Aircraft	A-10	11/8/2002	Neat	SDA	25	48	0.31	JP-8	gravity
32	Aircraft	A-10	11/8/2002	1:10	SDA	25	48	0.31	JP-8	gravity
33	Aircraft	A-10	11/8/2002	1:100	SDA	25	48	0.31	JP-8	gravity
34	Aircraft	A-10	11/8/2002	1:1000	SDA	25	48	0.31	JP-8	gravity
35	Aircraft	A-10	11/8/2002	Neat	BHIBLA	25	48	0.31	JP-8	gravity
36	Aircraft	A-10	11/8/2002	1:10	BHIBLA	25	48	0.31	JP-8	gravity
37	Aircraft	A-10	11/8/2002	1:100	BHIBLA	25	48	0.31	JP-8	gravity
38	Aircraft	A-10	11/8/2002	1:1000	BHIBLA	25	48	0.31	JP-8	gravity

— Indicates analysis omitted

		Sample Description					
#	Tank Composition	pH	Sample Color	General Description	Obvious Solids	TDS (mg/L)	Free Phase Water
1	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—
3	steel w/ epoxy lining	—	light yellow	clear	no	—	no
4	steel w/ epoxy lining	—	light yellow	clear	no	—	no
5	steel w/ epoxy lining	—	light yellow	clear	no	—	no
6	steel w/ epoxy lining	—	light yellow	clear	no	—	no
7	steel w/ epoxy lining	—	light yellow	clear	no	—	no
8	steel w/ epoxy lining	—	light yellow	clear	no	—	no
9	steel w/ epoxy lining	—	light yellow	clear	no	—	no
10	steel w/ epoxy lining	—	light yellow	clear	no	—	no
11	steel w/ epoxy lining	—	light yellow	clear	no	—	no
12	steel w/ epoxy lining	—	light yellow	clear	no	—	no
13	steel w/ epoxy lining	—	light yellow	clear	no	—	no
14	steel w/ epoxy lining	—	light yellow	clear	no	—	no
15	aluminum	6.6	light yellow	clear	yes	—	yes
16	aluminum	6.6	light yellow	clear	yes	—	yes
17	aluminum	6.6	light yellow	clear	yes	—	yes
18	aluminum	6.6	light yellow	clear	yes	—	yes
19	aluminum	6.6	light yellow	clear	yes	—	yes
20	aluminum	6.6	light yellow	clear	yes	—	yes
21	aluminum	6.6	light yellow	clear	yes	—	yes
22	aluminum	6.6	light yellow	clear	yes	—	yes
23	aluminum	6.6	light yellow	clear	yes	—	yes
24	aluminum	6.6	light yellow	clear	yes	—	yes
25	aluminum	6.6	light yellow	clear	yes	—	yes
26	aluminum	6.6	light yellow	clear	yes	—	yes
27	aluminum	—	yellow	clear	no	—	no
28	aluminum	—	yellow	clear	no	—	no
29	aluminum	—	yellow	clear	no	—	no
30	aluminum	—	yellow	clear	no	—	no
31	aluminum	—	yellow	clear	no	—	no
32	aluminum	—	yellow	clear	no	—	no
33	aluminum	—	yellow	clear	no	—	no
34	aluminum	—	yellow	clear	no	—	no
35	aluminum	—	yellow	clear	no	—	no
36	aluminum	—	yellow	clear	no	—	no
37	aluminum	—	yellow	clear	no	—	no
38	aluminum	—	yellow	clear	no	—	no

	Light Microscopy				Colony Description		Quantity Data		
#	Gram +/-	Organism Shape	Length of Cells (μm)	Width of Cells (μm)	Colony Type	Colony Color	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours
1	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	0	0	0
4	—	—	—	—	—	—	0	0	0
5	—	—	—	—	—	—	0	0	0
6	—	—	—	—	—	—	0	0	0
7	—	—	—	—	—	—	0	0	0
8	—	—	—	—	—	—	0	0	0
9	—	—	—	—	—	—	0	0	0
10	—	—	—	—	—	—	0	0	0
11	—	varied	varied	varied	round	white	0	0	5 days to grow
12	—	—	—	—	—	—	0	0	0
13	—	—	—	—	—	—	0	0	0
14	—	—	—	—	—	—	0	0	0
15	—	—	—	—	—	—	0	0	0
16	—	varied	varied	varied	round	pink	0	1	0
17	—	varied	varied	varied	irregular	white	0	0	5 days to grow
18	neg	coccus	1 diameter	—	round	pink	0	0	5 days to grow
19	—	—	—	—	—	—	0	0	0
20	—	—	—	—	—	—	0	0	0
21	—	—	—	—	—	—	0	0	0
22	—	—	—	—	—	—	0	0	0
23	—	—	—	—	—	—	0	0	0
24	—	—	—	—	—	—	0	0	0
25	—	—	—	—	—	—	0	0	0
26	—	varied	varied	varied	irregular	white	0	0	5 days to grow
27	neg	coccus	1 diameter	—	round	white	0	1	0
28	—	—	—	—	—	—	0	0	0
29	—	—	—	—	—	—	0	0	0
30	neg	coccus	1 diameter	—	round	pink	12	575	120,000
31	—	—	—	—	—	—	0	0	0
32	—	—	—	—	—	—	0	0	0
33	—	—	—	—	—	—	0	0	0
34	—	—	—	—	—	—	0	0	0
35	—	—	—	—	—	—	0	0	0
36	—	—	—	—	—	—	0	0	0
37	—	—	—	—	—	—	0	0	0
38	pos	coccus	1 diameter	—	round	white	1	3	10,000

				Identification Data				
				FAME analysis			DNA gene sequence	
#	Bacteria vs. Fungi	Spores	Digital Photo	Sim Index	> .1 S. I. Separation	GC Best Match	DNA % ID	DNA Best Match
1	—	—	—	—	—	Library match not attempted	—	—
2	—	—	—	—	—	Library match not attempted	—	—
3	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—
11	bacteria	no	DMATANEATBHI	—	—	No match found	—	—
12	—	—	—	—	—	—	—	—
13	—	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—
16	bacteria	no	DMATR10TSBA	—	—	Library match not attempted	—	—
17	bacteria	no	DMATR100TSBA	—	—	—	—	—
18	bacteria	no	DMATR1000TSBA	—	—	—	—	—
19	—	—	—	—	—	—	—	—
20	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—
22	—	—	—	—	—	—	—	—
23	—	—	—	—	—	—	—	—
24	—	—	—	—	—	—	—	—
25	—	—	—	—	—	—	—	—
26	bacteria	no	DMATR1000BHI	—	—	No match found	—	—
27	bacteria	no	DMAACNEATTSBA	—	—	—	—	—
28	—	—	—	—	—	—	—	—
29	—	—	—	—	—	—	—	—
30	bacteria	no	DMAAC1000TSBA	—	—	—	—	—
31	—	—	—	—	—	—	—	—
32	—	—	—	—	—	—	—	—
33	—	—	—	—	—	—	—	—
34	—	—	—	—	—	—	—	—
35	—	—	—	—	—	—	—	—
36	—	—	—	—	—	—	—	—
37	—	—	—	—	—	—	—	—
38	bacteria	no	DMAAC1000BHI	—	—	No match found	—	—

Appendix J: Worksheet / Sample Data – Hill AFB (KHIF)

	Site Information									
#	**Sample**	**Type / Number**	**Date Sampled**	**Dilution**	**Medium**	**Temp °C**	**% Relative Humidity**	**Inches Rainfall (last 30 days)**	**Fuel Type**	**Sample Method**
1	Neg Control	40	11/12/2002	Neat	TSBA	6	56	1.09	JP-8	gravity
2	Pos Control	40	11/12/2002	1:10	TSBA	6	56	1.09	JP-8	gravity
3	Tank	40	11/12/2002	1:100	TSBA	6	56	1.09	JP-8	gravity
4	Tank	40	11/12/2002	1:1000	TSBA	6	56	1.09	JP-8	gravity
5	Tank	40	11/12/2002	Neat	SDA	6	56	1.09	JP-8	gravity
6	Tank	40	11/12/2002	1:10	SDA	6	56	1.09	JP-8	gravity
7	Tank	40	11/12/2002	1:100	SDA	6	56	1.09	JP-8	gravity
8	Tank	40	11/12/2002	1:1000	SDA	6	56	1.09	JP-8	gravity
9	Tank	40	11/12/2002	Neat	BHIBLA	6	56	1.09	JP-8	gravity
10	Tank	40	11/12/2002	1:10	BHIBLA	6	56	1.09	JP-8	gravity
11	Tank	40	11/12/2002	1:100	BHIBLA	6	56	1.09	JP-8	gravity
12	Tank	40	11/12/2002	1:1000	BHIBLA	6	56	1.09	JP-8	gravity
13	Tank	34	11/12/2002	Neat	TSBA	6	56	1.09	JP-8	gravity
14	Tank	34	11/12/2002	1:10	TSBA	6	56	1.09	JP-8	gravity
15	Truck	34	11/12/2002	1:100	TSBA	6	56	1.09	JP-8	gravity
16	Truck	34	11/12/2002	1:1000	TSBA	6	56	1.09	JP-8	gravity
17	Truck	34	11/12/2002	Neat	SDA	6	56	1.09	JP-8	gravity
18	Truck	34	11/12/2002	1:10	SDA	6	56	1.09	JP-8	gravity
19	Truck	34	11/12/2002	1:100	SDA	6	56	1.09	JP-8	gravity
20	Truck	34	11/12/2002	1:1000	SDA	6	56	1.09	JP-8	gravity
21	Truck	34	11/12/2002	Neat	BHIBLA	6	56	1.09	JP-8	gravity
22	Truck	34	11/12/2002	1:10	BHIBLA	6	56	1.09	JP-8	gravity
23	Truck	34	11/12/2002	1:100	BHIBLA	6	56	1.09	JP-8	gravity
24	Truck	34	11/12/2002	1:1000	BHIBLA	6	56	1.09	JP-8	gravity
25	Truck	F-16	11/12/2002	Neat	TSBA	6	56	1.09	JP-8 +100	gravity
26	Truck	F-16	11/12/2002	1:10	TSBA	6	56	1.09	JP-8 +100	gravity
27	Aircraft	F-16	11/12/2002	1:100	TSBA	6	56	1.09	JP-8 +100	gravity
28	Aircraft	F-16	11/12/2002	1:1000	TSBA	6	56	1.09	JP-8 +100	gravity
29	Aircraft	F-16	11/12/2002	Neat	SDA	6	56	1.09	JP-8 +100	gravity
30	Aircraft	F-16	11/12/2002	1:10	SDA	6	56	1.09	JP-8 +100	gravity
31	Aircraft	F-16	11/12/2002	1:100	SDA	6	56	1.09	JP-8 +100	gravity
32	Aircraft	F-16	11/12/2002	1:1000	SDA	6	56	1.09	JP-8 +100	gravity
33	Aircraft	F-16	11/12/2002	Neat	BHIBLA	6	56	1.09	JP-8 +100	gravity
34	Aircraft	F-16	11/12/2002	1:10	BHIBLA	6	56	1.09	JP-8 +100	gravity
35	Aircraft	F-16	11/12/2002	1:100	BHIBLA	6	56	1.09	JP-8 +100	gravity
36	Aircraft	F-16	11/12/2002	1:1000	BHIBLA	6	56	1.09	JP-8 +100	gravity
37	Aircraft	40	11/12/2002	Neat	TSBA	6	56	1.09	JP-8	gravity
38	Aircraft	40	11/12/2002	1:10	TSBA	6	56	1.09	JP-8	gravity

— Indicates analysis omitted

		Sample Description					
#	Tank Composition	pH	Sample Color	General Description	Obvious Solids	TDS (mg/L)	Free Phase Water
1	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—
3	steel w/ epoxy lining	—	light yellow	clear	no	—	no
4	steel w/ epoxy lining	—	light yellow	clear	no	—	no
5	steel w/ epoxy lining	—	light yellow	clear	no	—	no
6	steel w/ epoxy lining	—	light yellow	clear	no	—	no
7	steel w/ epoxy lining	—	light yellow	clear	no	—	no
8	steel w/ epoxy lining	—	light yellow	clear	no	—	no
9	steel w/ epoxy lining	—	light yellow	clear	no	—	no
10	steel w/ epoxy lining	—	light yellow	clear	no	—	no
11	steel w/ epoxy lining	—	light yellow	clear	no	—	no
12	steel w/ epoxy lining	—	light yellow	clear	no	—	no
13	steel w/ epoxy lining	—	light yellow	clear	no	—	no
14	steel w/ epoxy lining	—	light yellow	clear	no	—	no
15	aluminum	6.3	light yellow	clear	no	—	yes
16	aluminum	6.3	light yellow	clear	no	—	yes
17	aluminum	6.3	light yellow	clear	no	—	yes
18	aluminum	6.3	light yellow	clear	no	—	yes
19	aluminum	6.3	light yellow	clear	no	—	yes
20	aluminum	6.3	light yellow	clear	no	—	yes
21	aluminum	6.3	light yellow	clear	no	—	yes
22	aluminum	6.3	light yellow	clear	no	—	yes
23	aluminum	6.3	light yellow	clear	no	—	yes
24	aluminum	6.3	light yellow	clear	no	—	yes
25	aluminum	6.3	light yellow	clear	no	—	yes
26	aluminum	6.3	light yellow	clear	no	—	yes
27	aluminum	—	light yellow	clear	yes	—	no
28	aluminum	—	light yellow	clear	yes	—	no
29	aluminum	—	light yellow	clear	yes	—	no
30	aluminum	—	light yellow	clear	yes	—	no
31	aluminum	—	light yellow	clear	yes	—	no
32	aluminum	—	light yellow	clear	yes	—	no
33	aluminum	—	light yellow	clear	yes	—	no
34	aluminum	—	light yellow	clear	yes	—	no
35	aluminum	—	light yellow	clear	yes	—	no
36	aluminum	—	light yellow	clear	yes	—	no
37	aluminum	—	light yellow	clear	yes	—	no
38	aluminum	—	light yellow	clear	yes	—	no

