



Microbial Identification System

Version 6.2

MIS Operating Manual
September 2012



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Sherlock Software and Libraries

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Chapter 1

Getting Started

Overview

The Sherlock Microbial Identification System (MIS), developed and marketed by MIDI, Inc., Newark, DE, USA analyzes and identifies microorganisms isolated in pure culture on artificial media. Sherlock uses a sample preparation procedure and gas chromatography (GC) to yield qualitatively and quantitatively reproducible fatty acid composition profiles. This sophisticated chromatographic system was developed for microbiologists; thus, chromatographic experience is not essential for operation. The Sherlock software calibrates and monitors the system to ensure proper functioning.

Fatty acids extracted from unknown microorganisms are automatically quantified and identified by the Sherlock software to determine the fatty acid composition. The fatty acid profile is then compared to a “library” (of profiles of reference strains) stored in the computer, to determine the identity of the unknown.

Hardware and Software Installation

This manual is intended to contain all information necessary for successful operation of the Sherlock system. It is assumed that a technician, qualified by MIDI Inc., has properly installed and connected all the components of your chromatographic system. Specific hardware installation procedures are not included in this manual. Consult the Agilent manuals accompanying the individual components for more information involving operation of the hardware without use of the Sherlock MIS.

The MIDI Sherlock software package includes *Installation Instructions* that can be used as a guide through the installation process for the Sherlock software. The instructions are supplied in electronic form on the Sherlock installation CD-ROM. **Please see the insert found in the CD-ROM case for instructions on how to select and print the installation instructions.**

Alternatively, MIDI Inc. can provide a full validation for your system. A technician qualified by MIDI Inc. will execute the Installation Qualification / Operation Qualification (IQ/OQ) protocol, yielding a signed report of system qualification. A Performance Qualification (PQ) protocol is also available.

Your software is shipped with a Security Module that needs to be connected to the computer’s USB port. The Sherlock software and libraries are locked to the serial number of the module and may not operate without it.

Note: If your computer requires service, or is replaced, be sure to retain the Security Module and connect it to any replacement system. Loss of the security module may require the purchase of new Sherlock software!

Agilent Technologies Gas Chromatograph (GC)

The Sherlock MIS Software can be used on one of these different hardware configurations: an Agilent 6890 GC, with an automatic liquid sampler, injector, and 100-vial tray (see Figure 1-1); an Agilent 6850 GC with an automatic liquid sampler, injector, and 8 or 27-vial turret (see Figure 1-2); or an Agilent 7890 GC with automatic liquid sampler, injector and 100-vial tray. The 6890 GCs can be dual or single tower. A Dell or Hewlett-Packard/Compaq computer with Agilent ChemStation software (version A.10.01 or above, or version B.01.01 or above) installed is also required. For specific information and requirements regarding any of these hardware components, please refer to the accompanying Installation Qualification included with your system. This manual assumes that a qualified MIDI Inc. or Agilent Technologies engineer has properly installed and connected all the components of your chromatographic unit during a standard installation.

Figure 1-1
Agilent GC 6890 Series



Environmental Considerations

Figure 1-2
Agilent GC 6850 Series

The chromatographic unit will operate within temperatures of 10-40°C (50-104°F) and 20-80% relative humidity (See Chapter 1 of each Agilent GC component manual for detailed environmental information); however, an environment comfortable for human habitation (reasonably constant temperature and humidity conditions) is recommended for optimum performance and instrument lifetime. Avoid exposure to corrosive substances and dust. **Do not use the Sherlock GC column for other applications.**

As a general rule, when not running samples, the hardware may be left on, with the carrier and auxiliary gases flowing and the oven set at 170°C or lower. Serious column damage will occur if the oven is hot and there is no hydrogen flow through the column. The chromatographic system will need less maintenance and operate more reliably if it is routinely left on.



Computer and Software

The Sherlock MIS runs on an IBM-compatible personal computer. The computer should be used as a dedicated instrument controller. All Sherlock files are located in the top-level directory named C:\SHERLOCK.

Minimum requirements:

- Personal computer with a processor running at 600 Mhz or higher, with a minimum of 128 Mb RAM (256 Mb preferred) and at least an 8 Gb hard drive. (For use with Sherlock DNA, a 1.6 Ghz processor and 512 Mb RAM are minimums.)
- Windows XP Professional or Windows 2000
- Agilent Technologies A.10.01 or higher ChemStation Software (including B.01.01 and higher)
- CD-ROM drive
- 1024x768 VGA Color Display
- LAN or HPIB Interface Board
- USB port
- Mouse

Figure 1-3
Agilent GC 78900 Series



Sherlock Standard Methods and Libraries

The Sherlock MIS is capable of identifying a wide range of microorganisms. Screening isolates for physiological or biochemical grouping before analysis is not required. Each library has been carefully developed by collecting well-characterized strains of reference cultures from microbiologists specializing in many areas, including:

- | | |
|--------------------|------------------------|
| • Medical/Clinical | • Sterility Control |
| • Environmental | • Plant and Soil |
| • Industrial | • Food |
| • Veterinary | • Drinking/Waste Water |

Reference strains are cultured and processed under carefully controlled conditions. Each library entry is a computer-generated composite of the well-characterized reference strains of each species or subspecies group of organisms, taking into consideration strain-to-strain and experimental variability.

In order to identify based on a particular library, one must process the sample with an associated method. The method determines the analytical parameters for chromatographic analysis as well as the peak naming for individual fatty acids and various control parameters for calibration.

Methods come in four types: **Standard**, **Rapid**, **Sensitive**, and **Instant**. Standard methods are the older form used by the Sherlock system for many years. Recent improvement in chromatographic equipment has allowed the development of Rapid methods. These methods are equivalent in identification ability to the associated standard method but run approximately three times faster and are twice as sensitive. Because of this improved sensitivity, Rapid methods require a different calibration mixture than Standard methods. Sensitive methods are as sensitive as Rapid methods but do not have the speed improvement. Sensitive methods use the Rapid calibration mix as well. Rapid method names start with the letter “R” and Sensitive method names start with the letter “S”.

The most recent additions are the *Instant FAME™* libraries and methods. These methods include a simpler and faster (under 3 minutes) extraction procedure that uses MIDI supplied reagents, consumables, and equipment. The new protocol is more sensitive and requires only 2.5 - 3.0 mg of log-phase cell growth rather than 20 - 40 mg required for the previous methods. Instant method names start with the letter “I”. They are covered in detail in the ***Instant FAME™ User’s Guide***.

Table 1-1 lists the usual libraries available for the Sherlock MIS and their associated methods.

Table 1-1: Sherlock Libraries and Methods

Package	Library	Method(s)	Description
AEROBE	TSBA6	TSBA6	Aerobes, 28°C, 24hr, on Trypticase Soy Broth Agar
	RTSBA6	RTSBA6	
	ITSA1	ITSA1	Environmental aerobes, 30°C, 24hr, on Trypticase Soy Agar
	CLIN6	CLIN6	Clinical Aerobes, 35°C, 24hr, on Blood Agar, Chocolate, etc.
	RCLIN6	RCLIN6	
	IBA1	IBA1	
	BTR3	BTR3	Bioterrorism Clinical Aerobes, 35°C, 24hr, on Blood Agar, Chocolate, etc.
	RBTR3	RBTR3	
	M17H10	MYCO6 SMYCO6	Mycobacteria, 35°C, 5-10% CO ₂ , on Middlebrook 7H10 Agar with OADC enrichment
	IMYC1	IMYC1	
	IR2A1	IR2A1	Water/stressed organisms, 30°C, 24hr, on R2A.

Package	Library	Method(s)	Description
ANAEROBE	BHIBLA	ANAER6 SAENER6	Anaerobes, 35°C, 48hr, on BHIBLA plates in Gas Packs
	MOORE6	MOORE6 SMOORE6	VPI Broth-grown Anaerobe Library, 35°C, in PYG Broth
YEAST	YST28	YEAST6 SYEAST6	Yeasts, 28°C, 24hr, SAB Dextrose Agar
	YSTCLN	YSTCLN6 SYSTCLN6	Yeasts, 28°C, 24hr, SAB Dextrose Agar
	ITY1	ITY1	Yeasts, 30°C, 24hr, TSA
	FUNGI	FUNGI6 SFUNGI6	Fungi, 28°C, 2-5 days, in SAB Dextrose Broth, 150 RPM shake culture
	ACTIN1	ACTIN6 SACTIN6	Actinomycetes, 28°C, 3-10 days, in Trypticase Soy Broth, 150 RPM shake culture

Sherlock Limitations

Sherlock can identify only those microorganisms for which fatty acid composition profiles of a representative number of correctly named reference strains have been determined and entered into one of Sherlock's libraries. The library entries have been determined by analyzing reference strains grown under controlled culture conditions. These culture conditions and sample preparation procedures must be followed.

Fatty acid profiles correlate with DNA homology, which is used to define the taxonomy at the species level. In some families, such as Enterobacteriaceae, several species are closely related and the taxonomy has been based primarily on biochemical reactions. The library entries of closely related species show some overlap with close second and third choices. The Composition Report produced by Sherlock assists the user in these cases by printing messages such as "confirm with other tests."

User-Generated Libraries

The Library Generation Software (LGS) option may be purchased separately to extend the power of your system. LGS supports gas chromatographic method development and enables you to make your own searchable library entries from your unique samples.

Optional Software Components

A variety of optional software components are available for Sherlock. For example, the Electronic Records and Signatures component enables compliance with FDA regulation 21 CFR part 11.

Overview of the Sherlock Operation

The Sherlock system is completely controlled by the computer. After sample extracts have been prepared (discussed in Chapter 2 - Preparing Extracts), the labeled sample bottles are inserted into the automatic liquid sampler tray. Information about each sample in the tray is entered into the Sherlock Sample Table using the computer keyboard.

When the information about each sample has been entered into the Sample Table, a batch can be started. A batch is a run of one or more sample extracts on the GC along with associated calibration and QC samples. When a batch is started, the following occurs:

- The sample's Method (instrument set points, calibration instructions, and sample tray location) is downloaded to the ChemStation which sets the GC parameters and controls the injection by the automatic liquid sampler.
- The system automatically runs calibration samples at the beginning of the batch and at intervals throughout the batch.
- The sample bottle for the first sample is retrieved from the tray and loaded into the turret by the automatic sampler. The sampler controller commands the injector to inject a small portion of the extract into the GC.
- A capillary column installed in the GC oven separates the fatty acid methyl esters (FAMEs) present in the mix as they pass through the column to the detector. The flame ionization detector burns the FAMEs, creating a proportional signal that is stored in ChemStation.
- When the run is complete, the retention time, response, and area / height ratio of each peak are calculated by the ChemStation and transmitted to Sherlock for further processing. Peaks in the chromatogram are identified by fatty acid Equivalent Chain Length (ECL) value and name.
- When peak naming is complete, Sherlock searches the library associated with the Method to identify the microorganism. The library search uses both the peak name and the peak percent to match with known profiles stored in the library. Following the library search, the computer prints the Composition Report, which includes the peak naming, the library classification results and the chromatogram.

The computer system automatically sets the operating parameters of the chromatographic unit. There is no need to manually enter the GC parameters or manipulate the keyboard of the GC when the system is running samples. The system will automatically recalibrate within a preset interval. The user is permitted to enter sample information into the Sample Table while samples are being processed. This allows for continuous operation of the Sherlock MIS.

Chapter 2

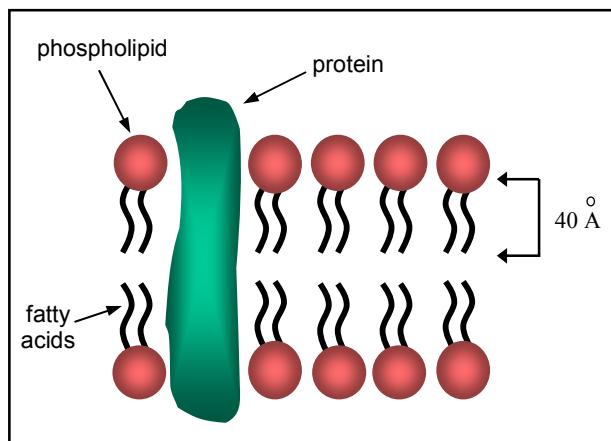
Preparing Extracts

Overview

PLEASE NOTE: This chapter is only for the Standard, Rapid, and Sensitive methods. Refer to the separate “*Instant FAME™ User’s Guide*” for instructions for preparing Instant FAME extracts.

The fatty acids of microorganisms are part of the structure of the cell membrane (Figure 2-1). Cells alter the fatty acid composition of their lipids to maintain membrane fluidity with varying environmental conditions. It is essential to control the selection of a culture medium and the time and temperature of incubation before comparing fatty acid compositions with the Sherlock Libraries. Media preparation steps and incubation conditions for two of the Aerobe package of libraries, TSBA6 and CLIN6, are included in this chapter. The Mycobacteria, Yeast package and Anaerobe package libraries require alternate media, incubation conditions, and/or sample preparation procedures. These are described in this chapter, as well as in Appendix B.

Figure 2-1
Cellular Membrane Bilayer



Note: Throughout this manual, when a method or library is mentioned, such as TSBA6 or MOORE6, the documentation also applies to the rapid or sensitive version, RTSBA6 and SMOORE6 respectively. If there are differences between versions, they will be explicitly described.

Sherlock relies on qualitative (which compounds are present) and quantitative (area percentages) analysis of the fatty acid composition of organisms. The following sample preparation procedures discussed in this chapter must be used to ensure reproducibility of results.

Typical Extraction Procedure

Day 1

- Label plates
- Streak plates (including quality control organisms)
- Incubate overnight

Day 2

- Fill out extraction log sheet

- Label extraction tubes (screw top culture tubes)
- Prepare GC-ready extract from each plate
- Label GC vials (sample bottles)

This chapter discusses sample processing, through the preparation of GC-ready sample extracts. It begins with the important topics of growth medium selection, glassware reuse and cleaning, and reagent preparation. Each of the steps in the extraction is then discussed in detail. There are five basic steps in the preparation of GC-ready extracts from cell cultures for fatty acid composition analysis. Table 2-1 gives a summary of the purpose of each of the five steps.