	Light Microscopy				Colony Description		Quantity Data		
#	Gram +/-	Organism Shape	Length of Cells (μm)	Width of Cells (μm)	Colony Type	Colony Color	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours
1	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	0	0	0
4	—	—	—	—	—	—	0	0	0
5	—	varied	varied	varied	round	white	0	6	0
6	—	—	—	—	—	—	0	0	0
7	—	—	—	—	—	—	0	0	0
8	—	—	—	—	—	—	0	0	0
9	—	—	—	—	—	—	0	0	0
10	—	—	—	—	—	—	0	0	0
11	—	—	—	—	—	—	0	0	0
12	—	varied	varied	varied	irregular	white	0	185	0
13	—	—	—	—	—	—	0	0	0
14	—	—	—	—	—	—	0	0	0
15	—	—	—	—	—	—	0	0	0
16	—	—	—	—	—	—	0	0	0
17	—	—	—	—	—	—	0	0	0
18	—	—	—	—	—	—	0	0	0
19	—	—	—	—	—	—	0	0	0
20	—	—	—	—	—	—	0	0	0
21	—	—	—	—	—	—	0	0	0
22	—	—	—	—	—	—	0	0	0
23	—	—	—	—	—	—	0	0	0
24	—	—	—	—	—	—	0	0	0
25	—	—	—	—	—	—	0	0	0
26	—	varied	varied	varied	irregular	white	0	98	0
27	—	—	—	—	—	—	0	0	0
28	—	—	—	—	—	—	0	0	0
29	—	—	—	—	—	—	0	0	0
30	—	—	—	—	—	—	0	0	0
31	—	—	—	—	—	—	0	0	0
32	—	—	—	—	—	—	0	0	0
33	—	—	—	—	—	—	0	0	0
34	—	—	—	—	—	—	0	0	0
35	—	—	—	—	—	—	0	0	0
36	—	—	—	—	—	—	0	0	0
37	—	—	—	—	—	—	0	0	0
38	—	—	—	—	—	—	0	0	0

				Identification Data				
				FAME analysis			DNA gene sequence	
#	Bacteria vs. Fungi	Spores	Digital Photo	Sim Index	> .1 S. I. Separation	GC Best Match	DNA % ID	DNA Best Match
1	—	—	—	—	—	Library match not attempted	—	—
2	—	—	—	—	—	Library match not attempted	—	—
3	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—
5	bacteria	no	HIFTA100TSBA	—	—	—	—	—
6	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—
12	bacteria	no	HIFTA10BHI	—	—	No match found	—	—
13	—	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—	—
18	—	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—
20	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—
22	—	—	—	—	—	—	—	—
23	—	—	—	—	—	—	—	—
24	—	—	—	—	—	—	—	—
25	—	—	—	—	—	—	—	—
26	bacteria	no	HIFTR1000BHI	—	—	Library match not attempted	—	—
27	—	—	—	—	—	—	—	—
28	—	—	—	—	—	—	—	—
29	—	—	—	—	—	—	—	—
30	—	—	—	—	—	—	—	—
31	—	—	—	—	—	—	—	—
32	—	—	—	—	—	—	—	—
33	—	—	—	—	—	—	—	—
34	—	—	—	—	—	—	—	—
35	—	—	—	—	—	—	—	—
36	—	—	—	—	—	—	—	—
37	—	—	—	—	—	—	—	—
38	—	—	—	—	—	—	—	—

Appendix K: Worksheet / Sample Data – Wright-Patterson AFB (KFFO)

	Site Information									
#	Sample	Type / Number	Date Sampled	Dilution	Medium	Temp °C	% Relative Humidity	Inches Rainfall (last 30 days)	Fuel Type	Sample Method
1	Neg Control	—	—	—	—	—	—	—	—	—
2	Pos Control	—	—	—	—	—	—	—	—	—
3	Tank 1	F/S 1	11/9/2002	Neat	TSBA	6	97	3.1	JP-8	gravity
4	Tank 1	F/S 1	11/9/2002	1:10	TSBA	6	97	3.1	JP-8	gravity
5	Tank 1	F/S 1	11/9/2002	1:100	TSBA	6	97	3.1	JP-8	gravity
6	Tank 1	F/S 1	11/9/2002	1:1000	TSBA	6	97	3.1	JP-8	gravity
7	Tank 1	F/S 1	11/9/2002	Neat	SDA	6	97	3.1	JP-8	gravity
8	Tank 1	F/S 1	11/9/2002	1:10	SDA	6	97	3.1	JP-8	gravity
9	Tank 1	F/S 1	11/9/2002	1:100	SDA	6	97	3.1	JP-8	gravity
10	Tank 1	F/S 1	11/9/2002	1:1000	SDA	6	97	3.1	JP-8	gravity
11	Tank 1	F/S 1	11/9/2002	Neat	BHIBLA	6	97	3.1	JP-8	gravity
12	Tank 1	F/S 1	11/9/2002	1:10	BHIBLA	6	97	3.1	JP-8	gravity
13	Tank 1	F/S 1	11/9/2002	1:100	BHIBLA	6	97	3.1	JP-8	gravity
14	Tank 1	F/S 1	11/9/2002	1:1000	BHIBLA	6	97	3.1	JP-8	gravity
15	Tank 2	F/S 2	11/9/2002	Neat	TSBA	6	97	3.1	JP-8	gravity
16	Tank 2	F/S 2	11/9/2002	1:10	TSBA	6	97	3.1	JP-8	gravity
17	Tank 2	F/S 2	11/9/2002	1:100	TSBA	6	97	3.1	JP-8	gravity
18	Tank 2	F/S 2	11/9/2002	1:1000	TSBA	6	97	3.1	JP-8	gravity
19	Tank 2	F/S 2	11/9/2002	Neat	SDA	6	97	3.1	JP-8	gravity
20	Tank 2	F/S 2	11/9/2002	1:10	SDA	6	97	3.1	JP-8	gravity
21	Tank 2	F/S 2	11/9/2002	1:100	SDA	6	97	3.1	JP-8	gravity
22	Tank 2	F/S 2	11/9/2002	1:1000	SDA	6	97	3.1	JP-8	gravity
23	Tank 2	F/S 2	11/9/2002	Neat	BHIBLA	6	97	3.1	JP-8	gravity
24	Tank 2	F/S 2	11/9/2002	1:10	BHIBLA	6	97	3.1	JP-8	gravity
25	Tank 2	F/S 2	11/9/2002	1:100	BHIBLA	6	97	3.1	JP-8	gravity
26	Tank 2	F/S 2	11/9/2002	1:1000	BHIBLA	6	97	3.1	JP-8	gravity
27	Tank 3	Unloading	11/9/2002	Neat	TSBA	6	97	3.1	JP-8	gravity
28	Tank 3	Unloading	11/9/2002	1:10	TSBA	6	97	3.1	JP-8	gravity
29	Tank 3	Unloading	11/9/2002	1:100	TSBA	6	97	3.1	JP-8	gravity
30	Tank 3	Unloading	11/9/2002	1:1000	TSBA	6	97	3.1	JP-8	gravity
31	Tank 3	Unloading	11/9/2002	Neat	SDA	6	97	3.1	JP-8	gravity
32	Tank 3	Unloading	11/9/2002	1:10	SDA	6	97	3.1	JP-8	gravity
33	Tank 3	Unloading	11/9/2002	1:100	SDA	6	97	3.1	JP-8	gravity
34	Tank 3	Unloading	11/9/2002	1:1000	SDA	6	97	3.1	JP-8	gravity
35	Tank 3	Unloading	11/9/2002	Neat	BHIBLA	6	97	3.1	JP-8	gravity
36	Tank 3	Unloading	11/9/2002	1:10	BHIBLA	6	97	3.1	JP-8	gravity
37	Tank 3	Unloading	11/9/2002	1:100	BHIBLA	6	97	3.1	JP-8	gravity
38	Tank 3	Unloading	11/9/2002	1:1000	BHIBLA	6	97	3.1	JP-8	gravity

— Indicates analysis omitted

	Site Information									
#	Sample	Type / Number	Date Sampled	Dilution	Medium	Temp °C	% Relative Humidity	Inches Rainfall (last 30 days)	Fuel Type	Sample Method
39	Tank 4	Bldg 159	9/1/2002	Neat	TSBA	28	63	3.53	JP-8	gravity
40	Tank 4	Bldg 159	9/1/2002	1:10	TSBA	28	63	3.53	JP-8	gravity
41	Tank 4	Bldg 159	9/1/2002	1:100	TSBA	28	63	3.53	JP-8	gravity
42	Tank 4	Bldg 159	9/1/2002	1:1000	TSBA	28	63	3.53	JP-8	gravity
43	Tank 4	Bldg 159	9/1/2002	Neat	SDA	28	63	3.53	JP-8	gravity
44	Tank 4	Bldg 159	9/1/2002	1:10	SDA	28	63	3.53	JP-8	gravity
45	Tank 4	Bldg 159	9/1/2002	1:100	SDA	28	63	3.53	JP-8	gravity
46	Tank 4	Bldg 159	9/1/2002	1:1000	SDA	28	63	3.53	JP-8	gravity
47	Tank 4	Bldg 159	9/1/2002	Neat	BHIBLA	28	63	3.53	JP-8	gravity
48	Tank 4	Bldg 159	9/1/2002	1:10	BHIBLA	28	63	3.53	JP-8	gravity
49	Tank 4	Bldg 159	9/1/2002	1:100	BHIBLA	28	63	3.53	JP-8	gravity
50	Tank 4	Bldg 159	9/1/2002	1:1000	BHIBLA	28	63	3.53	JP-8	gravity
51	Truck	821	11/22/2002	Neat	TSBA	-1	91	3.08	JP-8	gravity
52	Truck	821	11/22/2002	1:10	TSBA	-1	91	3.08	JP-8	gravity
53	Truck	821	11/22/2002	1:100	TSBA	-1	91	3.08	JP-8	gravity
54	Truck	821	11/22/2002	1:1000	TSBA	-1	91	3.08	JP-8	gravity
55	Truck	821	11/22/2002	Neat	SDA	-1	91	3.08	JP-8	gravity
56	Truck	821	11/22/2002	1:10	SDA	-1	91	3.08	JP-8	gravity
57	Truck	821	11/22/2002	1:100	SDA	-1	91	3.08	JP-8	gravity
58	Truck	821	11/22/2002	1:1000	SDA	-1	91	3.08	JP-8	gravity
59	Truck	821	11/22/2002	Neat	BHIBLA	-1	91	3.08	JP-8	gravity
60	Truck	821	11/22/2002	1:10	BHIBLA	-1	91	3.08	JP-8	gravity
61	Truck	821	11/22/2002	1:100	BHIBLA	-1	91	3.08	JP-8	gravity
62	Truck	821	11/22/2002	1:1000	BHIBLA	-1	91	3.08	JP-8	gravity
63	Aircraft	134	11/22/2002	Neat	TSBA	-1	91	3.08	JP-8	gravity
64	Aircraft	134	11/22/2002	1:10	TSBA	-1	91	3.08	JP-8	gravity
65	Aircraft	134	11/22/2002	1:100	TSBA	-1	91	3.08	JP-8	gravity
66	Aircraft	134	11/22/2002	1:1000	TSBA	-1	91	3.08	JP-8	gravity
67	Aircraft	134	11/22/2002	Neat	SDA	-1	91	3.08	JP-8	gravity
68	Aircraft	134	11/22/2002	1:10	SDA	-1	91	3.08	JP-8	gravity
69	Aircraft	134	11/22/2002	1:100	SDA	-1	91	3.08	JP-8	gravity
70	Aircraft	134	11/22/2002	1:1000	SDA	-1	91	3.08	JP-8	gravity
71	Aircraft	134	11/22/2002	Neat	BHIBLA	-1	91	3.08	JP-8	gravity
72	Aircraft	134	11/22/2002	1:10	BHIBLA	-1	91	3.08	JP-8	gravity
73	Aircraft	134	11/22/2002	1:100	BHIBLA	-1	91	3.08	JP-8	gravity
74	Aircraft	134	11/22/2002	1:1000	BHIBLA	-1	91	3.08	JP-8	gravity

— Indicates analysis omitted

		Sample Description					
#	Tank Composition	pH	Sample Color	General Description	Obvious Solids	TDS (mg/L)	Free Phase Water
1	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—
3	steel w/ epoxy lining	6.0	dark yellow	turbid	yes	80	yes
4	steel w/ epoxy lining	6.0	dark yellow	turbid	yes	80	yes
5	steel w/ epoxy lining	6.0	dark yellow	turbid	yes	80	yes
6	steel w/ epoxy lining	6.0	dark yellow	turbid	yes	80	yes
7	steel w/ epoxy lining	6.0	dark yellow	turbid	yes	80	yes
8	steel w/ epoxy lining	6.0	dark yellow	turbid	yes	80	yes
9	steel w/ epoxy lining	6.0	dark yellow	turbid	yes	80	yes
10	steel w/ epoxy lining	6.0	dark yellow	turbid	yes	80	yes
11	steel w/ epoxy lining	6.0	dark yellow	turbid	yes	80	yes
12	steel w/ epoxy lining	6.0	dark yellow	turbid	yes	80	yes
13	steel w/ epoxy lining	6.0	dark yellow	turbid	yes	80	yes
14	steel w/ epoxy lining	6.0	dark yellow	turbid	yes	80	yes
15	steel w/ epoxy lining	6.2	dark yellow	turbid	yes	140	yes
16	steel w/ epoxy lining	6.2	dark yellow	turbid	yes	140	yes
17	steel w/ epoxy lining	6.2	dark yellow	turbid	yes	140	yes
18	steel w/ epoxy lining	6.2	dark yellow	turbid	yes	140	yes
19	steel w/ epoxy lining	6.2	dark yellow	turbid	yes	140	yes
20	steel w/ epoxy lining	6.2	dark yellow	turbid	yes	140	yes
21	steel w/ epoxy lining	6.2	dark yellow	turbid	yes	140	yes
22	steel w/ epoxy lining	6.2	dark yellow	turbid	yes	140	yes
23	steel w/ epoxy lining	6.2	dark yellow	turbid	yes	140	yes
24	steel w/ epoxy lining	6.2	dark yellow	turbid	yes	140	yes
25	steel w/ epoxy lining	6.2	dark yellow	turbid	yes	140	yes
26	steel w/ epoxy lining	6.2	dark yellow	turbid	yes	140	yes
27	steel w/ epoxy lining	5.6	dark yellow	turbid	yes	240	yes
28	steel w/ epoxy lining	5.6	dark yellow	turbid	yes	240	yes
29	steel w/ epoxy lining	5.6	dark yellow	turbid	yes	240	yes
30	steel w/ epoxy lining	5.6	dark yellow	turbid	yes	240	yes
31	steel w/ epoxy lining	5.6	dark yellow	turbid	yes	240	yes
32	steel w/ epoxy lining	5.6	dark yellow	turbid	yes	240	yes
33	steel w/ epoxy lining	5.6	dark yellow	turbid	yes	240	yes
34	steel w/ epoxy lining	5.6	dark yellow	turbid	yes	240	yes
35	steel w/ epoxy lining	5.6	dark yellow	turbid	yes	240	yes
36	steel w/ epoxy lining	5.6	dark yellow	turbid	yes	240	yes
37	steel w/ epoxy lining	5.6	dark yellow	turbid	yes	240	yes
38	steel w/ epoxy lining	5.6	dark yellow	turbid	yes	240	yes

		Sample Description					
#	Tank Composition	pH	Sample Color	General Description	Obvious Solids	TDS (mg/L)	Free Phase Water
39	steel w/ epoxy lining	5.4	dark yellow	turbid	yes	410	yes
40	steel w/ epoxy lining	5.4	dark yellow	turbid	yes	410	yes
41	steel w/ epoxy lining	5.4	dark yellow	turbid	yes	410	yes
42	steel w/ epoxy lining	5.4	dark yellow	turbid	yes	410	yes
43	steel w/ epoxy lining	5.4	dark yellow	turbid	yes	410	yes
44	steel w/ epoxy lining	5.4	dark yellow	turbid	yes	410	yes
45	steel w/ epoxy lining	5.4	dark yellow	turbid	yes	410	yes
46	steel w/ epoxy lining	5.4	dark yellow	turbid	yes	410	yes
47	steel w/ epoxy lining	5.4	dark yellow	turbid	yes	410	yes
48	steel w/ epoxy lining	5.4	dark yellow	turbid	yes	410	yes
49	steel w/ epoxy lining	5.4	dark yellow	turbid	yes	410	yes
50	steel w/ epoxy lining	5.4	dark yellow	turbid	yes	410	yes
51	steel w/ epoxy lining	6.6	light yellow	clear	yes	—	yes
52	steel w/ epoxy lining	6.6	light yellow	clear	yes	—	yes
53	steel w/ epoxy lining	6.6	light yellow	clear	yes	—	yes
54	steel w/ epoxy lining	6.6	light yellow	clear	yes	—	yes
55	steel w/ epoxy lining	6.6	light yellow	clear	yes	—	yes
56	steel w/ epoxy lining	6.6	light yellow	clear	yes	—	yes
57	steel w/ epoxy lining	6.6	light yellow	clear	yes	—	yes
58	steel w/ epoxy lining	6.6	light yellow	clear	yes	—	yes
59	steel w/ epoxy lining	6.6	light yellow	clear	yes	—	yes
60	steel w/ epoxy lining	6.6	light yellow	clear	yes	—	yes
61	steel w/ epoxy lining	6.6	light yellow	clear	yes	—	yes
62	steel w/ epoxy lining	6.6	light yellow	clear	yes	—	yes
63	aluminum	—	light yellow	clear	no	—	no
64	aluminum	—	light yellow	clear	no	—	no
65	aluminum	—	light yellow	clear	no	—	no
66	aluminum	—	light yellow	clear	no	—	no
67	aluminum	—	light yellow	clear	no	—	no
68	aluminum	—	light yellow	clear	no	—	no
69	aluminum	—	light yellow	clear	no	—	no
70	aluminum	—	light yellow	clear	no	—	no
71	aluminum	—	light yellow	clear	no	—	no
72	aluminum	—	light yellow	clear	no	—	no
73	aluminum	—	light yellow	clear	no	—	no
74	aluminum	—	light yellow	clear	no	—	no

	Light Microscopy				Colony Description		Quantity Data		
#	Gram +/-	Organism Shape	Length of Cells (μm)	Width of Cells (μm)	Colony Type	Colony Color	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours
1	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	0	0	0
4	—	—	—	—	—	—	0	0	0
5	—	—	—	—	—	—	0	0	0
6	—	—	—	—	—	—	0	0	0
7	—	—	—	—	—	—	0	0	0
8	—	—	—	—	—	—	0	0	0
9	—	—	—	—	—	—	0	0	0
10	—	—	—	—	—	—	0	0	0
11	—	—	—	—	—	—	0	0	0
12	neg	bacillus	1	0.5	irregular	white	4	6	400
13	neg	bacillus	1	0.5	irregular	white	102	180	102,000
14	—	varied	varied	varied	irregular	white	49	61	490,000
15	—	—	—	—	—	—	0	0	0
16	—	—	—	—	—	—	0	0	0
17	—	—	—	—	—	—	0	0	0
18	—	—	—	—	—	—	0	0	0
19	—	—	—	—	—	—	0	0	0
20	—	—	—	—	—	—	0	0	0
21	—	—	—	—	—	—	0	0	0
22	—	—	—	—	—	—	0	0	0
23	—	—	—	—	—	—	0	0	0
24	pos	bacillus	1	0.5	irregular	white	5	7	500
25	pos	bacillus	1	0.5	irregular	white	19	26	19,000
26	pos	bacillus	1	0.5	irregular	white	35	42	350,000
27	neg	bacillus	1	0.5	round	white	1	2	10
28	neg	bacillus	1	0.5	round	white	1	2	100
29	—	—	—	—	—	—	0	0	0
30	—	—	—	—	—	—	0	0	0
31	—	—	—	—	—	—	0	0	0
32	—	—	—	—	—	—	0	0	0
33	—	—	—	—	—	—	0	0	0
34	—	—	—	—	—	—	0	0	0
35	—	—	—	—	—	—	0	0	0
36	neg	bacillus	1	0.5	irregular	white	107	245	10,700
37	—	—	—	—	—	—	0	0	0
38	neg	bacillus	1	0.5	irregular	white	190	295	1,900,000

	Light Microscopy				Colony Description		Quantity Data		
#	Gram +/-	Organism Shape	Length of Cells (μm)	Width of Cells (μm)	Colony Type	Colony Color	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours
39	—	—	—	—	—	—	0	0	0
40	—	varied	varied	varied	round	white	1	2	100
41	—	—	—	—	—	—	0	0	0
42	—	—	—	—	—	—	0	0	0
43	—	—	—	—	—	—	0	0	0
44	—	—	—	—	—	—	0	0	0
45	—	—	—	—	—	—	0	0	0
46	—	—	—	—	—	—	0	0	0
47	—	—	—	—	—	—	0	0	0
48	—	—	—	—	—	—	0	0	0
49	—	varied	varied	varied	irregular	white	95	148	950,000
50	—	—	—	—	—	—	0	0	0
51	—	—	—	—	—	—	0	0	0
52	—	—	—	—	—	—	0	0	0
53	—	—	—	—	—	—	0	0	0
54	—	—	—	—	—	—	0	0	0
55	—	—	—	—	—	—	0	0	0
56	—	—	—	—	—	—	0	0	0
57	—	—	—	—	—	—	0	0	0
58	—	—	—	—	—	—	0	0	0
59	—	—	—	—	—	—	0	0	0
60	pos	bacillus	1	0.5	irregular	white	110	204	11,000
61	—	—	—	—	—	—	0	0	0
62	—	—	—	—	—	—	0	0	0
63	—	—	—	—	—	—	0	0	0
64	—	—	—	—	—	—	0	0	0
65	—	—	—	—	—	—	0	0	0
66	—	—	—	—	—	—	0	0	0
67	—	—	—	—	—	—	0	0	0
68	—	—	—	—	—	—	0	0	0
69	—	—	—	—	—	—	0	0	0
70	—	—	—	—	—	—	0	0	0
71	—	varied	varied	varied	irregular	white	32	51	320
72	—	varied	varied	varied	irregular	white	45	62	4,500
73	—	—	—	—	—	—	0	0	0
74	—	—	—	—	—	—	0	0	0

				Identification Data				
				FAME analysis			**DNA gene sequence**	
#	**Bacteria vs. Fungi**	**Spores**	**Digital Photo**	**Sim Index**	**> .1 S. I. Separation**	**GC Best Match**	**DNA % ID**	**DNA Best Match**
1	—	—	—	—	—	Library match not attempted	—	—
2	—	—	—	0.491	yes	*Stenotrophomonas-maltophilia*	—	—
3	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—
12	bacteria	no	FFOTan110BHI	—	—	Library match not attempted	—	—
13	bacteria	no	FFOTan1100BHI	—	—	No match found	—	—
14	fungi	no	FFOTan11000BHI	—	—	No match found	—	—
15	—	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—	—
18	—	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—
20	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—
22	—	—	—	—	—	—	—	—
23	—	—	—	—	—	—	—	—
24	bacteria	no	FFOTan210BHI	—	—	No match found	—	—
25	bacteria	no	FFOTan2100BHI	—	—	No match found	—	—
26	bacteria	no	FFOTan21000BHI	—	—	Library match not attempted	—	—
27	bacteria	no	FFOTan3NEATTSBA	0.435	yes	*Bacillus-cereus-GC subroup A*	—	—
28	bacteria	no	FFOTan310TSBA	0.897	yes	*Bacillus-licheniformis*	—	—
29	—	—	—	—	—	—	—	—
30	—	—	—	—	—	—	—	—
31	—	—	—	—	—	—	—	—
32	—	—	—	—	—	—	—	—
33	—	—	—	—	—	—	—	—
34	—	—	—	—	—	—	—	—
35	—	—	—	—	—	—	—	—
36	bacteria	no	FFOTan310BHI	—	—	No match found	—	—
37	—	—	—	—	—	—	—	—
38	bacteria	no	FFOTan31000BHI	—	—	No match found	—	—