Chapter 3 discusses computer processing of the samples, including loading the sample tray with the extracts, logging the samples into the computer, and carrying out the actual chromatographic processing under computer control.

*Table 2-1
Five Steps to Prepare Extracts*

Step	Purpose
Harvesting	Removal of cells from culture media
Saponification	Lysis of the cells to liberate fatty acids from the cellular lipids
Methylation	Formation of fatty acid methyl esters (FAMEs)
Extraction	Transfer of the FAMEs from the aqueous phase to the organic phase
Base Wash	Aqueous wash of the organic extract prior to chromatographic analysis

Growth Medium Selection

For each Sherlock Library, a standard medium has been selected for four reasons:

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

Note: There are slight, but significant differences in the chemical composition of the media from the different suppliers. These differences can cause quantitative shifts in the fatty acid profiles. It is imperative that you use material from the MIDI recommended suppliers in Appendix A and that preparation procedures are carefully followed.

TSBA Media for Aerobes (TSBA6 Library)

Trypticase soy broth agar (TSBA) plates are the standard media for aerobes. Use the following recipe for TSBA preparation. Follow the vendor recommendations in Appendix A.

- Combine in a 2-liter flask:

Trypticase soy broth	30 grams
Granulated agar	15 grams
Distilled water	1 liter

- Stir over heat until the mixture is boiling and the agar has dissolved.
- Autoclave for 15 minutes at 121°C and 15 psi.

Note: Do not overheat the media. Use an autoclave that has a predictable temperature/ time cycle.

- Cool to 60°C in a water bath.
- Dispense 20 to 25 ml of the melted agar aseptically into sterile 100x15 mm diameter petri dishes. The agar should be 2.5 to 3.2 mm deep.
- Allow agar to solidify at room temperature. If plates are to be stored for a period of time, they should be allowed to stand for 24 hours prior to packing in sterile sleeves. They may then be refrigerated until needed.

Note on commercially prepared TSBA plate media: TSBA can be purchased from BBL through lab suppliers. Request the specific BBL catalog number given in Appendix A. TSBA media sold by other suppliers may not give equivalent results. Customers are urged to perform comparative studies with well-characterized ATCC strains to determine if the media are comparable.

MRSA Media for Aerobic Lactobacilli (TSBA6 Library)

Follow the vendor recommendations in Appendix A. MRS Lactobacilli broth agar (MRSA) plates are made from the following recipe:

- Combine in a 2-liter flask:

Lactobacilli MRS broth	55 grams
Granulated agar	15 grams
Distilled water	1 liter

- Stir over heat until the mixture is boiling and the agar has dissolved.

- Autoclave for 15 minutes at 121°C and 15 psi.

Note: Do not overheat the media. Use an autoclave with a predictable temperature/ time cycle.

- Cool to around 50°C in a water bath.
- Dispense 20 to 25 ml of the melted agar aseptically into sterile 100x15mm diameter petri dishes. The agar should be 2.5 to 3.2 mm deep.
- Allow agar to solidify at room temperature. Then repack in sterile sleeves and store refrigerated until needed.
- For anaerobic growth conditions, pre-reduce the plates in jars (use glove box) under anaerobic atmosphere for 24 hours before use.

Blood Agar for Clinical Aerobes (CLIN6 Library)

Clinical isolates are grown on TSA with 5% defibrinated sheep blood at 35°C. This medium is available commercially (see Appendix A).

Most of the organisms in the CLIN6 Library were grown at 35°C without CO₂. If necessary to obtain sufficient growth, we used 5% CO₂. The use of CO₂ is noted in the name field that accompanies the Library Search. A few organisms were grown with and without CO₂. If CO₂ had a significant effect on the profile, a separate Library entry was made for each growth condition.

If you grow a clinical organism in a CO₂ environment (since we do not), the profile may change slightly, but you should still match our Library entries, all other variables being equal.

Chocolate Agar for Clinical Aerobes (CLIN6 Library)

A few clinical isolates require this agar. It is similar to blood agar, but the sheep blood is hemolyzed. It is commercially available (see Appendix A).

Media for *Campylobacter* species (CLIN6 Library)

Known or suspected *Campylobacter* species should be grown on either Chocolate or TSA Blood Agar at 35°C in a CampyPak® (see Appendix A).

Buffered Charcoal Yeast Extract (CYE) Agar (CLIN6 Library)

Legionella species require this medium. It is commercially available (see Appendix A).

MI7H10 Media for Mycobacteria

Cultures are grown on Middlebrook 7H10 plates plus Middlebrook OADC Enrichment, in 5-10% CO₂ at 35°C until growth is adequate. Grow *Mycobacterium marinum* at 30°C. The extraction procedure for mycobacteria is modified to reduce the frequency of liner changes. The problem is due to a material that makes the extract appear cloudy (see Appendix B).

BHIBLA Media for Plate-Grown Anaerobes (BHIBLA Library)

All plate-grown anaerobes, including lactobacilli, are grown on supplemented brain heart infusion with blood (BHIBLA) plates or Brucella blood agar plates. Pre-poured plates are available commercially (see Appendix B).

PYG Media for Broth-Grown Anaerobes (MOORE6 Library)

See Appendix B of the Operating Manual for complete instructions for broth-grown anaerobes for use with the MOORE6 Method. Tubed media for this analysis are available commercially (see Appendix B).

SAB Media for Yeasts (YST28 & YSTCLN Libraries)

Yeast cultures are grown on Sabouraud Dextrose Agar at 28°C for 24 hours. The medium is prepared according to the label directions and aseptically dispensed into sterile 100x15 mm Petri dishes. This media is available commercially (see Appendix B).

Yeast Culture Techniques

These organisms have only a few fatty acids. Therefore, it is imperative that they are grown on the same media used for database building. Yeasts appear to work well if they are given three subcultures after arriving at the bench. Lyophilized cultures may require four subcultures following reactivation.

Trypticase Soy Broth for Actinomycetes (ACTIN1 Library)

See Appendix B of the Operating manual for complete instructions for broth-grown Actinomycetes. Actinomycetes are grown in 20 ml of Trypticase Soy Broth on a shaker rotating at 150 rpm. This media is available commercially (see Appendix B).

SAB Media for Fungi

See Appendix B of the Operating Manual for complete instructions for growing filamentous Fungi. When starting Fungi on plates, use Sabouraud Dextrose Agar. When preparing flasks for fungal growth, use 40 ml Sabouraud Dextrose Broth. Both of these media are available commercially (see Appendix B). Fungi are growth at 28°C on a shaker rotating at 150 rpm.

R2A Agar for Water Isolates

Some isolates from water will not grow on rich media, such as TSBA. R2A is a “minimal” medium that can be used instead. As of this printing, there is not a database for organisms grown on R2A. If you use this medium, you must generate your own Library. The medium is commercially available. Call MIDI Technical Support for suggestions and for help with building your library.

Glassware Reuse and Cleaning

A few precautions are required to eliminate possible sample contamination by dirty glassware.

- Culture tubes and screw caps are reusable and are easily cleaned by soaking in a high quality biological cleaner, followed by a thorough rinse with clean distilled water.
- Reagent bottles must have Teflon®-lined caps. Bottles should be cleaned and rinsed with distilled water before preparation of reagents. The bottle containing Reagent 3 (extraction solvent) **must be totally dried** before reagent preparation.
- Pipetting systems should have only Teflon or glass parts in contact with the reagents. Avoid the use of rubber pipette bulbs.
- The tips of disposable Pasteur pipettes packaged with foam pads should be heated or flamed prior to use to eliminate contaminating peaks in the chromatographic analysis.

Before analyzing unknown samples, test the sample preparation procedure for cleanliness and the purity of the reagents by running a reagent blank (complete procedure without cells added).

Reagent Preparation

Four reagents are required to saponify the cells, esterify, extract and base wash the fatty acid extract. Prepare the reagents in clean, brown, labeled 1-liter bottles. Place a Teflon-coated stir bar in each bottle to aid in mixing. Only Teflon and glass should come in contact with the reagents. Bottles can be reused without rinsing. Auto-pipettes increase the speed of preparation but should be primed and checked for proper operation before each batch of samples. Check the calibration by dispensing reagents into a graduated cylinder. The following recipes prepare reagents to process 300 samples and may be proportionally adjusted to suit the laboratory sample throughput. Reagent preparation for the VPI Anaerobe Broth method and library are included in Appendix B.

Caution: Reagents 1 and 4 are caustic and Reagent 2 is acidic; wear safety glasses and gloves at all times. The hexane and methyl tert-butyl ether (MTBE) in Reagent 3 are flammable. Extinguish all flames and heat sources before use. Handle in a chemical fume hood.

Reagent 1 - Saponification Reagent

- Combine water and methanol. Add NaOH pellets to the solution while stirring. Stir until the pellets dissolve.

Sodium hydroxide (certified ACS)	45 grams
Methanol (HPLC grade)	150 ml
Deionized distilled water	150 ml

Reagent 2 - Methylation Reagent

- Add acid to methanol while stirring.

Note: The hydrochloric acid must have a certified concentration. Only HCl shipped in glass bottles is acceptable. Consult your vendor for packaging information. Please refer to the supplier recommendation in Appendix A. If 6.00N HCl is not available, consult your manufacturer's label for the concentration of that lot. Confirm that the final solution is 6.00N by titrating against a standard base solution.

6.00N Hydrochloric Acid	325 ml
Methanol (HPLC grade)	275 ml

Reagent 3 - Extraction Solvent

- Add the MTBE to the hexane and stir well.

Hexane (HPLC Grade)	200 ml
Methyl tert-butyl ether (HPLC Grade)	200 ml

Reagent 4 - Base Wash

- Add NaOH pellets to the water while stirring. Stir until the pellets are dissolved.

Sodium hydroxide (certified ACS)	10.8 g
Deionized distilled water	900 ml

Additional Reagents

- Saturated NaCl: Dissolve 40g ACS NaCl in 100 ml dI H₂O.

Reagent Storage and Shelf Life

It is recommended that reagents be prepared fresh every 30 days. They can be stored at room temperature in bottles supplied with Teflon-lined caps. Store the pipetter in a clean container. All reagents should be labeled properly including the date of preparation and the expiration date of one month. The purity of reagents should be confirmed by preparing a reagent control blank (procedure without cells) with every batch of samples.

When not in use, remove the pipetter from the Reagent 1 (saponification reagent) bottle and rinse (by pumping) with clean, distilled water to prevent seizing of the plunger. Cap the reagent bottle with a clean Teflon-lined screw cap.

Reagent 3, the extraction solvent, is flammable and should be stored properly in a chemical fume hood or vented solvent cabinet.

Labeling Plates

This section discusses the first step in a typical extraction procedure. The first step is to label and date your agar plates. The label can contain your laboratory accession number, and the source or suspected identity of the microorganism. The label information should be used in two other places:

1. Label information should be put on the Extraction Log Sheet (this is recommended, not mandatory). A sample Extraction Log Sheet is shown later in this chapter (Figure 2-3).
2. When you are ready to run your samples, enter the same information into the “Name” field of the computer’s Sample Table. It is also a good idea when entering samples into the Sample Table to place any pertinent comments in the Name field to get a permanent record in the data file for future reference (Max # characters = 42).

Table 2-2: Selected Validation Organisms

Aerobes	ATCC Number	
Gram Negative	TSBA6	CLIN6
<i>Acinetobacter baumanii</i>	19606	19606
<i>Acinetobacter calcoaceticus</i>	23055	---
<i>Burkholderia (ceno)cepacia</i>	25609	25609
<i>Citrobacter freundii</i>	8090	---
<i>Escherichia coli</i>	11775	---
* <i>Pseudomonas aeruginosa</i>	27853, 9027	27853, 9027
* <i>Stenotrophomonas maltophilia</i>	13637	13637
Gram Positive	TSBA6	CLIN6
<i>Corynebacterium striatum</i>	6940	6940
<i>Enterococcus faecalis</i>	19433, 4083	19433
<i>Kocuria rosea</i>	186	---
<i>Kocuria varians</i>	15306, 15396	---
<i>Staphylococcus aureus</i>	12600	12600, 25923
<i>Staphylococcus epidermidis</i>	14990	---
Bacillus Species	TSBA6	CLIN6
<i>Bacillus cereus</i>	14579	14579
<i>Bacillus circulans</i>	4513	---
<i>Bacillus licheniformis</i>	14580	---
<i>Bacillus sphaericus</i>	14577	---
<i>Geobacillus stearothermophilus</i>	12980	---
<i>Bacillus subtilis</i>	6633	6633

* indicates the suggested daily QC organism

Bacillus subtilis 6633 is a particularly important organism to use during validation. This organism is extremely sensitive and will often be the first indicator of an issue with sample preparation.

Quality Control

Each sample batch should contain a known strain as a positive control and a reagent blank containing no bacteria.

A positive control (QC) should be processed to check the complete procedure. This known reference strain with a valid library entry should be processed with each batch of samples. The recommended organism for the TSBA6 library is *Stenotrophomonas maltophilia* (ATCC 13637). This organism's fatty acid profile is complex and is diagnostic for problems with all stages of sample preparation. This QC analysis should give a consistent, high Similarity Index (see PQ Table in chapter 7).

A negative control (reagent blank) should be processed with each batch of samples. This blank is run through the complete procedure but no cells are added. This QC analysis should contain no named peaks.

The list in Table 2-2 is presented only as a suggested or representative list of strains that could be used in a more involved microbiological validation protocol.

General Guidelines for Selecting ATCC Cultures for Validation

If the type strain is a Preceptrol® culture, that strain is probably very typical of the population and would make a good choice.