				Identification Data				
				FAME analysis			**DNA gene sequence**	
#	**Bacteria vs. Fungi**	**Spores**	**Digital Photo**	**Sim Index**	**> .1 S. I. Separation**	**GC Best Match**	**DNA % ID**	**DNA Best Match**
39	—	—	—	—	—	—	—	—
40	fungi	no	FFOTan410TSBA	0.253	yes	*Bacillus-cereus-GC subroup A*	—	—
41	—	—	—	—	—	—	—	—
42	—	—	—	—	—	—	—	—
43	—	—	—	—	—	—	—	—
44	—	—	—	—	—	—	—	—
45	—	—	—	—	—	—	—	—
46	—	—	—	—	—	—	—	—
47	—	—	—	—	—	—	—	—
48	—	—	—	—	—	—	—	—
49	fungi	no	FFOTan41000BHI	—	—	No match found	—	—
50	—	—	—	—	—	—	—	—
51	—	—	—	—	—	—	—	—
52	—	—	—	—	—	—	—	—
53	—	—	—	—	—	—	—	—
54	—	—	—	—	—	—	—	—
55	—	—	—	—	—	—	—	—
56	—	—	—	—	—	—	—	—
57	—	—	—	—	—	—	—	—
58	—	—	—	—	—	—	—	—
59	—	—	—	—	—	—	—	—
60	bacteria	no	FFOTR10BHI	—	—	No match found	—	—
61	—	—	—	—	—	—	—	—
62	—	—	—	—	—	—	—	—
63	—	—	—	—	—	—	—	—
64	—	—	—	—	—	—	—	—
65	—	—	—	—	—	—	—	—
66	—	—	—	—	—	—	—	—
67	—	—	—	—	—	—	—	—
68	—	—	—	—	—	—	—	—
69	—	—	—	—	—	—	—	—
70	—	—	—	—	—	—	—	—
71	fungi	no	FFOACNEATBHI	—	—	No match found	—	—
72	fungi	no	FFOAC10BHI	—	—	Library match not attempted	—	—
73	—	—	—	—	—	—	—	—
74	—	—	—	—	—	—	—	—

E02C105.90A [1207] WP TANK 4 1:10 TSBA

Volume: DATA File: E02C105.90A Seq Counter: 9 ID Number: 1207
Type: Samp Bottle: 43 Method: TSBA40
Created: 12/10/02 5:33:16 PM
Sample ID: WP TANK 4 1:10 TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.462	2.704E+8	0.023	----	7.076	SOLVENT PEAK	----	< min rt	
1.544	20368	0.021	----	7.270		----	< min rt	
1.615	1593	0.023	----	7.438		----	< min rt	
2.201	548	0.026	----	8.829		----	< min rt	
3.054	625	0.028	1.177	10.607	11:0 ISO	0.29	ECL deviates 0.001	Reference -0.001
3.773	2334	0.025	1.110	11.607	12:0 ISO	1.02	ECL deviates -0.002	Reference -0.005
4.089	1590	0.026	1.088	12.002	12:0	0.68	ECL deviates 0.002	Reference -0.001
4.716	41982	0.028	1.055	12.613	13:0 ISO	17.36	ECL deviates -0.001	Reference -0.005
4.807	3560	0.028	1.050	12.702	13:0 ANTEISO	1.47	ECL deviates 0.000	Reference -0.004
5.884	14158	0.031	1.009	13.618	14:0 ISO	5.60	ECL deviates -0.001	Reference -0.005
6.365	12185	0.034	0.994	14.002	14:0	4.75	ECL deviates 0.002	Reference -0.002
7.044	487	0.035	0.977	14.475	Sum In Feature 1	0.19	ECL deviates -0.003	15:1 ISO I/13:0 3OH
7.173	430	0.030	----	14.565		----		
7.257	79632	0.034	0.972	14.624	15:0 ISO	30.34	ECL deviates 0.001	Reference -0.003
7.384	10497	0.035	0.969	14.713	15:0 ANTEISO	3.99	ECL deviates 0.000	Reference -0.005
7.660	661	0.033	0.963	14.905	15:1 w5c	0.25	ECL deviates 0.002	
7.798	1298	0.042	0.960	15.001	15:0	0.49	ECL deviates 0.001	Reference -0.003
7.985	636	0.042	0.957	15.120	14:0 ISO 3OH	0.24	ECL deviates 0.001	
8.177	1026	0.034	----	15.242		----		
8.403	3019	0.039	0.949	15.385	16:1 w7c alcohol	1.12	ECL deviates -0.002	
8.555	7324	0.039	0.947	15.482	Sum In Feature 2	2.72	ECL deviates 0.002	16:1 ISO I/14:0 3OH
8.779	10286	0.047	0.943	15.624	16:0 ISO	3.80	ECL deviates -0.003	Reference -0.008
8.989	690	0.039	0.940	15.757	16:1 w11c	0.25	ECL deviates 0.000	
9.084	953	0.037	0.938	15.817	Sum In Feature 3	0.35	ECL deviates -0.005	16:1 w7c/15 iso 2OH
9.149	22777	0.039	0.937	15.858	Sum In Feature 3	8.37	ECL deviates 0.006	15:0 ISO 2OH/16:1w7c
9.374	8196	0.038	0.934	16.001	16:0	3.00	ECL deviates 0.001	Reference -0.004
9.724	2411	0.039	0.929	16.213	15:0 2OH	0.88	ECL deviates -0.006	
10.010	7695	0.046	0.925	16.385	ISO 17:1 w10c	2.79	ECL deviates -0.003	
10.135	11612	0.046	0.924	16.461	ISO 17:1 w5c	4.20	ECL deviates 0.000	
10.267	1707	0.043	0.922	16.540	17:1 ANTEISO A	0.62	ECL deviates 0.000	
10.417	13060	0.041	0.920	16.630	17:0 ISO	4.71	ECL deviates 0.000	Reference -0.005
10.569	1508	0.042	0.918	16.722	17:0 ANTEISO	0.54	ECL deviates -0.001	Reference -0.006
13.604	5645	0.046	----	18.512		----		
13.768	370	0.037	----	18.608		----		
15.962	415	0.055	----	19.905		----		
16.528	264	0.060	----	20.240		----	> max rt	
16.805	293	0.103	----	20.404		----	> max rt	
17.037	1210	0.106	----	20.541		----	> max rt	
17.263	473	0.070	----	20.675		----	> max rt	
17.838	495	0.168	----	21.015		----	> max rt	
17.938	119	0.039	----	21.075		----	> max rt	
18.270	1752	0.030	----	21.271		----	> max rt	
----	487	---	----	----	Summed Feature 1	0.19	15:1 ISO H/13:0 3OH	13:0 3OH/15:1 i I/H
----	-----	---	----	----		----	15:1 ISO I/13:0 3OH	
----	7324	---	----	----	Summed Feature 2	2.72	12:0 ALDE ?	unknown 10.928
----	-----	---	----	----		----	16:1 ISO I/14:0 3OH	14:0 3OH/16:1 ISO I
----	23729	---	----	----	Summed Feature 3	8.72	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c

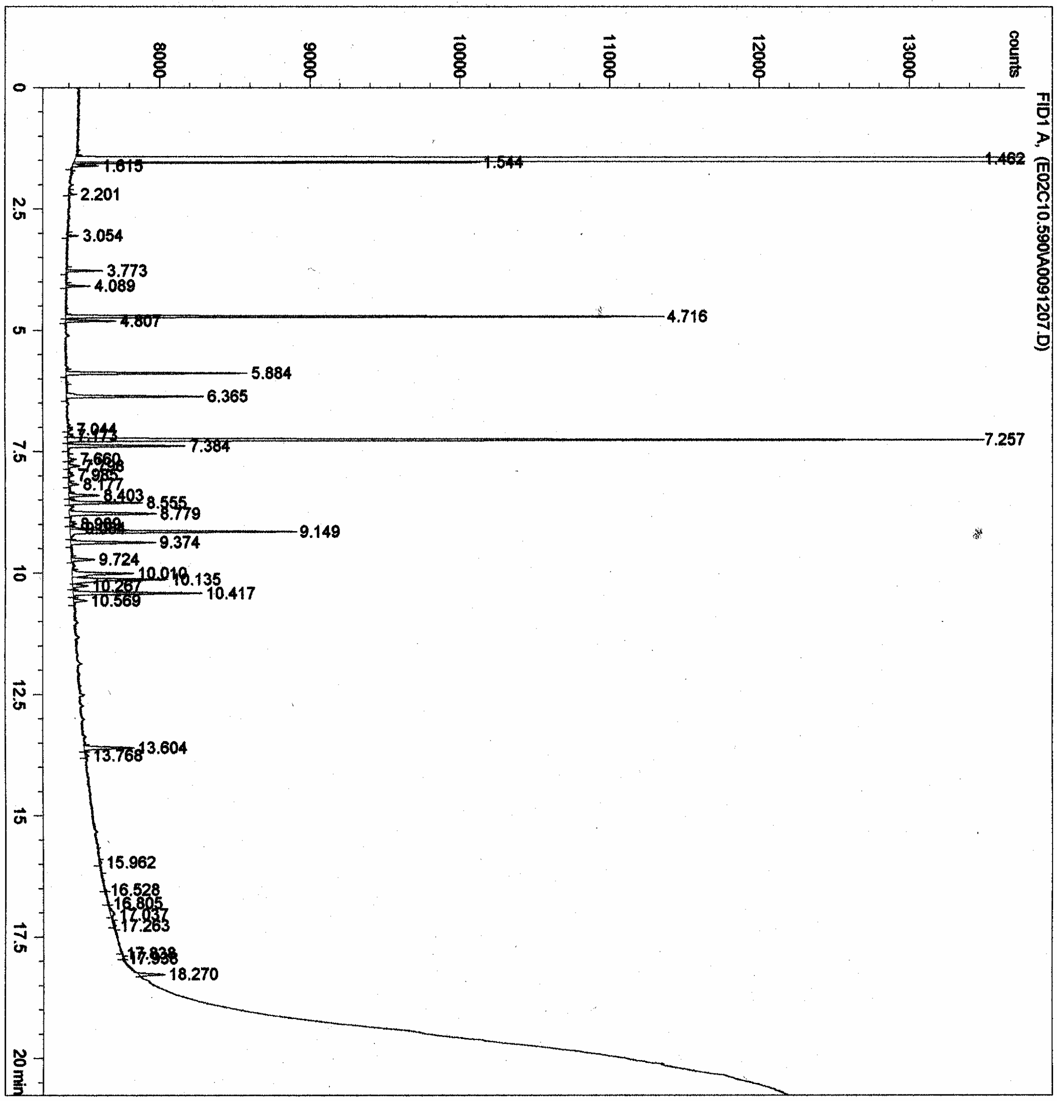
ECL Deviation: 0.002 Reference ECL Shift: 0.004 Number Reference Peaks: 14
Total Response: 268768 Total Named: 260883
Percent Named: 97.07% Total Amount: 255143

Matches:

Library	Sim Index	Entry Name
TSBA40 4.10	0.253	Bacillus-cereus-GC subgroup A*

```
Data File C:\SHERLOCK\RAW\E02C10.590\A0091207.D                              Sample Name:
    Sherlock Id: WP TANK 4 1:10 TSBA

    --------------------------------------------------------------------
    Injection Date  : 12/10/02 5:37:24 PM
    Sample Name     :  1207                          Location : Vial 43
    Acq. Operator   :
                                                  Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 12/10/02 5:33:16 PM
    ====================================================================
```



```
*** End of Report ***
```

E02C105.90A [1206] WP TANK 3 1:10 TSBA

Volume: DATA File: E02C105.90A Seq Counter: 8 ID Number: 1206
Type: Samp Bottle: 42 Method: TSBA40
Created: 12/10/02 5:07:41 PM
Sample ID: WP TANK 3 1:10 TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.462	2.737E+8	0.023	----	7.068	SOLVENT PEAK	----	< min rt	
1.549	3153	0.023	----	7.275		----	< min rt	
3.780	498	0.028	1.110	11.610	12:0 ISO	0.22	ECL deviates 0.001	Reference 0.005
5.884	2804	0.033	1.009	13.616	14:0 ISO	1.15	ECL deviates -0.003	Reference -0.006
6.364	1280	0.036	0.994	14.001	14:0	0.52	ECL deviates 0.001	Reference -0.003
7.257	80873	0.034	0.972	14.624	15:0 ISO	31.90	ECL deviates 0.001	Reference -0.004
7.387	84521	0.035	0.969	14.715	15:0 ANTEISO	33.24	ECL deviates 0.002	Reference -0.003
8.402	3646	0.038	0.949	15.385	16:1 w7c alcohol	1.40	ECL deviates -0.002	
8.783	10987	0.037	0.943	15.627	16:0 ISO	4.20	ECL deviates 0.000	Reference -0.005
8.987	6071	0.038	0.940	15.756	16:1 w11c	2.31	ECL deviates -0.001	
9.374	8543	0.038	0.934	16.001	16:0	3.24	ECL deviates 0.001	Reference -0.004
9.756	761	0.043	0.929	16.232	15:0 2OH	0.29	ECL deviates 0.013	
10.009	5403	0.041	0.925	16.384	ISO 17:1 w10c	2.03	ECL deviates -0.004	
10.158	4251	0.041	0.923	16.474	Sum In Feature 4	1.59	ECL deviates -0.002	17:1 ISO I/ANTEI B
10.417	20755	0.040	0.920	16.630	17:0 ISO	7.75	ECL deviates 0.000	Reference -0.005
10.571	27243	0.040	0.918	16.722	17:0 ANTEISO	10.15	ECL deviates -0.001	Reference -0.005
16.312	466	0.157	----	20.116		----	> max rt	
16.369	97	0.035	----	20.149		----	> max rt	
16.878	440	0.156	----	20.451		----	> max rt	
----	4251	---	----	----	Summed Feature 4	1.59	17:1 ISO I/ANTEI B	17:1 ANTEISO B/i I

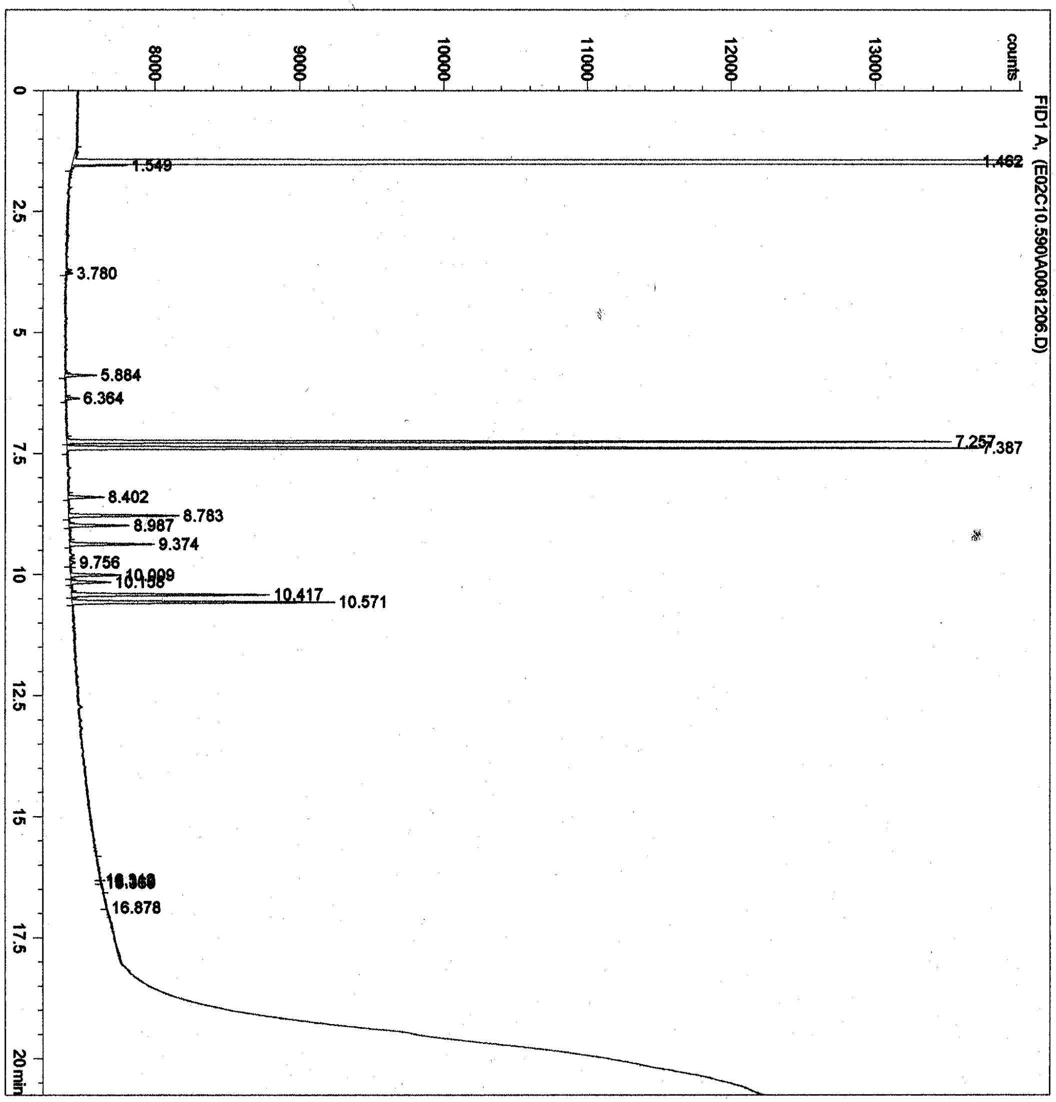
ECL Deviation: 0.004 Reference ECL Shift: 0.004 Number Reference Peaks: 9
Total Response: 257636 Total Named: 257636
Percent Named: 100.00% Total Amount: 246456

Matches:

Library	Sim Index	Entry Name
TSBA40 4.10	0.897	Bacillus-licheniformis* (Bacillus subtilis group)
	0.537	Bacillus-subtilis*
CLIN40 4.00	0.332	Bacillus-subtilis
	0.294	Staphylococcus-lentus*
	0.251	Bacillus-licheniformis

```
Data File C:\SHERLOCK\RAW\E02C10.590\A0081206.D                          Sample Name:
    Sherlock Id: WP TANK 3 1:10 TSBA

    ---------------------------------------------------------------------
    Injection Date  : 12/10/02 5:11:48 PM
    Sample Name     :  1206                        Location : Vial 42
    Acq. Operator   :
                                                 Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 12/10/02 5:07:42 PM
    =====================================================================
```



*** End of Report ***

E02C105.90A [1205] WP TANK 3 NEAT TSBA

Volume: DATA File: E02C105.90A Seq Counter: 7 ID Number: 1205
Type: Samp Bottle: 41 Method: TSBA40
Created: 12/10/02 4:42:03 PM
Sample ID: WP TANK 3 NEAT TSBA

Profile:

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.462	2.712E+8	0.023	----	7.076	SOLVENT PEAK	----	< min rt	
1.615	1043	0.024	----	7.439		----	< min rt	
2.203	541	0.029	----	8.832		----	< min rt	
3.054	440	0.023	1.177	10.606	11:0 ISO	0.23	ECL deviates 0.000	Reference -0.001
3.773	1690	0.027	1.110	11.607	12:0 ISO	0.84	ECL deviates -0.002	Reference -0.004
4.090	1195	0.026	1.088	12.003	12:0	0.58	ECL deviates 0.003	Reference 0.000
4.716	33403	0.028	1.055	12.613	13:0 ISO	15.69	ECL deviates -0.001	Reference -0.004
4.807	2427	0.028	1.050	12.702	13:0 ANTEISO	1.13	ECL deviates 0.000	Reference -0.003
5.884	10130	0.031	1.009	13.617	14:0 ISO	4.55	ECL deviates -0.002	Reference -0.005
6.365	9371	0.034	0.994	14.002	14:0	4.15	ECL deviates 0.002	Reference -0.002
7.257	72819	0.035	0.972	14.624	15:0 ISO	31.52	ECL deviates 0.001	Reference -0.003
7.385	8195	0.035	0.969	14.713	15:0 ANTEISO	3.54	ECL deviates 0.000	Reference -0.004
7.662	491	0.032	0.963	14.906	15:1 w5c	0.21	ECL deviates 0.003	
7.801	1055	0.040	0.960	15.003	15:0	0.45	ECL deviates 0.003	Reference -0.001
8.177	1071	0.039	----	15.241		----		
8.403	2869	0.041	0.949	15.385	16:1 w7c alcohol	1.21	ECL deviates -0.002	
8.556	5588	0.039	0.947	15.482	Sum In Feature 2	2.36	ECL deviates 0.002	16:1 ISO I/14:0 3OH
8.779	9089	0.049	0.943	15.624	16:0 ISO	3.82	ECL deviates -0.003	Reference -0.008
8.988	751	0.036	0.940	15.756	16:1 w11c	0.31	ECL deviates -0.001	
9.085	933	0.037	0.938	15.818	Sum In Feature 3	0.39	ECL deviates -0.004	16:1 w7c/15 iso 2OH
9.150	20659	0.040	0.937	15.858	Sum In Feature 3	8.62	ECL deviates 0.006	15:0 ISO 2OH/16:1w7c
9.375	7528	0.039	0.934	16.001	16:0	3.13	ECL deviates 0.001	Reference -0.003
9.725	2742	0.039	0.929	16.213	15:0 2OH	1.13	ECL deviates -0.006	
10.011	9567	0.046	0.925	16.385	ISO 17:1 w10c	3.94	ECL deviates -0.003	
10.136	12046	0.046	0.924	16.460	ISO 17:1 w5c	4.95	ECL deviates -0.001	
10.266	1338	0.044	0.922	16.539	17:1 ANTEISO A	0.55	ECL deviates -0.001	
10.417	14434	0.040	0.920	16.630	17:0 ISO	5.91	ECL deviates 0.000	Reference -0.005
10.569	1356	0.043	0.918	16.722	17:0 ANTEISO	0.55	ECL deviates -0.001	Reference -0.006
12.736	598	0.047	0.897	18.001	18:0	0.24	ECL deviates 0.001	Reference -0.005
13.605	3742	0.045	----	18.512		----		
17.024	879	0.100	----	20.533		----	> max rt	
17.263	360	0.058	----	20.674		----	> max rt	
17.618	383	0.114	----	20.884		----	> max rt	
17.702	154	0.052	----	20.934		----	> max rt	
17.951	526	0.135	----	21.081		----	> max rt	
18.271	1666	0.031	----	21.271		----	> max rt	
----	5588	---	----	----	Summed Feature 2	2.36	12:0 ALDE ?	unknown 10.928
----	-----	---	----	----		----	16:1 ISO I/14:0 3OH	14:0 3OH/16:1 ISO I
----	21593	---	----	----	Summed Feature 3	9.01	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c

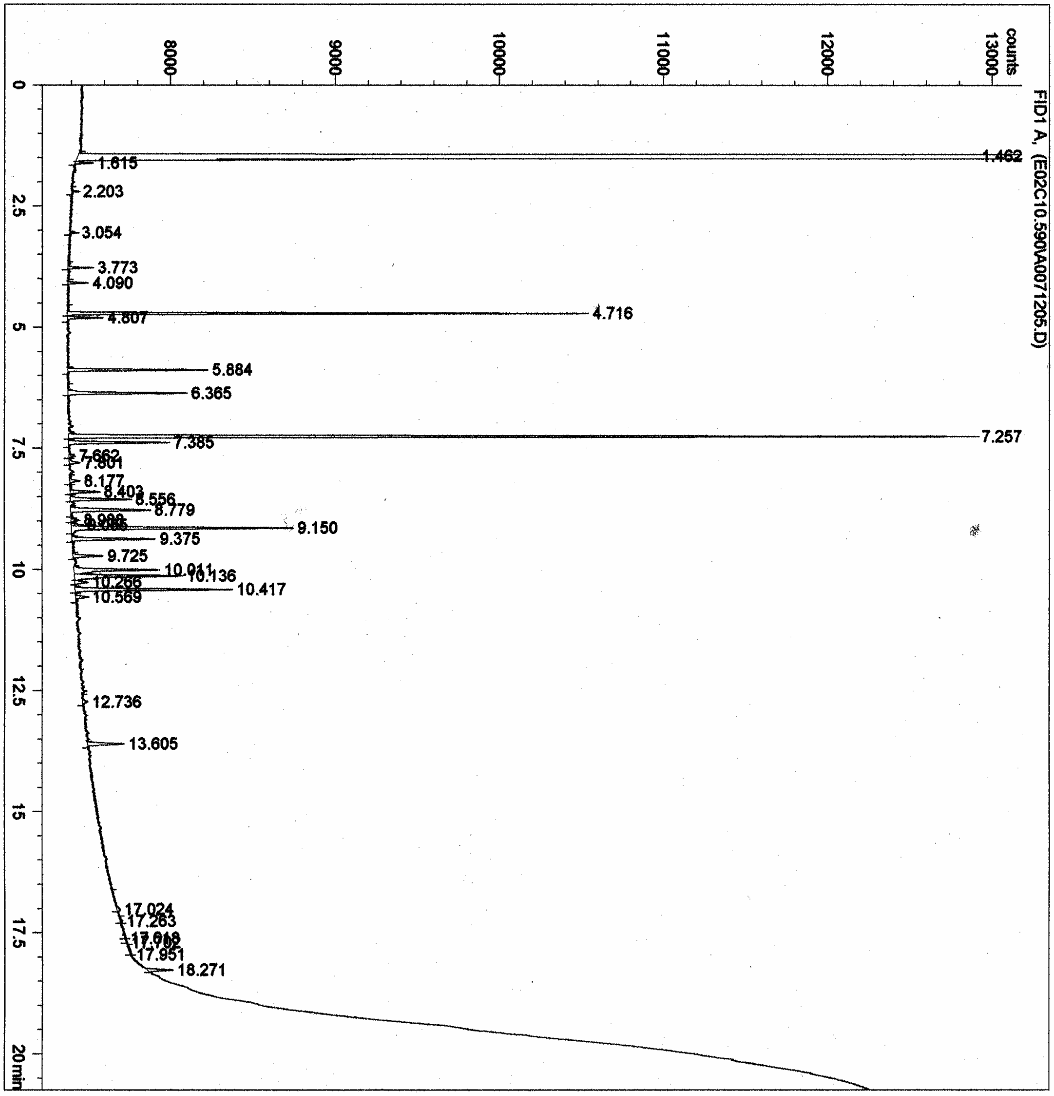
ECL Deviation: 0.003 Reference ECL Shift: 0.004 Number Reference Peaks: 15
Total Response: 235526 Total Named: 230713
Percent Named: 97.96% Total Amount: 224618