If the strain has been deposited for use with a particular FDA, CFR, Pharmacopoeia, or phage host test, the strain may be atypical of the species. Use caution before selecting that strain. Avoid patent strains. Boldfaced organisms in Table 2-2 can be used for TSBA6 and CLIN6 library validation. An * indicates the suggested daily QC organism.

Streaking Plates

NOTE: Instant FAME™ uses a different technique (see the Instant FAME™ User's Guide)

The Quadrant Streak pattern is recommended for culturing cells on plates for identification by Sherlock MIS (see Fig. 2-2). The goal of this pattern is to create four densities of cells and to verify culture purity. Quadrant 3 typically will contain cells in the late log phase of growth. MIDI recommends two subcultures on the media to be used before analysis.

Select a well-isolated colony or small cross section of growth from the isolation plate. The first subculture will help assess the growth rate on the desired media and thus the inoculum size. For slow growing organisms it may be necessary to inoculate multiple plates.

With a sterile inoculation loop, transfer the cells to the plate. Spread the cells over the area of quadrant 1 touching the entire ring of the loop to the media so that the region is heavily inoculated. Inoculate quadrant 2 by rotating the loop 90° and passing the loop edge through the corner of quadrant 1 twice, as shown in the drawing. Then streak the rest of quadrant 2 with parallel lines without reentering quadrant 1.

Inoculate quadrant 3 by rotating the loop 90° and passing the edge of the loop through the corner of quadrant 2 twice. Then streak the rest of quadrant 3 with parallel lines without reentering quadrant 2.

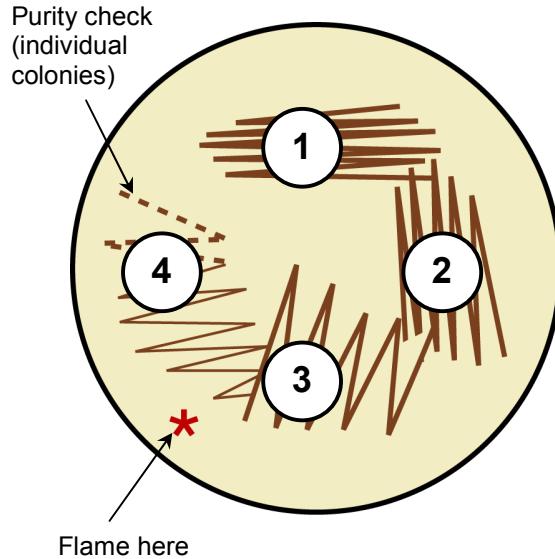
Sterilize and cool your inoculation loop. The loop can be cooled by plunging it into the agar plate in an area without any cell colonies.

Inoculate quadrant 4 by passing the edge of the loop through the corner of quadrant 3 twice. Then streak the rest of quadrant 4 with parallel lines without reentering quadrant 3. This streaking pattern results in ample material for analysis, while confirming the presence of a single colony type or pure culture.

Incubation

The most stable fatty acid compositions are from cultures in the late log phase. The Sherlock libraries have been developed by selecting incubation conditions that are the most favorable for a

Figure 2-2
Quadrant Streak Technique



majority of microorganisms. The following standard culture conditions have been used for most of the library entries. Exceptions are noted in the library listings in Appendix B.

Use a small, high-quality incubator in which growth conditions can be controlled. Use of a larger incubator that is shared with other users increases the risk of not maintaining proper conditions. Do not leave any disinfecting agents in the incubator as very low levels of these chemicals in the atmosphere can retard the growth of organisms on agar plates.

Aerobe Incubation

The standard incubation conditions for aerobes (TSBA6) are the following:

- $28 \pm 2^{\circ}\text{C}$ temperature
- 24 ± 2 hours time

The clinical aerobes (CLIN6) require incubation at $35 \pm 1^{\circ}\text{C}$.

Slow-Growing Organisms

Although qualitatively stable, the fatty acid compositions of pre-log phase cultures may not be quantitatively reproducible. Analyses of 24-hour cultures of slow-growing isolates can result in low similarity index values or misidentification. For accurate speciation of such cultures, extended incubation may be necessary to obtain quantitative reproducibility and to achieve sufficient cell mass for analysis. It may be necessary to inoculate several plates to achieve sufficient cell mass.

Fastidious Organisms

Not all microorganisms can be cultured with the standard conditions. Fastidious organisms may require enriched media or specific atmospheric conditions.

The appropriate culture conditions for these organisms are listed next to the entries in the library listings (see *Chapter 5-Viewing Installed Libraries*).

Sherlock can only identify these organisms if the indicated culture conditions are used.

Closely related groups of fastidious organisms have been incubated under the same culture conditions for generation of the library entries. The conditions used for library generation of fastidious organisms will be similar to those conditions necessary for primary isolation of the culture.

Extraction Log Sheet

Figure 2-3
Extraction Log Sheet

After plates have been incubated for 24 hours, it is recommended that you fill out an Extraction Log Sheet (see Fig 2-3). This will provide you with a laboratory record matching extraction numbers to auto sampler tray positions. You can vary the format to suit the needs of your laboratory. Recall that the "Sample ID" entry in the Extraction Log Sheet should match the labeling on the plate in which the culture was grown. The same text should be used when you enter the sample into the computer's Sample Table (discussed in the next chapter). Also note that at this point in time the GC auto sampler tray position is not yet known, so it is left blank.

Labeling Culture Tubes

After filling out an entry in the Extraction Log Sheet for each plate, you need to label the culture tubes into which the cell cultures will be harvested. You should label each culture tube with the extraction number from the Extraction Log Sheet.

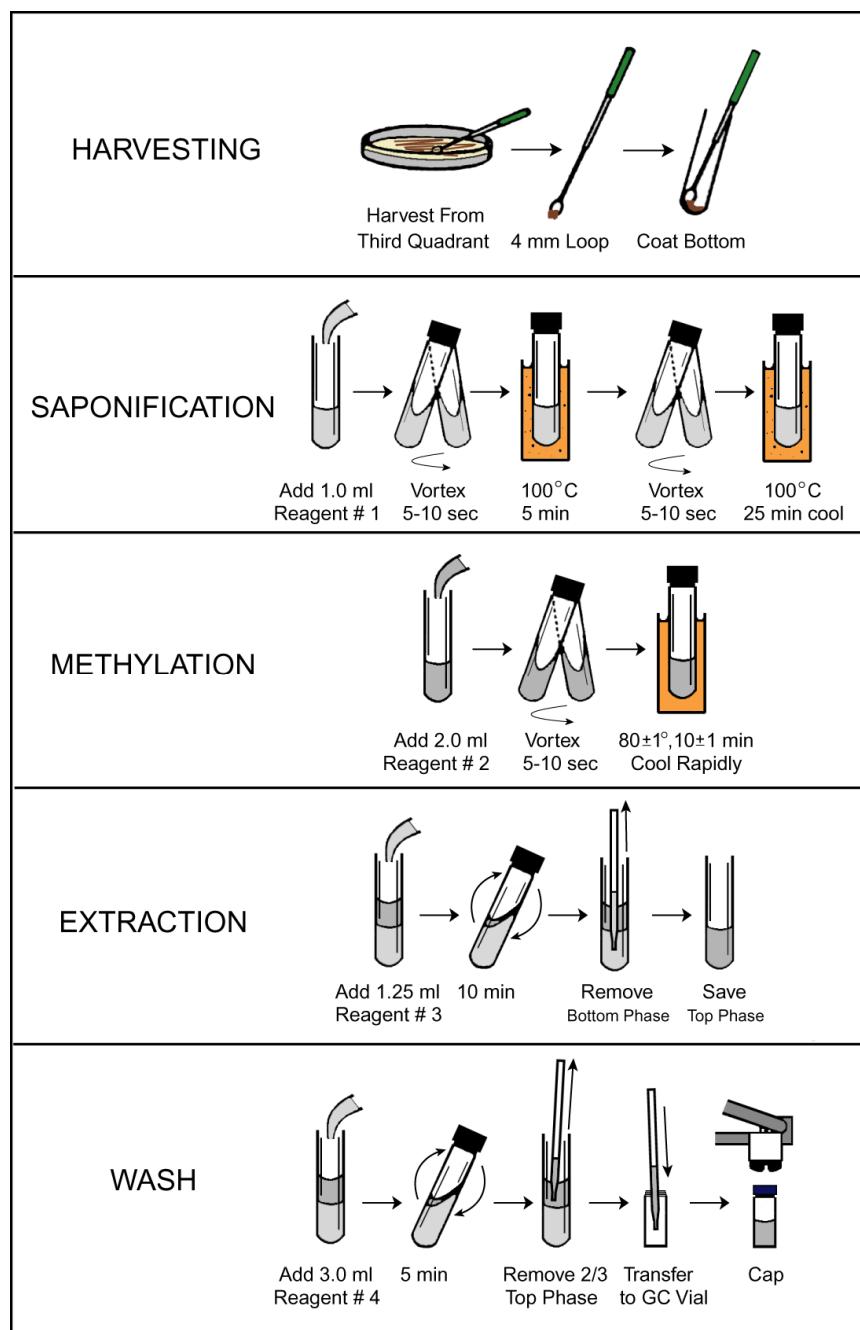
Note: Use a laboratory-marking pen to write directly on the glass. Self-adhesive labels will not readily adhere to the culture tubes in a boiling water bath.

EXTRACTED BY:		DATA ENTERED BY:	
Name:	Date:	Name:	Date:
Sample ID	GC# Position	Sample ID	GC# Position
1 QC-BLANK		31	
2		32	
3		33	
4		34	
5		35	
6		36	
7		37	
8		38	
9		39	
10		40	
11		41	
12		42	
13		43	
14		44	
15		45	
16		46	
17		47	
18		48	
19		49	
20		50	
REAGENT LOT #			
21	Reagent #1		
22	Reagent #2		
23	Reagent #3		
24	Reagent #4		
25	Saturated NaCl/H ₂ O		
26	WATERBATH TEMP.		
27	Waterbath 1 (100°C)		
28	Waterbath 2 (80°C)		
29	Time in	am/pm	
30	Time out	am/pm	
QC Runs _____			
Other Runs _____			

Preparing Extracts: Five Basic Steps

Each sample is processed in a single test tube and as many as 50 samples can be prepared conveniently in a batch. A summary of the processing activities associated with each step is shown in Figure 2-4.

Figure 2-4
Extract Preparation Activities



1. Harvesting

NOTE: Instant FAME™ uses a different technique (see the Instant FAME™ User's Guide)

The Quadrant Streak method is designed to dilute the inoculum so that quadrant 4 will contain well-isolated colonies to serve as a check for purity. Colonies should be harvested from the most dilute quadrant exhibiting confluent growth (late log phase) along the streaking axis. This area of harvesting typically yields the most stable fatty acid compositions since the inoculum has been diluted enough to result in abundant growth of colonies without a limiting nutrient supply.

Note: The optimum area for harvesting usually occurs in quadrant 3.

Failure to use cells from the correct quadrant on the plate can result in poor library matches. Cells from quadrant 3 are preferred; however, it may be necessary to use cells from quadrant 2 or quadrant 1 with slower growing organisms.

Remove the cultured cells from the plate by gently scraping the surface of the culture medium with a sterile 4 mm inoculating loop. A back-and-forth motion while slightly rocking the loop is useful in picking up the cells.

For non-Rapid and non-Sensitive methods, one heaping 4 mm loopful (approx. 40 mg) of live wet cells is ample material for processing. For the Rapid and Sensitive methods, approximately 20 mg of live wet cells is appropriate; this amount is half of a heaping loopful. Some cultures will not adhere well to the metal inoculating loop. A plastic inoculating loop, a Pasteur pipette melted into a small loop, or a smaller diameter wire loop can be used to harvest such cultures. Be sure to obtain about the same total amount of cells as a heaping 4 mm loopful would provide. This can be judged by observing the total area counts in the final report or by weighing a few samples for practice.

Note: Make sure that the caps fit the tubes in the next step before proceeding and the cap is Teflon-coated.

Figure 2-5
Harvesting Procedure

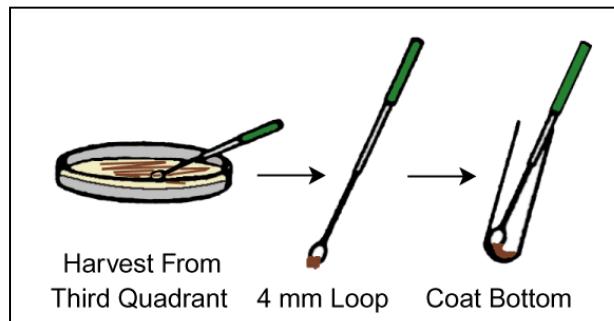
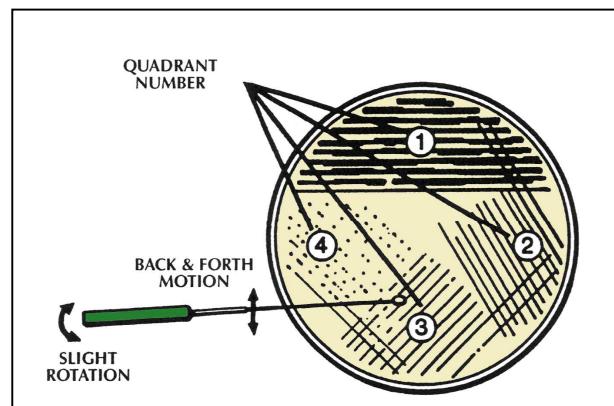


Figure 2-6
Removing Cells From Plate



- Insert the loop with the cells into a clean, dry 13 mm x 100 mm screw cap culture tube. Wipe the cells off the loop and onto the lower inner surface of the culture tube within 10 mm of the bottom of the culture tube. Remove and sterilize the loop.
- If you have not already done so, be sure to label the culture tube with the extraction number (from the Extraction Log Sheet) corresponding to the harvested plate. Use a laboratory-marking pen to write directly on the glass. Culture tubes can be reused if properly cleaned. Harvest cells from each plate in the batch before proceeding to the saponification step.