Matches:

Library	**Sim Index**	**Entry Name**
TSBA40 4.10	0.435	Bacillus-cereus-GC subgroup A*
	0.250	Bacillus-mycoides-GC subgroup A (Bacillus cereus group)
	0.240	Bacillus-thuringiensis canadensis sv.**
	0.224	Bacillus-thuringiensis kenyae sv.**
CLIN40 4.00	0.178	Bacillus-mycoides

```
Data File C:\SHERLOCK\RAW\E02C10.590\A0071205.D                         Sample Name:
    Sherlock Id: WP TANK 3 NEAT TSBA

    ====================================================================
    Injection Date  : 12/10/02 4:46:13 PM
    Sample Name     :  1205                        Location : Vial 41
    Acq. Operator   :
                                                 Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 12/10/02 4:42:03 PM
    ====================================================================
```



```
*** End of Report ***
```

Appendix L: Worksheet / Sample Data – Moody AFB (KVAD)

	Site Information									
#	Sample	Type / Number	Date Sampled	Dilution	Medium	Temp °C	% Relative Humidity	Inches Rainfall (last 30 days)	Fuel Type	Sample Method
1	Neg Control	—	—	—	—	—	—	—	—	—
2	Pos Control	—	—	—	—	—	—	—	—	—
3	Tank	Tank 1	12/5/2002	Neat	TSBA	7	75	2.89	JP-8	gravity
4	Tank	Tank 1	12/5/2002	1:10	TSBA	7	75	2.89	JP-8	gravity
5	Tank	Tank 1	12/5/2002	1:100	TSBA	7	75	2.89	JP-8	gravity
6	Tank	Tank 1	12/5/2002	1:1000	TSBA	7	75	2.89	JP-8	gravity
7	Tank	Tank 1	12/5/2002	Neat	SDA	7	75	2.89	JP-8	gravity
8	Tank	Tank 1	12/5/2002	1:10	SDA	7	75	2.89	JP-8	gravity
9	Tank	Tank 1	12/5/2002	1:100	SDA	7	75	2.89	JP-8	gravity
10	Tank	Tank 1	12/5/2002	1:1000	SDA	7	75	2.89	JP-8	gravity
11	Tank	Tank 1	12/5/2002	Neat	BHIBLA	7	75	2.89	JP-8	gravity
12	Tank	Tank 1	12/5/2002	1:10	BHIBLA	7	75	2.89	JP-8	gravity
13	Tank	Tank 1	12/5/2002	1:100	BHIBLA	7	75	2.89	JP-8	gravity
14	Tank	Tank 1	12/5/2002	1:1000	BHIBLA	7	75	2.89	JP-8	gravity
15	Truck	39	12/5/2002	Neat	TSBA	7	75	2.89	JP-8	gravity
16	Truck	39	12/5/2002	1:10	TSBA	7	75	2.89	JP-8	gravity
17	Truck	39	12/5/2002	1:100	TSBA	7	75	2.89	JP-8	gravity
18	Truck	39	12/5/2002	1:1000	TSBA	7	75	2.89	JP-8	gravity
19	Truck	39	12/5/2002	Neat	SDA	7	75	2.89	JP-8	gravity
20	Truck	39	12/5/2002	1:10	SDA	7	75	2.89	JP-8	gravity
21	Truck	39	12/5/2002	1:100	SDA	7	75	2.89	JP-8	gravity
22	Truck	39	12/5/2002	1:1000	SDA	7	75	2.89	JP-8	gravity
23	Truck	39	12/5/2002	Neat	BHIBLA	7	75	2.89	JP-8	gravity
24	Truck	39	12/5/2002	1:10	BHIBLA	7	75	2.89	JP-8	gravity
25	Truck	39	12/5/2002	1:100	BHIBLA	7	75	2.89	JP-8	gravity
26	Truck	39	12/5/2002	1:1000	BHIBLA	7	75	2.89	JP-8	gravity
27	Aircraft	C-130	12/5/2002	Neat	TSBA	7	75	2.89	JP-8	gravity
28	Aircraft	C-130	12/5/2002	1:10	TSBA	7	75	2.89	JP-8	gravity
29	Aircraft	C-130	12/5/2002	1:100	TSBA	7	75	2.89	JP-8	gravity
30	Aircraft	C-130	12/5/2002	1:1000	TSBA	7	75	2.89	JP-8	gravity
31	Aircraft	C-130	12/5/2002	Neat	SDA	7	75	2.89	JP-8	gravity
32	Aircraft	C-130	12/5/2002	1:10	SDA	7	75	2.89	JP-8	gravity
33	Aircraft	C-130	12/5/2002	1:100	SDA	7	75	2.89	JP-8	gravity
34	Aircraft	C-130	12/5/2002	1:1000	SDA	7	75	2.89	JP-8	gravity
35	Aircraft	C-130	12/5/2002	Neat	BHIBLA	7	75	2.89	JP-8	gravity
36	Aircraft	C-130	12/5/2002	1:10	BHIBLA	7	75	2.89	JP-8	gravity
37	Aircraft	C-130	12/5/2002	1:100	BHIBLA	7	75	2.89	JP-8	gravity
38	Aircraft	C-130	12/5/2002	1:1000	BHIBLA	7	75	2.89	JP-8	gravity

— Indicates analysis omitted

		Sample Description					
#	Tank Composition	pH	Sample Color	General Description	Obvious Solids	TDS (mg/L)	Free Phase Water
1	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—
3	steel w/ epoxy lining	6.6	brown	turbid	yes	30	yes
4	steel w/ epoxy lining	6.6	brown	turbid	yes	30	yes
5	steel w/ epoxy lining	6.6	brown	turbid	yes	30	yes
6	steel w/ epoxy lining	6.6	brown	turbid	yes	30	yes
7	steel w/ epoxy lining	6.6	brown	turbid	yes	30	yes
8	steel w/ epoxy lining	6.6	brown	turbid	yes	30	yes
9	steel w/ epoxy lining	6.6	brown	turbid	yes	30	yes
10	steel w/ epoxy lining	6.6	brown	turbid	yes	30	yes
11	steel w/ epoxy lining	6.6	brown	turbid	yes	30	yes
12	steel w/ epoxy lining	6.6	brown	turbid	yes	30	yes
13	steel w/ epoxy lining	6.6	brown	turbid	yes	30	yes
14	steel w/ epoxy lining	6.6	brown	turbid	yes	30	yes
15	aluminum	7.2	light yellow	clear	no	—	yes
16	aluminum	7.2	light yellow	clear	no	—	yes
17	aluminum	7.2	light yellow	clear	no	—	yes
18	aluminum	7.2	light yellow	clear	no	—	yes
19	aluminum	7.2	light yellow	clear	no	—	yes
20	aluminum	7.2	light yellow	clear	no	—	yes
21	aluminum	7.2	light yellow	clear	no	—	yes
22	aluminum	7.2	light yellow	clear	no	—	yes
23	aluminum	7.2	light yellow	clear	no	—	yes
24	aluminum	7.2	light yellow	clear	no	—	yes
25	aluminum	7.2	light yellow	clear	no	—	yes
26	aluminum	7.2	light yellow	clear	no	—	yes
27	aluminum	—	dark yellow	clear	yes	—	no
28	aluminum	—	dark yellow	clear	yes	—	no
29	aluminum	—	dark yellow	clear	yes	—	no
30	aluminum	—	dark yellow	clear	yes	—	no
31	aluminum	—	dark yellow	clear	yes	—	no
32	aluminum	—	dark yellow	clear	yes	—	no
33	aluminum	—	dark yellow	clear	yes	—	no
34	aluminum	—	dark yellow	clear	yes	—	no
35	aluminum	—	dark yellow	clear	yes	—	no
36	aluminum	—	dark yellow	clear	yes	—	no
37	aluminum	—	dark yellow	clear	yes	—	no
38	aluminum	—	dark yellow	clear	yes	—	no

	Light Microscopy				Colony Description		Quantity Data		
#	Gram +/-	Organism Shape	Length of Cells (μm)	Width of Cells (μm)	Colony Type	Colony Color	Col/0.1 mL 24 hours	Col/0.1 mL 48 hours	Col/mL 24 hours
1	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—
3	neg	bacillus	1	0.5	round	white	13	32	130
4	—	—	—	—	—	—	0	0	0
5	—	—	—	—	—	—	0	0	0
6	—	—	—	—	—	—	0	0	0
7	—	—	—	—	—	—	0	0	0
8	neg	bacillus	1	0.5	round	white	1	1	100
9	—	—	—	—	—	—	0	0	0
10	—	—	—	—	—	—	0	0	0
11	neg	bacillus	1	0.5	round	white	78	920	780
12	neg	bacillus	1	0.5	round	white	2	34	200
13	—	—	—	—	—	—	0	0	0
14	—	—	—	—	—	—	0	0	0
15	—	—	—	—	—	—	0	0	0
16	—	—	—	—	—	—	0	0	0
17	—	—	—	—	—	—	0	0	0
18	—	—	—	—	—	—	0	0	0
19	—	—	—	—	—	—	0	0	0
20	—	—	—	—	—	—	0	0	0
21	—	—	—	—	—	—	0	0	0
22	—	—	—	—	—	—	0	0	0
23	—	—	—	—	—	—	0	0	0
24	—	—	—	—	—	—	0	0	0
25	—	—	—	—	—	—	0	0	0
26	—	—	—	—	—	—	0	0	0
27		varied	varied	varied	round	white	1	2	10
28	—	—	—	—	—	—	0	0	0
29	—	—	—	—	—	—	0	0	0
30	—	—	—	—	—	—	0	0	0
31	—	varied	varied	varied	irregular	white	1	1	10
32	—	—	—	—	—	—	0	0	0
33	—	—	—	—	—	—	0	0	0
34	—	—	—	—	—	—	0	0	0
35	neg	bacillus	1	0.5	round	white	3	3	30
36	—	—	—	—	—	—	0	0	0
37	—	—	—	—	—	—	0	0	0
38	—	—	—	—	—	—	0	0	0

				Identification Data				
				FAME analysis			DNA gene sequence	
#	**Bacteria vs. Fungi**	**Spores**	**Digital Photo**	**Sim Index**	**> .1 S. I. Separation**	**GC Best Match**	**DNA % ID**	**DNA Best Match**
1	—	—	—	—	—	Library match not attempted	—	—
2	—	—	—	—	—	No match found	—	—
3	bacteria	no	VADTANEATTSBA	0.012	yes	*Flavobacterium-johnsoniae*	—	—
4	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—
8	bacteria	no	VADTA10SDA	—	—	No match found	—	—
9	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—
11	bacteria	no	VADTANEATBHI	—	—	No match found	—	—
12	bacteria	no	VADTA10BHI	—	—	No match found	—	—
13	—	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—	—
18	—	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—
20	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—
22	—	—	—	—	—	—	—	—
23	—	—	—	—	—	—	—	—
24	—	—	—	—	—	—	—	—
25	—	—	—	—	—	—	—	—
26	—	—	—	—	—	—	—	—
27	fungi	no	VADACNEATTSBA	0.443	no	*Bacillus-megaterium-GC subroup A*	—	—
28	—	—	—	—	—	—	—	—
29	—	—	—	—	—	—	—	—
30	—	—	—	—	—	—	—	—
31	fungi	no	VADACNEATSDA	—	—	Library match not attempted	—	—
32	—	—	—	—	—	—	—	—
33	—	—	—	—	—	—	—	—
34	—	—	—	—	—	—	—	—
35	bacteria	no	VADACNEATBHI	—	—	No match found	—	—
36	—	—	—	—	—	—	—	—
37	—	—	—	—	—	—	—	—
38	—	—	—	—	—	—	—	—

E02C165.65A [1234] MOODY TANK NEAT TSBA

Volume: DATA File: E02C165.65A Seq Counter: 6 ID Number: 1234
Type: Samp Bottle: 71 Method: TSBA40
Created: 12/16/02 3:40:49 PM
Sample ID: MOODY TANK NEAT TSBA

Profile: ONE OR MORE NAMED PEAKS REJECTED. SEE COMMENTS IN THE REPORT.