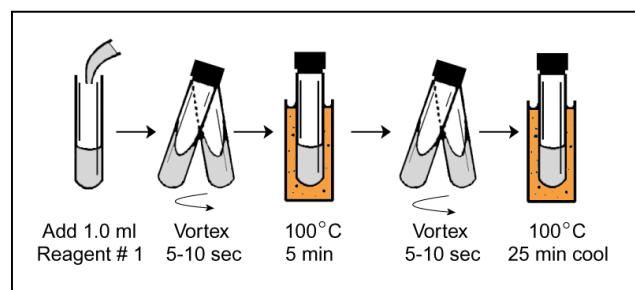
2. Saponification

A strong methanolic base combined with heat kills and lyses the cells. Fatty acids are cleaved from the cell lipids and are converted to their sodium salts.

Note: Use a boiling or circulating water bath. Heating blocks or other heating means do not have adequate heat transfer and temperature control for this step.

Figure 2-7
Saponification Procedure

- Pipette 1.0 ± 0.1 ml of Reagent 1, the methanolic base, into each of the culture tubes in the batch.
- Tightly seal each tube with a clean teflon-lined screw cap.
- Vortex the tube for 5-10 seconds.
- Place a rack of the batched sample tubes into a boiling or circulating water bath at 95°C-100°C.



Caution: Heating of sealed tubes builds pressure. Scratched or cracked glassware can burst. It is recommended that heating be done in a chemical safety hood or behind a plastic shield. Wear safety glasses at all times.

- After five minutes, remove the tubes from the boiling water and cool them slightly. DO NOT LOOSEN CAPS. Vortex each tube for 5-10 seconds. Return the tubes to the water bath.
- Check the tubes for leakage, as evidenced by the presence of bubbles rising in the tube. Retighten the caps of leaking tubes. If bubbling continues, the sample must be cooled to room temperature and then transferred to a new culture tube.
- Continue heating the tubes in the water bath for an additional 25 minutes.

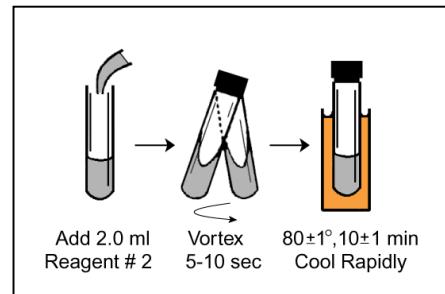
- After a total of 30 minutes of saponification in the water bath, remove and set the rack of tubes in a pan of cold tap water to cool. Ice-cold water is not recommended.

3. Methylation

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

- Uncap each tube in the batch, then add 2.0 ± 0.1 ml of reagent 2, the methylation reagent, to each tube.
- Tightly cap each tube and vortex the solution for 5-10 seconds.

*Figure 2-8
Methylation Procedure*



Because of an excess of reagents, a granular precipitate (salt) may form. Proceed with the following:

- Heat the tubes in an $80 \pm 1^\circ\text{C}$ water bath for 10 ± 1 minute.
- Remove and quickly cool to room temperature by placing tubes in a tray of cold tap water. Ice-cold water is not recommended. Shake the tubes to speed the cooling process.

Note: Excess time and temperature during this step can degrade cyclopropane-containing compounds, which can alter the fatty acid profiles and naming of the organism.

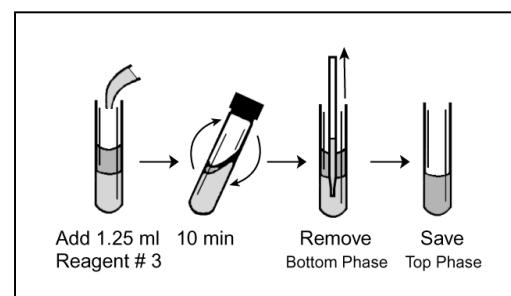
4. Extraction

Fatty acid methyl esters are removed from the acidic aqueous phase and transferred to an organic phase with a liquid-liquid extraction procedure.

Caution: Hexane/MTBE is flammable. Extinguish all flames and heat sources when using these solvents.

- Uncap each tube, then add 1.25 ± 0.1 ml of Reagent 3, the extraction solvent, to each tube.
- Tightly seal the tubes. Place batch of tubes in a laboratory rotator and gently mix end-over end for 10 minutes.
- Uncap each tube. Using a clean Pasteur pipette for each sample, remove and discard the aqueous (lower) phase.

*Figure 2-9
Extraction Procedure*

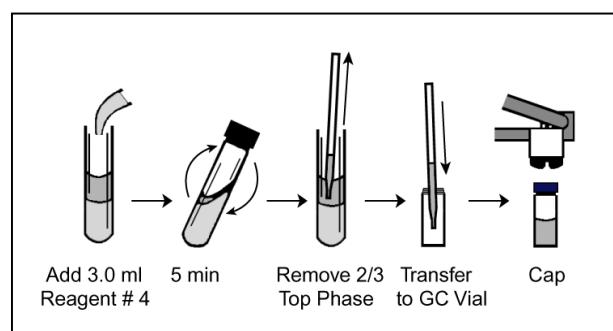


5. Base Wash

A dilute base solution is 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 tailing and loss of the hydroxy fatty acid methyl esters.

- Add 3.0 ± 0.1 ml of Reagent 4, the base wash, to each tube.
- Tightly cap and gently rotate the tubes end-over-end for 5 minutes.
- Brief centrifugation (three minutes at 2000 RPM) is recommended to clarify the interface between the phases when an emulsion is present.

Figure 2-10
Base Wash Procedure



Caution: *Test with extraction tubes and water before using reagents.*

Alternatively:

A few drops of a saturated ACS grade NaCl/water solution can be added to the tube to aid in breaking the emulsion. Hold the tube vertically and rotate it rapidly between the palms of the hands, and allow it to settle for a few minutes.

Transfer of Extract to Sample Vial

- Label the sampler vials for extract identification. You should use your accession number or some information from the “Sample ID” field from the Extraction Log Sheet that uniquely associates the sample bottle with an entry in the Extraction Log Sheet.

Note: *Screw cap vials are acceptable and readily available from lab supply companies. Use a laboratory-marking pen to label the sample vials. Adhesive labels will not reliably adhere to the sample bottles and may interfere with the operation of the automatic liquid sampler bottle gripper.*

- Uncap each tube. Using a clean Pasteur pipette for each sample, transfer about 2/3 of the organic (upper) phase from the tube and transfer to a clean GC sample vial. The interface between the two layers is sometimes difficult to see, and care must be taken not to transfer any of the aqueous (lower) phase into the auto sampler vial.
- Attach a cap onto the sampler vial.

- Ensure that the cap is tightly sealed. If using a crimp cap, try to rotate the cap while holding the bottle. It should slip at roughly the torque required to seal a small screw-topped bottle. If too loose, adjust the capping tool (stop screw in the handle) and recrimp the vial.

When all sample extracts have been transferred to sample vials, you are ready to load the automatic sampler and start running samples. The next chapter picks up from this point.

Delay During Sample Preparation

Ideally, sample processing should continue uninterrupted once cells have been harvested. If it is necessary to delay the sample preparation procedure, there are several steps at which the samples can be held with little effect on the fatty acid analysis.

- Following the harvesting step and before the addition of Reagent 1, samples can be held in the incubator for one or two hours or quickly frozen. If large numbers are being processed, take the plates out of the incubator in small groups and harvest the cells. Place the sample preparation tubes containing the cells in the bottom, back into the incubator, or freeze the tube, and pull another group of plates. When all plates have been processed, remove all the tubes from storage and proceed with the saponification step.
- Following methylation, the extraction step cannot be delayed. Proceed immediately with extraction.
- After extraction and removal of the aqueous phase, the organic phase can be held, refrigerated, overnight.
- Samples will degrade in contact with the Base Wash (Reagent 4). Remove the top phase promptly.
- The completed extract, in the crimped-top GC vial, can be refrigerated for several days before GC analysis.
- Following GC analysis, the extracts can be stored refrigerated for several days. Replace the pierced septum with a new one to minimize solvent loss through evaporation.

Chapter 3

Analyzing Samples

Overview

This chapter continues with the gas chromatographic processing of the samples. In this chapter we will look at loading the sample tray with the sample vials, logging the samples into the computer, and carrying out the actual sample processing under computer control.

Loading the Automatic Liquid Sampler

Figure 3-1
The Automatic Liquid Sampler

The Automatic Liquid Sampler for the 6890 and 7890 GCs are comprised of a sample tray and an automatic injector tower as pictured in Figure 3-1.

The 6850 includes an automatic injector tower a 27-vial turret. (A smaller 8-vial turret is also available.) Before samples can be run, the injector turret must be loaded with wash and waste bottles, and the sample tray or turret must be loaded with Calibration Standard and sample bottles.

Loading the Turret

WARNING: Instant FAME Reagent 3 should NOT be used as the wash solvent. This reagent will damage the column. Hexane wash solvent is supplied in the Instant FAME Refill Kit.

Standard, rapid, and sensitive methods – a full, fresh vial of hexane/MTBE or pure hexane wash solvent must be placed in the injector turret along with waste vials. Reagent 3, used in the extraction procedure for these methods, may be used as the wash solvent. For the 6890 and 7890 GC turrets, place the wash vial in the Solvent A position and place waste vials in the Waste A and Waste B positions. For the 6850 GC, 27-vial turret, place the wash vial in the Solvent A position and place waste vials in the Waste A and Waste C positions. For the 6850 GC, 8-vial turret, place the wash vial in the Solvent A position and place the waste vial in the position labeled WA.

Instant FAME methods – two full, fresh vials of hexane wash solvent (supplied with the *Instant FAME Refill Kit*) must be loaded in the injector turret along with waste vials. For the 6890 and 7890 GC turrets, place wash vials in the Solvent A and Solvent B positions and place waste vials in the Waste A and Waste B positions. For the 6850 GC, 27-vial turret, place wash vials in the



Solvent A and Solvent B positions and place waste vials in the Waste A and Waste C positions. For the 6850 GC, 8-vial turret, place wash vials in the Solvent A and Solvent B positions and place the waste vial in the position labeled WA.

The turrets can be moved by hand to gain access to the vials. Label the wash vials “SOLVENT WASH” and use these vials for this purpose only. This will prevent contamination of the clean solvent. The solvent wash vials must be cleaned and refilled before each batch of samples is run. After each injection, the syringe is cleaned using the wash solvent. The waste from cleaning the syringe is discarded in the waste vials. The waste vials must be emptied and cleaned before starting a batch. Instructions for doing this are:

- Remove the waste vials and the solvent wash vials.
- Remove the caps and dump the contents into an appropriate solvent waste disposal container.
- Clean all parts and bottles in a soap solution, rinse with water, and dry.
- Fill the SOLVENT WASH bottles to the neck with fresh solution.
 - **Standard, rapid, and sensitive** methods – use either Reagent 3 for these methods or use pure hexane.
 - **Instant FAME** methods – use the hexane wash solution supplied in the Instant FAME Refill Kit.
- Replace the diffusion caps and screw the tops on finger tight. Do not install a septum in the caps of these bottles; the wash solution can become contaminated.
- Place the bottles in the turret in their proper locations. The arm on the sample tray will pick a bottle from the tray and place it in the sample position of the turret when the batch starts.
 - **Standard, rapid, and sensitive** methods – use the Solvent A position
 - **Instant FAME** methods – use both Solvent A and Solvent B positions.

Note: If mixing *Instant FAME* in a batch with standard, rapid, or sensitive methods, follow the instructions for *Instant FAME*. The other methods can use the *Instant FAME* wash and waste vials.

Calibration Standard

All Sherlock methods require calibration. When a calibration analysis is completed, the computer checks 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** methods use Calibration Standard 1 (part number 1200-A); the **rapid, sensitive, and Instant FAME** methods use Rapid Calibration Standard (part number 1300-AA). Both calibration standards contain the same compounds and are automatically run twice at the beginning of every batch and automatically reanalyzed after every 11th sample injection. The straight chain C9:0 to C20:0 fatty acid methyl esters (FAMEs) are used by the system to quantitatively calibrate and compensate for peak area discrimination between low

and high boiling point fatty acids. Five hydroxy FAMEs are added to the mixture to detect injection port liner or column degradation, which can result in poor peak shape (tailing of the peaks) or an actual 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. Deviations from the expected values result in a failure to calibrate, and a warning message to the user.

A second function of the Calibration Standard is to provide accurate retention times for the straight chain FAMEs. These retention times are used to calculate Equivalent Chain Length (ECL) values by which peaks in subsequent analyses are named. The system calculates how much the calibration analysis has deviated from the expected retention times and reports the Root Mean Square (RMS) fit error. If a calibration run is invalid due to a high RMS fit error, Sherlock will print a message to warn the user and will repeat the calibration analysis. If the system fails to calibrate after two consecutive attempts, the error message will be repeated and the batch aborted.

The Calibration Standards are shipped in 2ml glass ampoules. Material Safety Data Sheets (MSDS) and instructions for use and storage are included with each order. The ampoules have an expiration date of approximately three years. Unopened ampoules may be stored at room temperature. After opening, the Calibration Standard should be refrigerated, but brought to room temperature before use. When an ampoule is opened, it should be divided into smaller volumes and stored until use. The ampoules are scored at the neck to facilitate opening as follows:

- Hold the ampoule vertically at arms length, with the bottle section in one hand, head section in the other, thumbs touching at the neck.
- Press out and away with thumbs open.
- Using a flamed glass (never plastic) pipette, transfer the contents to suitable glass auto sampler vials that contain low volume glass inserts. Cap until needed with Teflon-lined silicone caps.

Make sure to use a fresh vial of the Calibration Standard. Before using a bottle of standard, check the fluid level in the calibration bottle by holding the bottle upright. If the septum has been pierced on the Calibration Standard vial and evaporation has occurred, replace it with a fresh bottle before starting a batch. The Calibration Standards are usable for one day after the septum has been pierced (Do not reuse the Calibration Standards). Possible contamination from the septum cap can degrade performance or result in an inability to calibrate the system.

Loading the Sample Tray

In 6890 and 7890 GC systems, the sample tray must be loaded with at least one bottle of Calibration Standard, and up to 99 bottles containing sample extracts. In a single tower system with one method in use, sample tray position 1 is usually used for Calibration Standard. (A dual tower system would have Calibration Standard in the first two positions.) Typically, the calibration

standard will be followed by a Blank and a positive QC sample. The remaining positions will be used for samples.

There must be separate vials of Calibration Standard for each method used in the batch. Dual tower systems require two vials of Calibration Standard for each method.

Each bottle placed into the sample tray must be logged (entered) into the computer's Sample Table. This procedure is discussed in the next section. Before starting a batch, be certain that the descriptions logged into the Sample Table match the bottles in the sample tray.

Sherlock Sample Processor Configuration



The Sherlock *Sample Processor Configuration*, , is accessed from the *Sherlock Toolbox Icon*



on the desktop, or through **Start>Programs>Sherlock>Sherlock Toolbox**. (Other Toolbox items will be described in *Chapter 7- Routine Maintenance*).

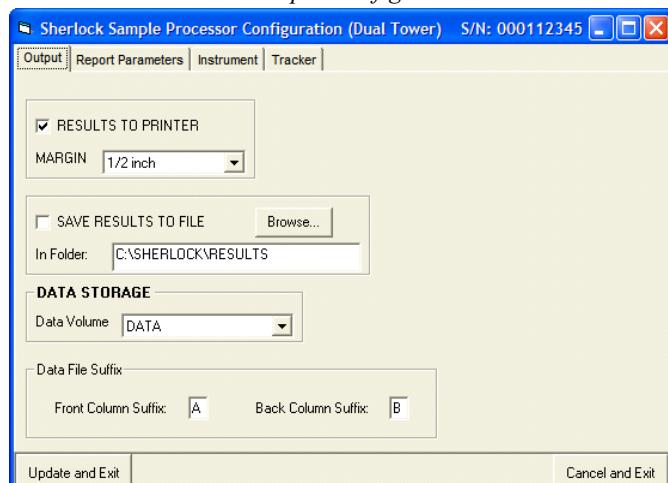
The *Sherlock Sample Processor Configuration* tool controls where Sherlock Sample Processor results are sent and stored, assigns important instrument control parameters and defines the level of detail in reports. The first screen that will appear is shown in Figure 3-2.

Figure 3-2
Sherlock Output Configuration

Note: When the *Sherlock Tracker* module is installed, a **Tracker** tab appears here (see *Sherlock Tracker manual*).

Results to Printer / Results to File

These sections determine how Sherlock Reports are stored while a Batch is running (refer to Figure 3-2). The **Results To Printer** checkbox determines whether chromatograms and Sherlock Reports will be printed after each sample and calibration are run. The Margin dropdown controls the margins for the text when printing during a batch. The options are $\frac{1}{4}$, $\frac{1}{2}$ or $\frac{3}{4}$ inch. If reports need to be placed into a 3-ring binder, choose the $\frac{3}{4}$ -inch option to avoid the loss of text from a hole punch.



The **Save Results To File** checkbox allows Sherlock reports to be saved to a formatted text file (the default directory for these reports is C:\Sherlock\Results). In Sherlock, chromatograms can be printed for each sample, after the sample match information. The file name is chosen automatically when this option is chosen. The user can print the files later using Microsoft® Word.

The **Update and Exit** button will save all changes made to the window, while **Cancel and Exit** will not save any changes.

To print the files in MS Word:

- Open MS Word.
- Open file in C:/Sherlock/Results/xxxxxxxxxx.rtf.
- From the File menu, go to Page Setup.
- Change the left and right margins to 0.25". In Paper Size, change the orientation to Portrait.
- The entire file or specific data can be chosen to print.

Data Storage

This controls the storage location of raw data in the Sherlock/DATA directory. There are eight storage locations, DATA and DATA1 through DATA7. This is where data is accessed for further manipulation by Sherlock functions. Data can be transferred to other data volumes within this directory or can be transferred from this directory to the archival system of the customer's choice (the default location is DATA).

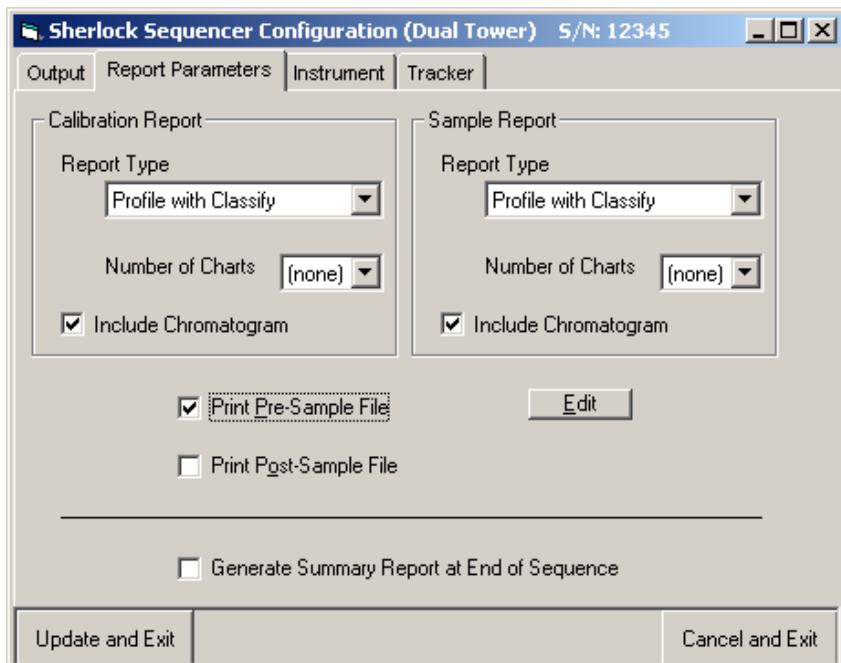
Data File Suffix

Typically, Sherlock data files end with "A" and "B" for the front and back column data files respectively. If one has multiple Sherlock systems, however, it is possible that the same data file name could be produced by both systems. By choosing a unique suffix letter, no name collision will occur and one can immediately identify which GC the batch was processed on based on the file name.

Report Parameters

This section controls the amount of sample identification detail that will appear on a Sherlock Sample or Calibration Report that is generated from the Sample Processor. Figure 3-3 shows the **Report Parameters** dialog box.

Figure 3-3
Sherlock Report Parameters



Under the **Report Type** dropdown menu, the following choices appear (see Figure 3-4):

MIDI strongly suggests using *the Profile with Classify option* for Calibrations and Samples.

Number of Charts (see Figure 3-3) refers to Comparison Charts. These graphical plots show how each fatty acid differs between the sample and the best library matches for this sample. There can be up to four comparison charts printed. See Chapter 4 for a more detailed explanation of Comparison Charts.

Including Chromatograms. By choosing this box, you can have the chromatogram associated with the sample printed at the end of each profile.

Figure 3-4
Report Types

Choices	Meaning
No reports	No report is generated
Brief	Will print only the sample name and any warning messages
Profile	Full page report that lists all the peaks in the chromatogram
Brief with classify	The sample name, any warning messages & the library search results
Profile with classify	Full-page report that lists all the peaks in the chromatogram and the library search results

Custom Headers and Footers on Sherlock Reports

The **Print Pre-Sample File** and **Print Post-Sample File** boxes allow you to print a custom text file before and after each sample report (such as Company information). When one of the boxes is checked, an **Edit** button will appear. Clicking on this button will allow you to enter as much custom information as needed.

Night Mode

When samples are run overnight, the best way to prevent the printer from running out of paper, is to put the Sample Processor in *Night Mode*. There are several ways to do this. One is to uncheck **Results to Printer** in Figure 3-2. The other method is to change the **Report Types** for the Calibration and the Sample to *No Reports*. Then, uncheck the **Include Chromatogram** boxes and click on the **Generate Summary Report at End of Batch** box. This latter method will print all sample run information (Brief with Classify) at the end of the batch, instead of after each sample



run. At a later time, through the Sherlock *CommandCenter*, you can generate other types of reports as needed.

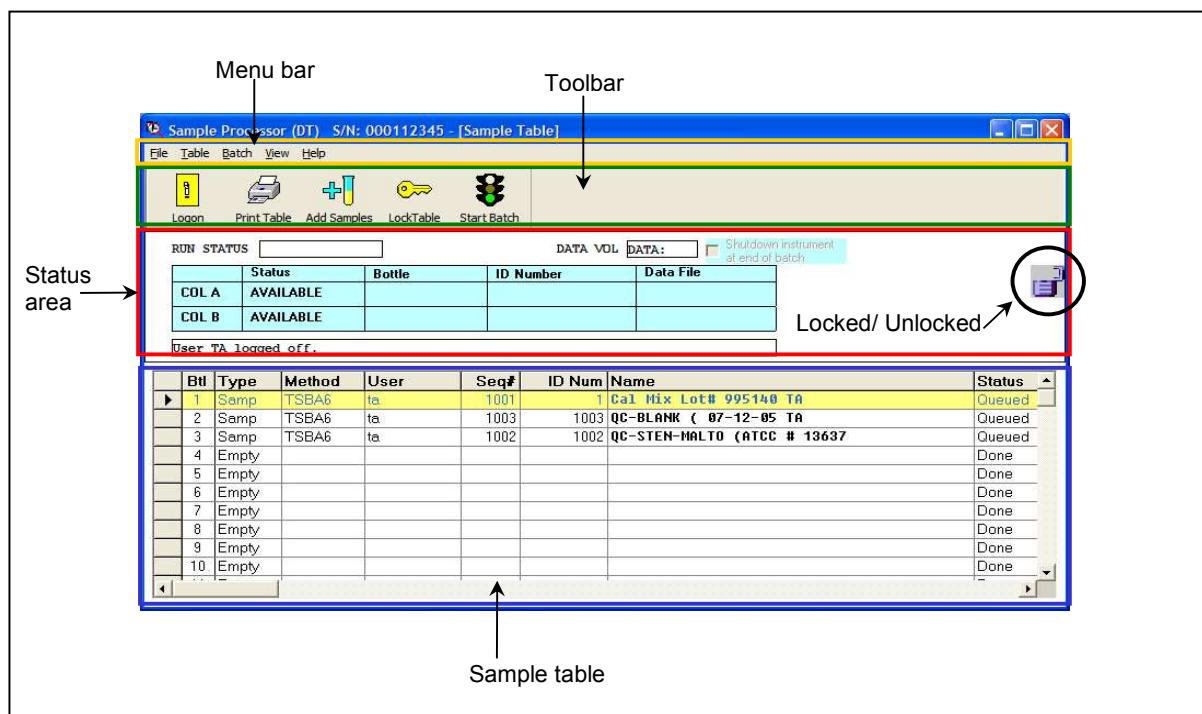
Instrument Parameters

This section will not need to be modified if the instrument was properly configured during installation. With a dual inlet system, ensure that the *GC Type* is set to **Dual**, even if only one tower is being used for Sherlock samples.

The Sherlock Sample Processor

The Sample Processor is accessed from the *Sample Processor Icon* on the desktop. Sherlock uses the Sample Processor as a sample table and as a link with the Agilent ChemStation for running batches of samples. The top menu and toolbars allow manipulation of the sample table. The middle windowpane gives Sherlock and ChemStation status information, currently running sample status and the data storage location. The bottom section is the sample table where the Calibration Standard and sample identification information is logged. Figure 3-5 shows the Sample Processor display.

Figure 3-5
Sherlock Sample Processor Screen



For the 6890 and 7890, there are 100 entries in the sample table, one for each tray position in the sample tray. The 6850 allows either 8 or 27 entries, depending on the turret size on the system. Before putting a sample bottle into the sample tray, the proper identification information must be logged into the corresponding bottle number in the sample table. The sample vial type can be a calibration sample, a sample extract to be processed for identification, a QC run, a blank or empty. In addition, the vial type can be labeled as STAT, which indicates a priority sample that will be run next.

Sherlock Electronic Records and Signatures (ERS)

In compliance with FDA regulation 21 CFR Part 11, MIDI has added Sherlock ERS to Sherlock. With this optional module enabled, users can control which laboratory personnel have access to data files, and who may create, view and review analyses by setting the user preferences.



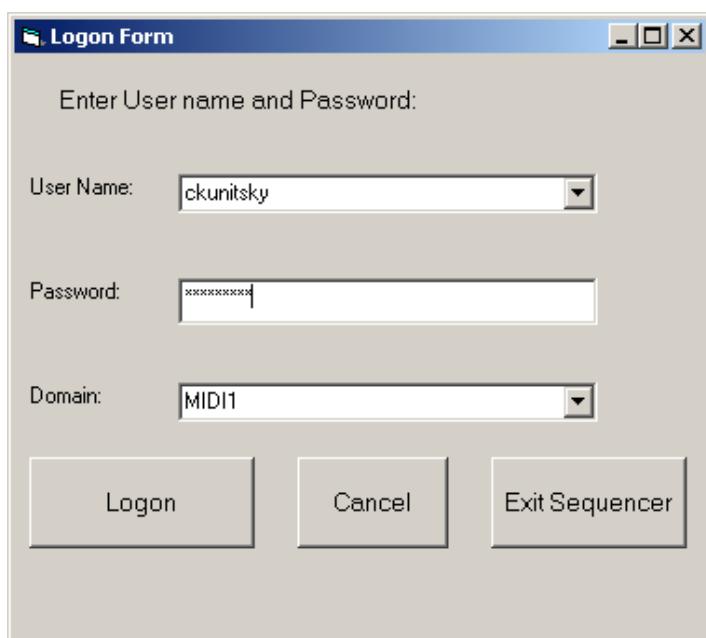
Note: If ERS is installed, the Logon Tool or Logoff Tool will be displayed in the Sample Processor Toolbar. In addition, the User field will appear in the Sample Table.