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.550	2.648E+8	0.047	----	7.068	SOLVENT PEAK	----	< min rt	
3.403	435	0.030	1.135	10.906	Sum In Feature 2	0.29	ECL deviates -0.008	12:0 ALDE ?
4.293	696	0.033	1.077	12.001	12:0	0.44	ECL deviates 0.001	Reference 0.000
5.583	2499	0.035	1.024	13.182	12:0 2OH	1.50	ECL deviates 0.005	
6.140	912	0.037	1.007	13.616	14:0 ISO	0.54	ECL deviates -0.003	Reference -0.006
6.633	2120	0.037	0.993	14.001	14:0	1.24	ECL deviates 0.001	Reference -0.002
7.542	42140	0.037	0.972	14.623	15:0 ISO	24.07	ECL deviates 0.000	Reference -0.004
7.675	20366	0.037	0.969	14.714	15:0 ANTEISO	11.60	ECL deviates 0.001	Reference -0.003
8.427	2742	0.042	0.955	15.209	14:0 2OH	1.54	ECL deviates 0.006	
8.709	955	0.045	0.951	15.385	16:1 w7c alcohol	0.53	ECL deviates -0.002	
8.889	7249	0.040	0.948	15.497	Sum In Feature 2	4.04	ECL deviates 0.009	14:0 3OH/16:1 ISO I
9.097	1844	0.042	0.945	15.627	16:0 ISO	1.02	ECL deviates 0.000	Reference -0.004
9.307	1276	0.041	0.941	15.758	16:1 w11c	0.71	ECL deviates 0.001	
9.404	35464	0.040	0.940	15.819	Sum In Feature 3	19.58	ECL deviates -0.003	16:1 w7c/15 iso 2OH
9.696	36219	0.040	0.936	16.001	16:0	19.91	ECL deviates 0.001	Reference -0.004
10.341	2251	0.052	0.928	16.387	ISO 17:1 w10c	1.23	ECL deviates -0.001	
10.490	839	0.043	0.926	16.476	Sum In Feature 4	0.46	ECL deviates 0.000	17:1 ISO I/ANTEI B
10.748	3214	0.042	0.924	16.630	17:0 ISO	1.74	ECL deviates 0.000	Reference -0.005
10.905	3112	0.043	0.922	16.724	17:0 ANTEISO	1.69	ECL deviates 0.001	Reference -0.004
11.185	5419	0.043	0.919	16.891	17:0 CYCLO	2.93	ECL deviates 0.003	Reference -0.002
11.362	433	0.045	0.918	16.996	17:0	0.23	ECL deviates -0.004	Reference -0.009
12.782	7112	0.044	0.907	17.823	18:1 w7c	3.79	ECL deviates 0.000	
13.086	1753	0.047	0.906	18.000	18:0	0.93	ECL deviates 0.000	Reference -0.006
14.975	398	0.095	----	19.106		----		
15.264	216	0.083	----	19.276		----		
15.945	680	0.276	----	19.678		----	> max ar/ht	
16.219	682	0.129	0.900	19.839	20:1 w7c	----	> max ar/ht	
16.332	201	0.065	----	19.906		----		
16.784	502	0.164	----	20.172		----	> max rt	
17.108	680	0.185	----	20.363		----	> max rt	
17.226	322	0.100	----	20.432		----	> max rt	
17.509	514	0.111	----	20.599		----	> max rt	
17.652	283	0.083	----	20.683		----	> max rt	
17.728	163	0.054	----	20.728		----	> max rt	
17.782	134	0.033	----	20.760		----	> max rt	
17.929	144	0.067	----	20.846		----	> max rt	
----	7683	---	----	----	Summed Feature 2	4.33	12:0 ALDE ?	unknown 10.928
----	-----	---	----	----		----	16:1 ISO I/14:0 3OH	14:0 3OH/16:1 ISO I
----	35464	---	----	----	Summed Feature 3	19.58	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c
----	839	---	----	----	Summed Feature 4	0.46	17:1 ISO I/ANTEI B	17:1 ANTEISO B/i I

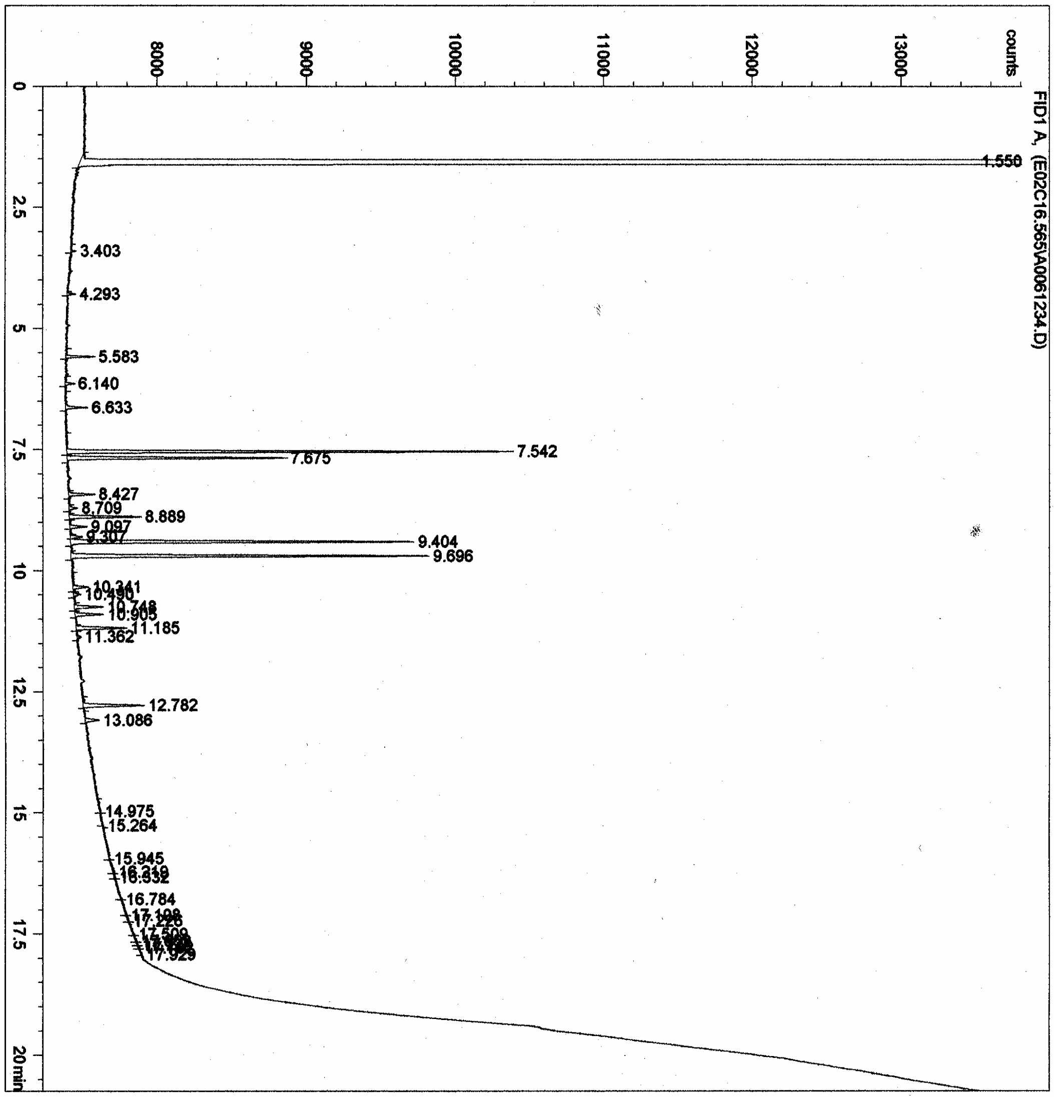
ECL Deviation: 0.004 Reference ECL Shift: 0.005 Number Reference Peaks: 12
Total Response: 181227 Total Named: 179050
Percent Named: 98.80% Total Amount: 170851
Profile: ONE OR MORE NAMED PEAKS REJECTED. SEE COMMENTS IN THE REPORT.

Matches:

Library	**Sim Index**	**Entry Name**
TSBA40 4.10	0.012	Flavobacterium-johnsoniae* (Cytophaga johnsonae)
CLIN40 4.00		(No Match)

```
Data File C:\SHERLOCK\RAW\E02C16.565\A0061234.D                          Sample Name:
    Sherlock Id: MOODY TANK NEAT TSBA

    =====================================================================
    Injection Date  : 12/16/02 3:45:09 PM
    Sample Name     :  1234                          Location : Vial 71
    Acq. Operator   :
                                                   Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 12/16/02 3:40:49 PM
    =====================================================================
```



*** End of Report ***

E02C165.65A [1235] MOODY AIRCRAFT NEAT TSBA

Volume: DATA File: E02C165.65A Seq Counter: 7 ID Number: 1235
Type: Samp Bottle: 72 Method: TSBA40
Created: 12/16/02 4:06:37 PM
Sample ID: MOODY AIRCRAFT NEAT TSBA

Profile: TOTAL RESPONSE LESS THAN 50000.0. CONCENTRATE AND RE-RUN.

RT	**Response**	**Ar/Ht**	**RFact**	**ECL**	**Peak Name**	**Percent**	**Comment1**	**Comment2**
1.551	2.613E+8	0.024	----	7.081	SOLVENT PEAK	----	< min rt	
4.937	515	0.035	1.048	12.614	13:0 ISO	2.05	ECL deviates 0.000	Reference -0.005
6.140	2818	0.033	1.007	13.618	14:0 ISO	10.79	ECL deviates -0.001	Reference -0.005
6.632	652	0.032	0.993	14.001	14:0	2.46	ECL deviates 0.001	Reference -0.003
7.542	11066	0.037	0.972	14.623	15:0 ISO	40.90	ECL deviates 0.000	Reference -0.004
7.673	10873	0.037	0.969	14.713	15:0 ANTEISO	40.07	ECL deviates 0.000	Reference -0.004
9.303	1044	0.039	0.941	15.754	16:1 w11c	3.74	ECL deviates -0.003	
15.215	288	0.085	----	19.242		----		
15.485	285	0.109	0.899	19.400	20:4 w6,9,12,15c	----	> max ar/ht	
15.692	253	0.075	----	19.522		----		
16.132	728	0.248	----	19.781		----	> max ar/ht	
16.324	156	0.069	----	19.893		----		
16.562	376	0.104	----	20.034		----	> max rt	
17.111	441	0.190	----	20.356		----	> max rt	
17.732	388	0.164	----	20.721		----	> max rt	

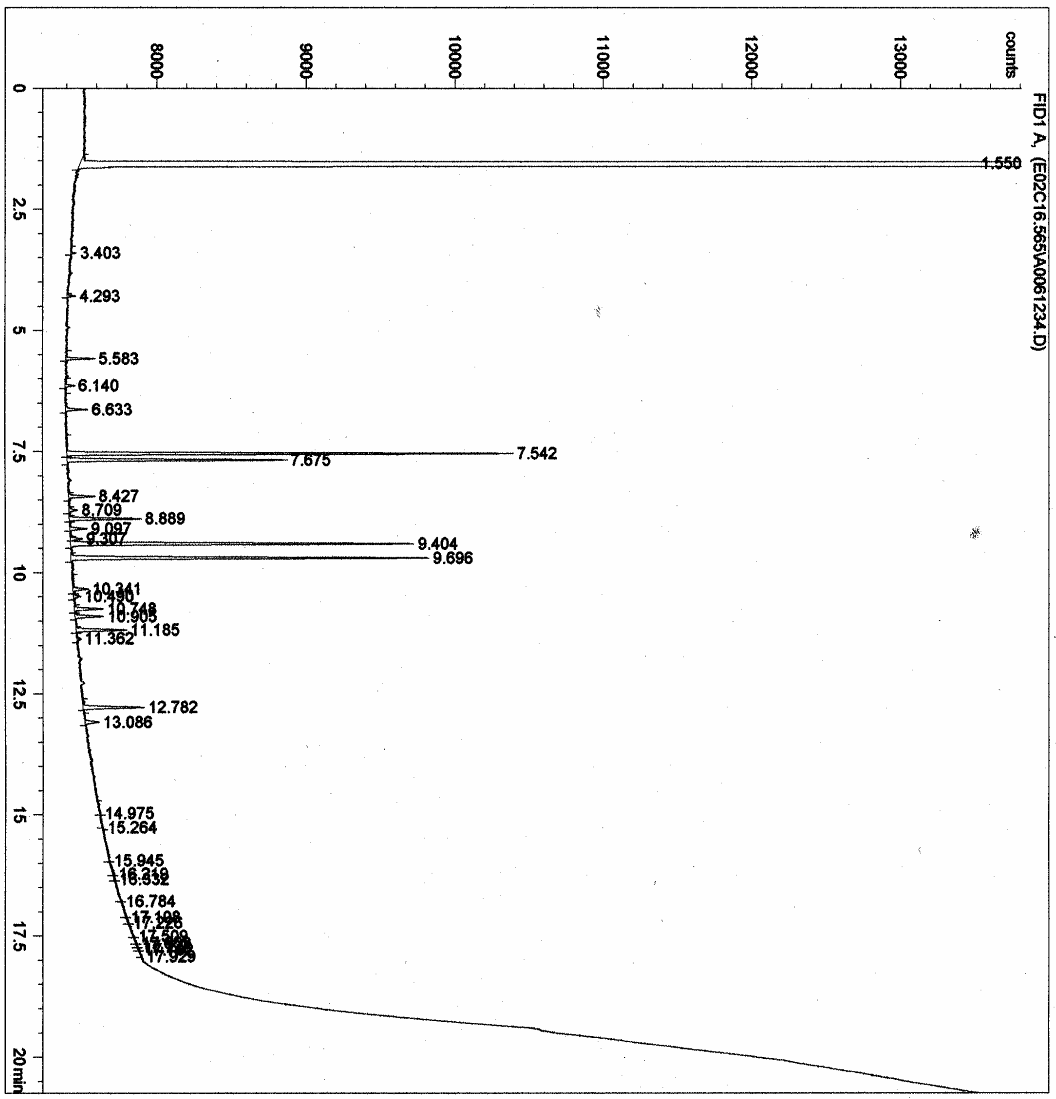
ECL Deviation: 0.002 Reference ECL Shift: 0.004 Number Reference Peaks: 5
Total Response: 28678 Total Named: 26967
Percent Named: 94.04% Total Amount: 26561
Profile: TOTAL RESPONSE LESS THAN 50000.0. CONCENTRATE AND RE-RUN.