Sherlock ERS requires users to sign onto the system to enter samples as well as to view entries and results (Figure 3-6). If the user tries to perform any action in the Sample Processor before logging on, the following dialog box will appear (below):



Sherlock ERS can be programmed to log off if the system remains inactive for a set period of time. Detailed information on Sherlock ERS can be found in the Sherlock ERS manual.

Figure 3-6
Sherlock ERS Logon



Sample Processor Menu Bar

File / Print

Allows printing the current sample table, optionally including result information. The sample table can be printed at any time, even when running samples. The table is printed as soon as the printer is available. The printer used is the default printer for your computer.

File / Exit

Allows exiting the Sample Processor. If a batch is running, it is strongly recommended that the user abort or stop the batch before exiting. Exiting the Sample Processor while a batch is running will leave the system in an unstable state, and requires a PC shutdown / restart.

Table – Clear Options

Each of the table clear options removes samples from the sample table by erasing the sample identification number and name, and changing the bottle type to **EMPTY**. When the system is running samples, the editor will not allow the removal of **CALIB**, or **RUNNING** bottles from the table.

Table / Clear All Samples

Removes all Queued or Done samples, but not calibration entries.

Table / Clear If Done

Removes samples that have already been analyzed (bottles with a status of **DONE**).

Table / Reset Table

Removes all samples and calibration entries, and establishes a new calibration entry.

Table / Clear Current Bottle

Removes information about the currently selected bottle.

***Note:** Table Clear functions must be used with caution as there is no Undo command in the Sample Processor and there is no way to restore a sample table.*

Table / Set Auto ID Number

The **Auto ID Number** feature should be used to assign and increment the Sequence Number field automatically for each sample run. Each sample will then have a unique identifying number. Sequence numbers determine the order that samples will be run, but see “**Stat**” below for a way to override this behavior. By default this number will also be used as the ID number. The user may change the ID numbers for samples as needed. ID numbers don’t have to be unique (but typically should be), and can be up to nine digits long. The ID numbers are saved with the sample in the Sherlock data file.

***Note:** The Sequence # (Seq #...) cannot be edited.*

Batch / Start Batch

This function will start the ChemStation automatically and begin sample processing. A calibration vial is always the first vial analyzed. This function is only available when the Sample Processor is not currently running a batch.

Batch / Stop at end of run

Stops the batch after the chromatographic analysis of the current sample is completed and the reports are printed.

Batch / Abort Batch

Stops the current analysis immediately. Note that the current analysis is lost. To prevent potential problems with ChemStation, it is prudent to wait until ChemStation has completely loaded before aborting. Further, if an injection has already been made for the current sample, raise the temperature of the oven to 300° C for five minutes to remove any lingering compounds from the GC.

View / Reset

Resets the sample table, showing all the columns. Use this option if resizing columns, and if you want to view library search results during a batch. The Sample Processor can show results of sample runs in the sample table. The right-most column of the table can show the similarity index for the library entry with the highest match achieved by that sample. If there are multiple high matches, the similarity index is preceded by a plus sign ("+"). You should always review the sample's report for proper interpretation of the results.

View / Reset No Results

Resets the sample table, hiding results columns. Use this option if resizing columns, and if you want to return to a standard state. This view is especially useful for initially setting up a sample table.

Sample Processor Tool Bar

Note: A description of all the Sample Processor icons is given in Table 3-1 at the end of the chapter.

Add Samples



This places the system in a mode that simplifies adding new samples to the sample table. When the **Add Samples** button is pressed, the Sample Table editor automatically advances to the next empty bottle position in the sample table below a selected position.

The sample type, method, and status will be entered automatically by the system based on the previous sample's information, while the user will enter sample name and pertinent sample information (Figure 3-5). The Seq # is automatically incremented and displayed.

This is the only correct way to add new samples to the Sample Processor. The **Add Samples**



button will change to **Done Adding** while the user is adding samples to the table (Figure 3-7). Press this tool to complete adding samples. See details below on **Adding Samples and Calibrations**.

Note: The pencil icon (see Figure 3-7) in the left side of the sample table indicates that changes are outstanding for the current sample but not permanent yet.

When editing the sample table, you can change the size of the columns by placing the cursor between two of the dark gray sample table headers. The cursor will change to the following shape:



You can then resize this column.

*Figure 3-7
Sample Addition Screen*

COL A	COL B	COL C	COL D	COL E	COL F	COL G
COL A	AVAILABLE					
COL B	AVAILABLE					

Btl	Type	Method	User	Seq#	ID Num	Name	Status
1	Samp	TSBA6	ta	1001	1	Cal Mix Lot# 995140 TA	Queued
2	Samp	TSBA6	ta	1003	1003	QC-BLANK (07-12-05 TA	Queued
3	Samp	TSBA6	ta	1002	1002	QC-STEN-MALTO (ATCC # 13637	Queued
4	Samp	TSBA6	TA	1004	1004		Queued
5	Empty						Done
6	Empty						Done
7	Empty						Done
8	Empty						Done
9	Empty						Done
10	Empty						Done



Lock Table / Unlock Table

This tool toggles the table between **locked** and **unlocked**. When the Sample Table is **locked**, no changes can be made inadvertently to the table. When adding or editing samples, the table must be unlocked.



Print Table

This button is identical in function to the **File / Print** menu item. The printer used is the default printer on your computer.



Start Batch

This button is identical in function to the **Batch / Start Batch** menu item.



Stop At End Of Run

This button is identical to the **Batch / Stop at end of run** menu item.



Abort Batch

This button is identical to the **Batch / Abort Batch** menu item.

Adding Samples and Calibrations

The first time the Sample Table is used, a calibration entry will already be in place. Every Sample Table must contain a calibration entry and vial of Calibration Standard. Sherlock will automatically calibrate prior to sample analysis and will recalibrate the system after every 11 samples. Samples may be added before or during the processing of samples by Sherlock.

When the **Add Samples** function is selected, the Sample Table editor automatically advances to the first empty bottle position. The next sequence number will be entered automatically by the system. The status of the new sample is set to **QUEUED**. Enter 1 to 42 characters for the sample identification in the **NAME** field. There must be at least one character entered as a sample name. Strike the **[Enter]** key to advance to the next empty bottle position. To stop adding samples, press the **Done Adding** button; the table can also be locked by clicking the **Lock Tool** to ensure no inadvertent changes are made.

Editing the Sample Table

Generally, to change an existing entry in the Sample Table, **Unlock** the table, click on the field to change, and select the choice from that drop down menu or type in the change (Figure 3-5). Sample Table entries can be edited at any time, even while samples are being run on the GC. Samples listed with a status of **RUNNING** are protected from edits. It is a good idea to **Lock** the table when finished editing.

***Note:** The Sequence # (Seq#) column is not editable.*

Changing the Type

Click on the **Type** field to select the choice for priority:

- **SAMPLE**: Normal priority.
- **STAT**: High priority sample, processed next.
- **CALIB**: A calibration bottle.
- **EMPTY**: No bottle in this sample tray position.
- **BLANK**: This bottle contains reagent blank.
- **QC**: This bottle contains a quality control sample.

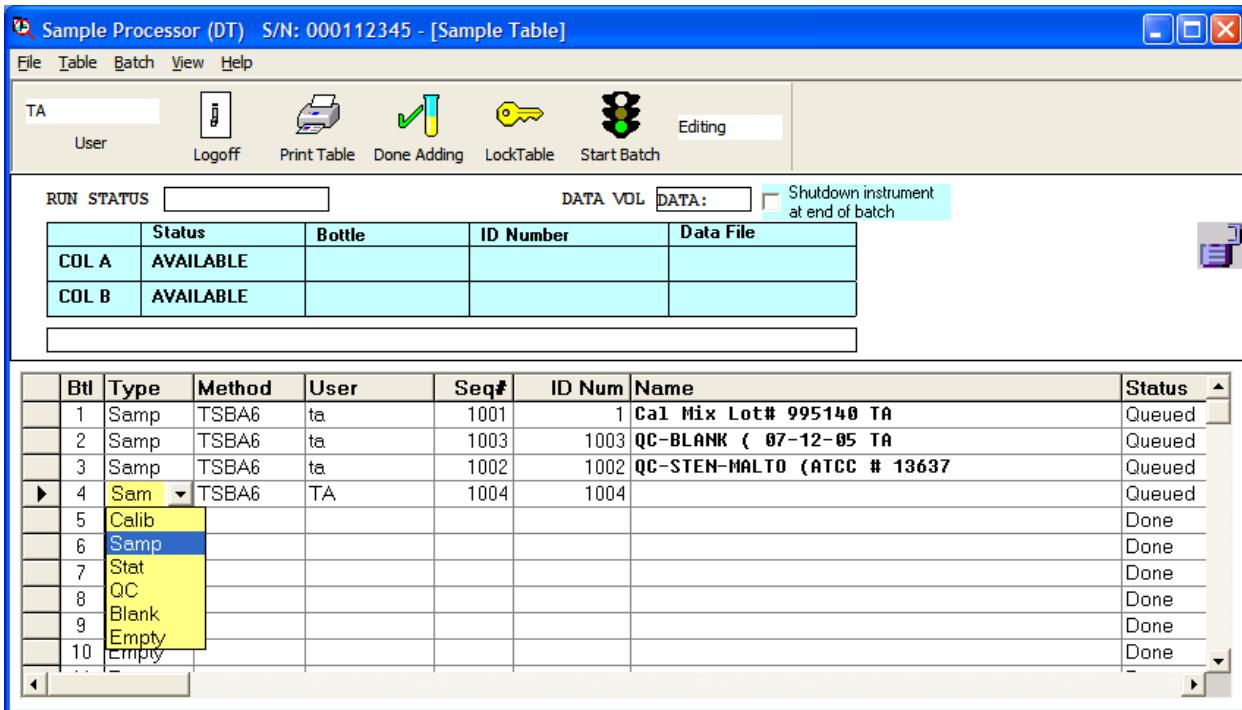
Changing the Sample Status

The status of the selected sample is either **QUEUED**, **DONE**, or **RUNNING**. To change from **QUEUED** to **DONE** or vice versa, click on the status field and choose from the drop down menu. Samples that have a status of **RUNNING** cannot be changed. If the user wants to process samples out of order, they can use the **STAT** function.

STAT samples are high priority samples that are processed before ordinary samples. Calibration analyses have priority over STAT samples. There must be at least one bottle of Calibration Standard in the sample tray. The user must also log the Calibration Standard into the Sample Table by specifying a sample type of **CALIB**. The Sequence Number field should automatically be set to 1 and cannot be changed.

***Note:** If multiple methods are loaded as part of a batch, (e.g. TSBA6, MOORE6, etc.) all samples of the first method will be run, regardless of the position on the Sample Table. However, if a sample from another method is selected as STAT, the system will finish the current run, recalibrate for the other method, and proceed to run all of the samples associated with the other method first.*

Figure 3-8
Editing Sample Table



Removing items from the Sample Table

If the table is not **Locked**, samples can be removed from the Sample Table by scrolling to the entry and choosing any of the Clear Table options or the Clear Current Bottle option from the menu bar.

Record Keeping

Enter enough data about the sample into the “Name” field so that anyone can go back a year later and know what was run. If the growth conditions were not standard for the Method being used, enter the nonstandard conditions in the name field. Also, enter any unusual observations that may aid in interpreting results. Consistency in the name is extremely important for record keeping as well as for cataloging samples and using the features of the CommandCenter for selecting samples. It is advantageous to organize name fields so that the user can look at groups of samples of interest. To compare groups of entries it will be necessary to create a system for making groups and subgroups very early in the data collection process. The name of a sample consists of two sections separated by an open parenthesis sign “(”. A total of 42 characters may be entered.

The section before the parenthesis should be broken up into fields using the hyphen “-“ character. We suggest using UN- in front of unknown samples. The fields should be from most general to most specific in information. For instance, an unknown sample from position 3 of lab 5 as part of project B might be coded “UN-PROJB-LAB5-POS3”.

The second section of the name begins with an open parenthesis sign “(”, and allows the user to enter discrete information about a sample, such as an identification number. If not using Electronic Records and Signatures, it is helpful to use the initials or some personal identifier for the person logging the sample into the computer. That person is verifying that the samples are placed in the correct positions on the auto sampler tray. It is not necessary to close the parenthesis or use hyphens after the open parenthesis. Once it “sees” the left parenthesis, the software looks no further for cataloging functions. While the user will be able to view this information in any file listing, it is for laboratory use only and the software does not use it. A full sample ID might look like “UN-PROJB-LAB5-POS3 (1234 GJ”

Starting a Batch

Sherlock is ready to analyze samples when the following are achieved:

- Verified that the proper printer parameters are in use, and that the printer has paper.

Note: See Pages 3-4 and 3-6 if immediate printing of results is not desired.

- Confirmed that each sample has the correct sample information logged into the sample table.
- Loaded the sample tray with the samples and the Calibration Standards corresponding to the sample table entries.
- Check that the wash bottle is full of Reagent 3.
- Confirm that there is sufficient gas to perform the analyses. For tanks, >200psi on dual stage regulator. For hydrogen generator, sufficient water and pressure (>60psi).
- Confirmed that ChemStation is Not open or running.

When the above steps have been taken, the system is ready to start. To start a batch, press on the



Start Batch Tool in the Toolbar. The sample table will be checked for common errors by the software.

- If prompted, make any necessary adjustments to the sample table.
- If no adjustments are needed, click on Start Batch.
- The system analyzes the first Calibration Standard, and if the system is working properly will proceed through the samples in the sample table.

While the system is running a batch, other commands are still active.

- Click on the sample table to make additions or edits to the sample table.

- Click on the Stop Batch icon if it is necessary to stop the operation of the system before completion of the batch.

Stopping a Batch

Once Sherlock has started a batch, the *Start Batch* Tool is replaced with the *Stop Batch* Tool



Note: This function is the preferred way to stop a batch before all samples have been analyzed. It is not possible to stop the Batch correctly using the ChemStation Abort function from the ChemStation menu bar. The GC will stop the run and return to a standby status, but Sherlock will not be able to proceed with the batch or communicate with ChemStation, and it will be difficult to close.

Note: Restart the computer if the Sample Processor gets disconnected from the ChemStation.

Two Sherlock menu options describing the options for correctly terminating a batch will appear:

- Click on **[Stop At End Run]** to complete the analysis in progress, print the results, and then stop the batch.
- Click on **[Abort Run]** to immediately stop the analysis in progress and stop the batch.

The **Abort Run** option can stop the current chromatographic run at any point within analysis. If the sample injection has occurred, any non-eluted compounds will remain on the chromatographic column.

Note: Never Abort Sherlock while ChemStation is loading. There will be time after ChemStation is loaded and before the first injection to halt the batch.

Instrument Shutdown

When a batch completes, the system will automatically shut down the instrument if the “Shutdown instrument at end of batch” checkbox is visible and checked. The Gas Chromatograph’s temperatures and flows will be lowered to save gases and energy.

The instrument shutdown feature is not available for the 7890 GC in Sherlock version 6.1.

Sample Processor Tools (Icons)

The icons associated with the Sample Processor are described in Table 3-1. Most of these *Tools* in the *Toolbar* can be accessed through the *Menu Bar*, but the *Tools* provide a shortcut to these features.

Table 3-1
Sherlock Sample Processor Tools

Name	Icon	Description
ERS Logon		With Electronic Records and Signatures (ERS) enabled, this button allows an authorized user to sign on.
ERS Logoff		Enables the user to log off from ERS.
Print Table		Used to print the sample table in the Sherlock Sample Processor.
Add Sample		Allows the user to add samples to the sample table.
Done Adding Sample		Press this button when done adding samples to the sample table.
Lock Sample Table		Allows the user to lock the sample table from editing.
Unlock Sample Table		Enables the user to unlock the sample table.
Start Batch		Allows the user to automatically load the Agilent ChemStation and begin sample processing.
Stop Batch		Stops the batch at the end of the current chromatographic run.
Abort Batch		Stops the current chromatographic run immediately.

Chapter 4

Interpreting Sherlock Reports

Overview

The **Sherlock Composition Report** consists of several sections, summarized below:

- **Pre-Sample File** (optional)
- **General Sample Information**- contain data location, batch and operator information, as well as the creation date and time.
- **Sample Profile Information**- contains the fatty acid composition of the sample. This section also contains the summary area, which is useful to troubleshoot the system.
- **Library Matches**- lists the results of comparing the fatty acid composition to the Sherlock Libraries.
- **Comparison Charts** (optional)- a graphical comparison of the current sample to the library entry matches.
- **Sample Chromatogram** (optional)- ChemStation chromatogram for the current sample.
- **Post-Sample File** (optional)

The first part of the Sherlock Composition Report will be shown in Figure 4-1 and the second half of the report will be shown in Figure 4-4. Each of the sections will be described after these figures.

***Note:** In Figures 4-1 and 4-4, the red text indicates the section of the Sherlock Composition Report.*

Figure 4-1
Sherlock Composition Report- First Half (red text shows sections)

Pre-Sample File (if included)

General Sample Information

Volume: DATA	File: E05A105.80A	Samp Ctr: 11	ID Number: 1049
Type: Samp	Bottle: 10	Method: CLIN6	
Created: 10/10/2005 5:59:20 PM		Created By: administrator	
Sample ID: PA-27853			

Sample Profile Information

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.572	4.662E+8	0.025	----	7.039	SOLVENT PEAK	----	< min rt	
3.005	752	0.026	1.147	10.000	10:0	0.12	ECL deviates 0.000	Reference 0.001
4.051	21604	0.028	1.050	11.418	10:0 3OH	3.23	ECL deviates -0.004	
4.571	25989	0.031	1.022	12.000	12:0	3.78	ECL deviates 0.000	Reference 0.001
5.067	485	0.032	1.005	12.440	11:0 3OH	0.07	ECL deviates 0.002	
5.931	39276	0.034	0.981	13.172	12:0 2OH	5.49	ECL deviates -0.005	
6.080	390	0.034	0.979	13.282	12:1 3OH	0.05	ECL deviates -0.006	
6.304	37276	0.035	0.975	13.449	12:0 3OH	5.17	ECL deviates -0.005	
7.046	2879	0.036	0.964	13.999	14:0	0.40	ECL deviates -0.001	Reference 0.001
9.915	63927	0.043	0.948	15.813	Sum In Feature 3	8.63	ECL deviates -0.009	16:1 w7c/16:1 w6c
10.225	218661	0.040	0.948	16.001	16:0	29.50	ECL deviates 0.001	Reference 0.002
11.311	445	0.044	0.947	16.631	17:0 iso	0.06	ECL deviates 0.001	Reference 0.002
11.585	2067	0.045	0.947	16.790	17:1 w8c	0.28	ECL deviates -0.002	
11.747	8563	0.047	0.947	16.884	17:0 cyclo	1.15	ECL deviates -0.004	
11.945	2943	0.043	0.947	17.000	17:0	0.40	ECL deviates 0.000	Reference 0.000
13.203	3105	0.045	0.947	17.716	Sum In Feature 5	0.42	ECL deviates -0.004	18:2 w6,9c/18:0 ante
13.291	10669	0.045	0.947	17.767	18:1 w9c	1.44	ECL deviates -0.002	
13.389	256140	0.047	0.947	17.822	Sum In Feature 8	34.55	ECL deviates -0.001	18:1 w7c
13.700	7291	0.049	0.947	17.999	18:0	0.98	ECL deviates -0.001	Reference -0.001
15.270	30200	0.045	0.945	18.898	19:0 cyclo w8c	4.07	ECL deviates -0.004	
16.118	1069	0.068	0.943	19.388	20:4 w6,9,12,15c	0.14	ECL deviates -0.007	
16.722	467	0.040	0.940	19.737	20:2 w6,9c	0.06	ECL deviates 0.005	
----	63927	---	----	----	Summed Feature 3	8.63	16:1 w7c/16:1 w6c	16:1 w6c/16:1 w7c
----	3105	---	----	----	Summed Feature 5	0.42	18:2 w6,9c/18:0 ante	18:0 ante/18:2 w6,9c
----	256140	---	----	----	Summed Feature 8	34.55	18:1 w7c	18:1 w6c

ECL Deviation: 0.004

Reference ECL Shift: 0.001

Number Reference Peaks: 7

Total Response: 735705

Total Named: 734201

Percent Named: 99.80%

Total Amount: 706575

Summary Area

Library Matches

Library	Sim Index	Entry Name
CLIN6 6.00	0.954	Pseudomonas-aeruginosa
	0.699	Pseudomonas-aeruginosa-mucoid strains

Post-Sample File (if included)

Pre-Sample and Post-Sample Files

See Pages 3-6 and 3-7 for more information about setting up this file.

General Sample Information

As specified by the user with **Sherlock Sample Processor Configuration**, the computer stores the chromatographic data in files on the selected data volume. For each batch of samples, a file name is automatically assigned by the computer and is printed at the top of the Composition Report. The area of the Sherlock Composition Report identifies the sample with the sample ID number, bottle number, sample name, and the date and time of the analysis. If the sample has been edited, the date and time of the last edit is also printed.

Sample Profile Information

Each peak from the chromatographic analysis is listed by retention time (RT), Response, and area/height ratio (AR/HT). Each detected peak in the chromatographic plot from the ChemStation should be listed in the printed report. Included in the composition report is the equivalent chain length (ECL), a linear interpolation of each peak's relative retention between two saturated straight-chain fatty acid methyl ester reference peaks. The Sherlock software compares the ECL of each peak in the analysis with the expected ECL of the fatty acids in the Peak Naming Table. The fatty acid name is printed in the Peak Name column. Peaks that do not correspond to ECL values of known fatty acid peaks are left unnamed and are not used in the library search.

After the peak areas are modified by the quantitative response factor and are normalized to 100%, the resulting weight percent is listed. The response factor adjustment, derived from running the quantitative Calibration Standard, corrects the area counts for long-term drift and instrument-to-instrument variation. The final two columns of the report are used to give additional information about the peak. The solvent area (Solvent Peak) is directly proportional to the amount of solvent (hexane/MTBE) injected. The solvent peak should be at least 15,000,000 area counts to ensure that a proper amount of extract was analyzed by the system. The solvent peak is not included in the weight percent calculation.

The Summary Section of the Sample Profile is useful to troubleshoot the system.

The total area count (Total Response) of peaks eluting at or between C9:0 and C20:0 is related to the total extracted fatty acids. Peaks eluting outside of this range are not named or used in the library search. The total area of all named peaks (Total Named) is listed with the percentage named (Percent Named). After correcting each peak's area by the response factor and summing, the total amount (Total Amount) is listed. For fatty acid analysis using MIDI Calibration Standard 1, this response should be between 1,000,000 and 1,600,000 (see Figure 4-5). A number of reference peaks (Number 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.

Some identified peaks are not used as part of the fatty acid profile. These “zeroed” peaks are assigned no percentage in the profile. Often these peaks are unknown compounds or compounds whose percentages are unreliable and would cause undesirable variance in the profile.

Note: Each analysis utilizes the quantitative calibration results. It is important to calibrate the system with fresh standards regularly.

Messages are printed in the Summary Section of the Composition Report to warn you of possible errors. The warning messages help you troubleshoot the system by suggesting chromatographic solutions. See Chapter 7 - Troubleshooting for detailed descriptions of the warning messages and probable solutions.

Sample Profile Messages

Messages are printed on the Composition Report to help you evaluate the analysis. Each peak in the composition report has a Name field and two Comment fields that are used to convey specific information about that individual peak. The most common messages are those that describe or identify chromatographic features. These messages are listed below with a brief description.

Column Overload

The maximum area count for a peak has been exceeded, which could result in peak misidentification. Dilute the sample with clean solvent (Reagent 3) and re-analyze the sample.

< min ar/ht

The peak width is narrower than expected for the fatty acid methyl esters. This indicates noise (spikes) from a dirty detector. See your GC manual for proper detector cleaning procedures.

> max ar/ht

The peak width is wider than the expected value for fatty acid methyl ester peaks. This indicates contamination of the analysis. Check the reagent control for the same peak (same ECL). If the problem persists, check the injection port liner for septum debris.

< min rt

The peak elutes before C9:0 so it is not identified as a fatty acid methyl ester of interest. The peak is not used in the library search.

>max rt

The peak elutes after C20:0 so it is not identified as a fatty acid methyl ester of interest. The peak is not used in the library search.

Reference

This peak is used as a reference peak to adjust the retention times of the other peaks within the analysis. The distance and direction (\pm in ECL units) that the system adjusted the peak are given.

< min response

In a calibration analysis, this peak is less than the acceptable area from the Calibration Control Parameters Table. Recalibrate using a fresh bottle of the Calibration Standard. If the error remains, check for a plugged syringe.

> max response

In a calibration analysis, the peak is greater than the expected percentage from the Peak Naming Table. If the bottle of Calibration Standard has been used for several days, recalibrate using a fresh bottle of the Calibration Standard. If the error remains, change the septum and the injection port liner or check the split flow.

Sum in Feature

The ECL value of the peak corresponds to one of the fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. This fatty acid comprises a portion of a *summed feature*. The total percentage of all the acids that are grouped as one feature is printed at the end of the fatty acid composition list.

Q-check

The peak response count in the Calibration Standard is less than or more than expected. Rerun the analysis. If the error remains, take the following actions one at a time and retry: Replace the Calibration Standard with a fresh bottle, change the injection port septum, change the injection port liner, and replace the capillary column. Consult MIDI technical support before changing the column to insure it is necessary.

Solvent peak

Each analysis will have the solvent (hexane/MTBE) peak identified. Since this peak elutes before C9:0, the <min rt message will appear in the comment field.

Summed Feature

The total calibrated area and area % of those peaks identified as a member of a summed feature are given at the end of the Composition listing.

Library Matches

Once a microorganism has been cultured, processed, and properly analyzed by Sherlock, its fatty acid composition can be matched with those of known organisms that are stored in the Standard Libraries. The Standard Library profiles have been carefully developed to take into account strain-to-strain and experimental variation. The Peak Naming Tables used are also designed with a built-

in ability to recognize those acids that may be related to other acids in an extract. Thus, if one acid is a precursor of another acid in a bacterium, the software can account for a decrease in one acid and an increase in the other. The library search is rapid. The naming of the unknown is available immediately upon completion of the gas chromatographic analysis. The Sherlock Library Search Report lists the most likely matches to the unknown composition, and provides a similarity index for each match.

Interpreting the Library Search

If the search results in more than one possible match, the suggested identities are listed in descending match quality. The matches are listed in order of similarity index, with the highest similarity index match listed first.

*Figure 4-2
The Library Search Result*

Library	Sim Index	Entry Name
CLIN6 6.00	0.667	Staphylococcus-warneri-GC subgroup B
	0.487	Staphylococcus-aureus-GC subgroup A
	0.471	Staphylococcus-aureus-GC subgroup B

Similarity Index (SI)

Many microbiology identification systems present results as a “probability” percentage. Thus, the system may report a 98% probability for the identification of an isolate. The basic assumption behind these “probabilities” is that species are well-defined groups of organisms with little variation in how they perform certain biochemical tests. Since comparisons have traditionally been made between two or more biochemical test systems, the comparisons were nothing more than how well the systems perform similar enzyme assays. Even when the identification is incorrect, the “probability” of the identification can be quite high and may be “confirmed” using a similar enzyme assay system.