Matches:

Library	**Sim Index**	**Entry Name**
TSBA40 4.10	0.443	Bacillus-megaterium-GC subgroup A
	0.392	Brevibacillus-laterosporus* (Bacillus)
	0.226	Brevibacillus-agri** (Bacillus)
CLIN40 4.00	0.289	Bacillus-megaterium

```
Data File C:\SHERLOCK\RAW\E02C16.565\A0061234.D                          Sample Name:
    Sherlock Id: MOODY TANK NEAT TSBA

    =====================================================================
    Injection Date  : 12/16/02 3:45:09 PM
    Sample Name     :  1234                          Location : Vial 71
    Acq. Operator   :
                                                   Inj Volume : 2 µl
    Method          : C:\HPCHEM\1\METHODS\$MIDI$A.M
    Last changed    : 12/16/02 3:40:49 PM
    =====================================================================
```



*** End of Report ***

Bibliography

Angeles-Chavez, C. and others. "New Strain of Anaerobic Bacteria and Its Association With Corrosion Pitting of X52 Pipeline Steel," *British Corrosion Journal* 36, No 4:292-296. 2001.

Angell, P. "Understanding Microbially Influenced Corrosion as Biofilm-Mediated Changes in Surface Chemistry," *Environmental Biotechnology* 10:269-272). 1999.

ASTM. "Standard Guide for Microbial Contamination in Fuels and Fuel Systems," The American Society for Testing and Materials, Designation D 6469-99, 1999.

Bacha, J. "Aviation Fuels Technical Review (FTR-3)," Chevron Products Company, 2000.

Bailey, C. A. and May, M. E. *Evaluation of Microbiological Test Kits for Hydrocarbon Fuel Systems*, Naval Research Laboratory, Washington, DC, February 1979.

Bakanauskas, S. *Bacterial Activity in JP-4 Fuel*, United States Department of Commerce, Project No. 7312. 1958.

Chesneau, H. L. "The Silent Fuel Killers (Stability and Microbiologicals)," *Proceedings of 2000 International Joint Power Generation Conference*. Miami Beach, Florida July 23-26, 2000.

Chevron Products Company. "Chevron Jet Fuel," Material Safety Data Sheet, MSDS No. 0513, Mar 2000.

Conidia Bioscience. *Hormoconis Resinae* in Aviation Kerosene. http://www.conidia.com/subfolder/science.html. 2002.

Del Rey Chemical Company. "Di Ethylene Glycol Monomethyl Ether," Material Safety Data Sheet, MSDS No. BNRSQ, Sep 1992.

Department of Defense. *Turbine Fuel, Aviation, Grades JP-4, JP-5, And JP-5/JP-8 ST.* Military Specification No. MIL-T-5624P. Washington: Government Printing Office, 1992.

de Schiapparelli, E. R. and de Meybaum, B. R. "Microbiological Corrosion in Terminal Storage Tanks for Aircraft Fuel," *National Association of Corrosion Engineers* 19:47-50. October 1980.

Echa Microbiological Ltd. *Testing Aircraft Fuel For Microorganisms – A Practical Guide To Using MicrobMonitor*^{2}, Cardiff UK, Technical Paper, 2002.

Ferrari, M. D. and others. “Occurrence of Heterotrophic Bacteria and Fungi In An Aviation Fuel Handling System And Its Relationship With Fuel Fouling,” *Revista Argentina de Microbiologia* 30:105-114. 1998.

Finefrock, V. H. and London, S. A. *Microbial Contamination of USAF JP-4 Fuels*, Technical Report AFAPL-TR-66-91 Aerospace Medical Research Laboratories. 1966.

Fox, A. and others. *Analytical Microbiology Methods: Chromatography and Mass Spectrometry*. New York: Plenum. 1990.

Gaylarde, C. C. and others. “Microbial Contamination of Stored Hydrocarbon Fuels and Its Control,” *Revista de Microbiologia* 30:01-10. 1999.

Geiss, K. T. and Frazier, J. M. “In Vitro Toxicities of Experimental Jet Fuel System Ice-Inhibiting Agents,” *The Science of the Total Environment* 274:209-218. 2001.

Gharaibeh, A. A. and Voorhees, K. J. “Characterization of Lipid Fatty Acids in Whole-Cell Microorganisms Using in Situ Supercritical Fluid Derivatization/Extraction and Gas Chromatography/Mass Spectrometry,” *Analytical Chemistry* 68:2805-2810. 1996.

Hucker, G. J. and Conn, H. J. *Methods of Gram Staining*, New York Agr. Exp Sta Technical Bulletin 93:1-37. 1927.

Johnson, K. J. and Synovec, R. E. “Pattern Recognition of Jet Fuels: Comprehensive GC x GC with ANOVA-Based Feature Selection and Principal Component Analysis,” *Chemometrics and Intelligent Laboratory Systems* 60:225-237. 2002.

Johnston, R. K. and others. *A Study of Existing Base Fuel Handling Procedures in Relation to Microbial Contamination*, Southwest Research Institute, San Antonio, Texas, Technical Documentary Report No. SEG TDR 64-29, AD 603852, June 1964.

Jung, C. M. and others. “Characterization of JP-7 Jet Fuel Degradation by the Bacterium *Nocardioides Luteus* Strain BAFB,” *Journal of Basic Microbiology* 42:127-131. 2002.

J. T. Baker Company. “Ethylene Glycol Monomethyl Ether,” Material Safety Data Sheet, MSDS No. E5300, Mar 2000.

Keresztes, Zs. and others. “The Influence of Biocides on the Microbiologically Influenced Corrosion of Mild Steel and Brass,” *Electrochimica Acta* 43, Nos. 1-2:77-85. 1998.

Mattie, D. R. and others. “Program Development in Military Toxicology Laboratories,” *Toxicology Letters* 79:291-295. 1995.

Maurice, L. Q. and others. “Advanced Aviation Fuels: A Look Ahead Via a Historical Perspective,” *Fuel* 80:747-756. 2001.

McClave, J. T. and others. *Statistics for Business and Economics* (8th Edition). NJ: Prentice Hall, Inc., 2001.

Merriam-Webster Online. *Merriam-Webster Collegiate Dictionary.* http://www.m-w.com/cgi-bin/dictionary?book=Dictionary&va=contaminates. 2002.

Meshako, C. E. and others. “Biodegradability and Microbial Toxicity of Aircraft Fuel System Icing Inhibitors,” *Environmental Toxicology* 14:383-390. 1999.

MIDI, Inc. *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*, Microbial Identification, Inc., Newark, DE, Technical Note #101. February 2001.

MIDI, Inc. “MIS Whole Cell Fatty Acid Analysis By Gas Chromatography,” Newark, DE, Training Manual. 2002.

MIDI, Inc. *Partial 16S rRNA Gene Alignment with GenBank,* Microbial Identification, Inc., Newark, DE, Alignment Report. 7 November 2002.

MIDI, Inc. *Report Interpretation Guide,* Microbial Identification, Inc., Newark, DE, Report Interpretation Guide. 2002.

MIS. “Sherlock® Microbial Identification System,” Version 4.0. Operating manual. 2001.

Mudry, T. Air Force Chemist, Air Force Petroleum Office, Aviano, Italy. Personal Correspondence. 26 October 2002.

Pardede, R. and Batts, B. D. “The Chemical Composition of a Jet Fuel Sediment Formed by Microbiological Action,” *Fuel* 75:1132-1138. 1996.

Spila, E. and others. “Determination of Organophosphate Contaminants in Jet Fuel,” *Journal of Chromatography* A 847:331-337. 1999.

Videla, H. “Microbially Induced Corrosion: an Updated Overview,” *International Biodeterioration & Biodegradation* 48:176-201. 2001.

Vita

Major Graef graduated in 1988 from the University of Missouri-Columbia where he received his Bachelor of Science degree in mathematics through the NROTC program.

After completion of The Basic School, Major Graef reported to Pensacola, Florida for flight training, being designated a Naval Flight Officer in April 1991. Following Electronic Warfare School, he then transferred to VAQ-129, NAS Whidbey Island, Washington, for training in the EA-6B Prowler as an Electronic Counter Measures Officer (ECMO).

In September 1992, Major Graef joined the "Seahawks" of VMAQ-4. His duties included the Director of Safety and Standardization, ECMO Training Officer, and maintenance division officer. He completed two Western Pacific deployments and one to NAS Sigonella, Italy in support of Operation DENY FLIGHT.

In December 1995, Major Graef transferred to Training Squadron EIGHTY-SIX, NAS Pensacola, Florida for duty as a flight instructor. While there, he received a Master's Degree in Management from Troy State University.

In 1999, Major Graef returned to MCAS Cherry Point, NC where he completed the Marine Corps Command and Staff College while assigned as the Electronic Warfare Officer of VMAQ-3 and the Executive Officer of Marine Wing Support Squadron 271.

In August 2001, he entered the Graduate School of Engineering and Management, Air Force Institute of Technology. Major Graef has accumulated over 2,300 flight hours and personal decorations include the Air Medal, the Navy and Marine Corps Commendation Medal with Combat "V" and two gold stars, and the Navy and Marine Corps Achievement Medal.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. REPORT DATE *(DD-MM-YYYY)*
25-03-2003

2. REPORT TYPE
Master's Thesis

3. DATES COVERED *(From – To)*
May 2002 – Mar 2003

4. TITLE AND SUBTITLE

AN ANALYSIS OF MICROBIAL CONTAMINATION IN MILITARY AVIATION FUEL SYSTEMS

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Graef, Harold W. Major, USMC

5d. PROJECT NUMBER
ENR # 2002-059

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES(S) AND ADDRESS(S)
Air Force Institute of Technology
Graduate School of Engineering and Environmental Management (AFIT/ENV)
2950 P Street, Building 640
WPAFB OH 45433-7765

8. PERFORMING ORGANIZATION REPORT NUMBER

AFIT/GEE/ENV/03-10

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AFRL/PRTG Bldg 490
Attn: Capt Michelle E. Rauch-Johnson
1790 Loop Road N DSN: 785-5345
WPAFB OH 45433 e-mail: michelle.rauch@wpafb.af.mil

10. SPONSOR/MONITOR'S ACRONYM(S)

AFRL/PRTG

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

APPROVED FOR PUBLIC RELEASE; DISTRIBUTION UNLIMITED.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Military aviation fuel systems can be an ideal environment for microorganisms. Microbial growth in hydrocarbon fuel systems arises because of the impracticality of keeping fuel tanks sterile and the inevitable presence of water from condensation. Microbial contaminants in aviation fuel systems are a concern because of their potential to degrade the fuel, accelerate tank corrosion, and threaten flight safety.

This research addresses the concern of using more environmentally friendly Fuel System Icing Inhibitors (FSII), which are also biocidal. Are significant levels of microorganisms growing in military aviation fuel systems, and if so, are there any common variables?

Forty aviation fuel samples were collected from fuel storage tanks (including flexible expeditionary fuel bladders), refueling trucks, and aircraft from 12 U.S. military bases. Samples were analyzed using peak naming and pattern recognition algorithms of sample extracts processed on a gas chromatograph.

Significant levels of microorganisms were found in military aviation fuel systems. 90% (36 of 40) of fuel samples produced microbial growth. Over 40% of the serial dilutions that produced microbial growth were characterized as moderately or heavily contaminated samples.

The microorganisms isolated were overwhelmingly Gram negative, anaerobic, bacilli with populations varying by orders of magnitude.

15. SUBJECT TERMS

Microbial identification, microbial contamination, Microbially Induced Corrosion (MIC), aviation fuel systems, Gas Chromatography (GC), ethylene glycol monomethyl ether (EGME).

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES
a. REPORT	b. ABSTRACT	c. THIS PAGE		
U	U	U	UU	236

19a. NAME OF RESPONSIBLE PERSON
Charles A. Bleckmann (ENV)

19b. TELEPHONE NUMBER *(Include area code)*
(937) 255-3636, ext 4721;
e-mail: Charles.Bleckmann@afit.edu