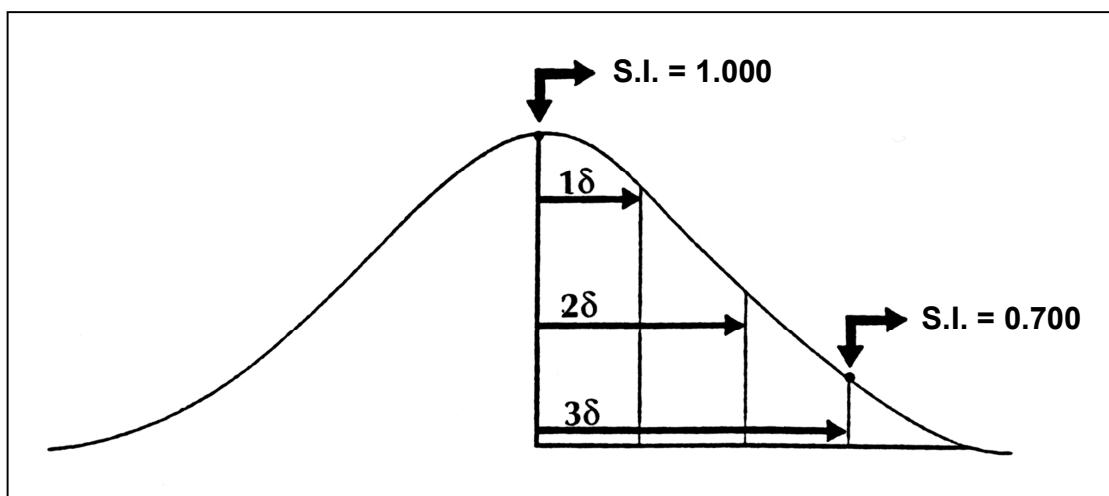
More recently developed techniques such as DNA homology and fatty acid analysis require other ways to express identification of microorganisms. The current standard for DNA homology is that above 70% DNA homology would indicate that two strains are of the same species.

The technique used by the Sherlock system is based on Similarity Index. The Similarity Index is a numerical value, which expresses how closely the fatty acid composition of an unknown compares with 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. This value

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. Thus, it is not a “probability” or percentage, but an expression of the relative distance from the population mean. An exact match of the fatty acid makeup of the unknown 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 Similarity Index will decrease in proportion to the cumulative variance between the composition of the unknown and the library entry.

The Similarity Index assumes that species of microorganisms have normal Gaussian distribution (the classic “bell shaped curve”) and that the mean of the population in any series of traits (e.g., fatty acid percentages) characterizes the group. Most of the population falls somewhere near the mean, but individuals will differ in composition and thus may show considerable variance from the mean. Another way of visualizing the Similarity Index is by looking at the Gaussian distribution of the population (in this case, the fatty acid composition). In Figure 4-3, the perfect mean percentage for all fatty acids in a single species entry (no variance on any fatty acid) is indicated by the line at the center. The Similarity Index for a strain that falls on this line in 1.000. As the variance increases, the strain falls further and further from the line, and the Similarity Index drops.

*Figure 4-3
Population Distribution in TSBA6 / RTSBA6 Library*



As one can see in Figure 4-3, in the TSBA6 / RTSBA6 Library, a strain with a Similarity Index of 0.700 falls within a three standard deviation window of the mean.

Interpretation Guidelines

Use the following guidelines when interpreting the Similarity Index. Samples with a similarity of 0.500 or higher with a separation of 0.100 between the first and second choice are considered good library comparisons. If the Similarity Index is between 0.300 and 0.500 and well separated from the second choice (>0.100 separation), it may be a good match, but an atypical strain (it would fall very far away from the mean on the normal distribution curve in Figure 4-3). Values lower than

0.300 suggest that we do not have the species in the database, but the software will indicate the most closely related species.

Sherlock Comparison Charts

A visual representation of the results of the library search is (optionally) given after the listings of the best possible matches and corresponding Similarity Indices. You can select 0, 1, 2, 3, or 4 charts as an option when searching libraries. You can view comparison charts automatically after each sample run (see Page 3-6 for information on how to turn on the comparison chart feature) or in Sherlock CommandCenter *Samples View* (See Chapter 5 - *Sherlock CommandCenter*, for information on how to view and print comparison charts). An example of a comparison chart is shown in the top of Figure 4-4. All fatty acids found in the extract and the library entries are listed in elution order on the left side of the chart. A scale of percentages is printed across the bottom of the chart. For each acid, the bar represents a ± 2 standard deviation window around the entry mean. The library entry mean-value for an acid is identified with bold vertical line. An oval is placed on the line opposite the fatty acid name indicating the amount of that acid in the sample. Examination of the chart may give the user a better understanding of the quality of the match than the Similarity Index; however, it must be remembered that the Similarity Index has been calculated using all the features and their cross correlation terms. The relationship of fatty acids to one another (cross correlation terms) is not evident in the comparison charts.

Sample Chromatogram

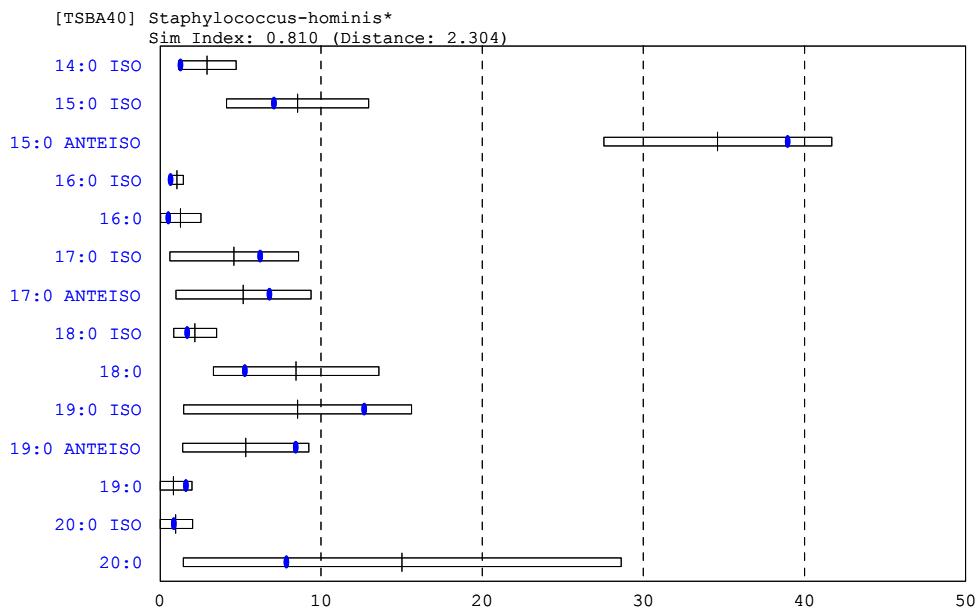
The ChemStation delivers not only the raw data but also the chromatogram to Sherlock (Figure 4-4). The chromatogram is a visual plot or trace of the electronic signal generated by the flame ionization detector (FID) as it burns the fatty acids eluting from the column. The raw data of the chromatogram is stored in a ChemStation file and can be reintegrated on-screen and reprinted if desired. Sherlock stores a file containing all peak retention times, response, and area/height ratios. In routine use of Sherlock, the chromatogram with the peak times plotted by the ChemStation normally need not be evaluated. However, you should be familiar with the contents of the plotted chromatogram to confirm proper operation of the system.

*Note: The sample chromatograms can also be viewed in the Sherlock CommandCenter *Samples View* (see Chapter 5) and in Sherlock ERS View (see ERS manual).*

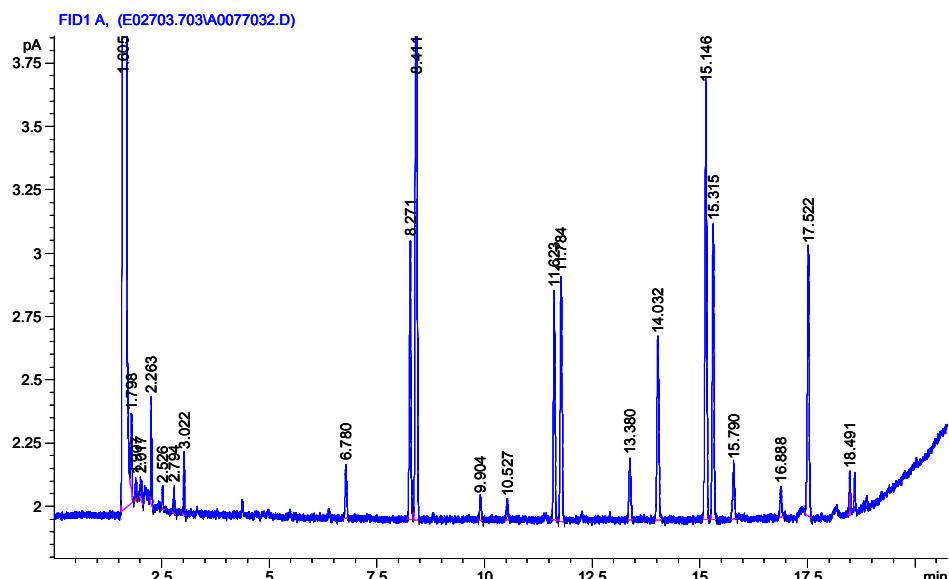
Fatty acids in the sample are separated by the column and identified by the retention time of each peak. Retention times are measured to a resolution of 0.001 minutes (0.0001 minutes for rapid and instant methods).

*Figure 4-4
Sherlock Composition Report- Second Half (red text shows sections)*

Comparison Charts



Sample Chromatogram



Post-Sample File (if one)

Calibration Reports

Calibration analyses are automatically run according to the Calibration Sequence Table as described in *Analyzing Samples, Chapter 3*. When a calibration analysis is scheduled, the computer checks the results against the Peak Naming Table for a specific number of peaks and a pattern of retention times and area percent amounts. If the analysis results in a report with peak data outside of the tolerance range set for the calibration, a warning is printed on the message line of the report and the calibration is repeated.

Note: Many potential calibration problems are subtle. Correct operation of the system still requires diligent operation and care.

Calibration Standard

For the Standard Aerobe, Anaerobe and Yeast Methods, Calibration Standard 1 (Microbial ID Part # 1200-A) is the first analysis of every Sherlock batch, and is automatically reanalyzed after every 11th sample injection. For the Rapid, Sensitive and *Instant* FAME methods, the Rapid Calibration Standard (Microbial ID Part # 1300-AA) should be used. Both standards have the same mix of compounds. The straight chain C9:0 to C20:0 fatty acid methyl esters are used by the system to quantitatively calibrate and compensate for peak area discrimination between the low and high boiling point fatty acids. Five hydroxy acids are added to the mixture to detect injection port liner or column degradation, which can result in poor peak shape (tailed hydroxy peaks) or an actual 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. Deviations from the expected values result in a failure to calibrate, and a warning message to the user.

A second function of the Calibration Standard is 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 analyses are named. The system calculates how much the calibration analysis has deviated from the expected relative retention times and reports the Root Mean Square (RMS) fit error. If a calibration run is invalid due to a high RMS fit error, Sherlock will print a message to warn the user, then will repeat the calibration analysis. If the system fails to calibrate after two consecutive attempts, the error message will be repeated and the batch aborted on this column.

If the library contains an entry for the Calibration Standard, the match factor (Similarity Index) can be used to monitor the overall performance of the system. The similarity index drops rapidly when the quantitative recovery of hydroxy fatty acids becomes unacceptable. If a Calibration returns with a No Match and a standard MIDI method and library is being used, that indicates a problem with quantitation. To prevent this, it is important to maintain a PQ Table as recommended in *Chapter 7 – Troubleshooting* and take corrective action . (If you have a custom library and the system returns with a no match, that library may not have an entry for the typical good calibration profile.)

The calibration report should be examined for signs of poor hydroxy fatty acid recovery. As the performance degrades to an unacceptable level, a small peak at 10.914 ECL usually appears, indicating the breakdown of the 14:0 3OH hydroxy peak to a 12:0 aldehyde. Also the 14:0 3OH peak may drop below 1.00% or the 16:0 2OH peak may drop below 2.05%, indicating poor performance. If any of the above cases are observed, corrective action should be taken. The injection port liner should be changed (see *Chapter 6 - Routine Maintenance* for details).

Acceptable Calibration

Shown below, Figure 4-5 is a calibration report of the hydroxy recovery in the range of acceptable operation. The Troubleshooting chapter (Chapter 6) also contains examples of a very good, a marginally acceptable, and a bad calibration report, and suggestions for corrective action.

*Figure 4-5
Calibration Report of Acceptable Recovery*

Volume: DATA	File: E05A174.32A	Samp Ctr: 1	ID Number: 1																																																																																																																																																																																																						
Type: Calib	Bottle: 1	Method: RTSBA6																																																																																																																																																																																																							
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<table border="1"> <thead> <tr> <th>RT</th><th>Response</th><th>Ar/Ht</th><th>RFact</th><th>ECL</th><th>Peak Name</th><th>Percent</th><th>Comment1</th><th>Comment2</th></tr> </thead> <tbody> <tr><td>0.7228</td><td>1.413E+9</td><td>0.014</td><td>----</td><td>6.6801</td><td>SOLVENT PEAK</td><td>----</td><td>< min rt</td><td></td></tr> <tr><td>1.0723</td><td>77342</td><td>0.012</td><td>1.237</td><td>9.0000</td><td>9:0</td><td>5.35</td><td></td><td></td></tr> <tr><td>1.2230</td><td>164529</td><td>0.011</td><td>1.167</td><td>10.0000</td><td>10:0</td><td>10.73</td><td>Peak match 0.0000</td><td></td></tr> <tr><td>1.4146</td><td>86928</td><td>0.010</td><td>1.108</td><td>11.0000</td><td>11:0</td><td>5.39</td><td>Peak match -0.0008</td><td></td></tr> <tr><td>1.4557</td><td>36597</td><td>0.010</td><td>1.099</td><td>11.1771</td><td>10:0 2OH</td><td>2.25</td><td>Peak match 0.0008</td><td></td></tr> <tr><td>1.5187</td><td>17649</td><td>0.010</td><td>1.085</td><td>11.4480</td><td>10:0 3OH</td><td>1.07</td><td>Peak match -0.0003</td><td></td></tr> <tr><td>1.6470</td><td>180972</td><td>0.009</td><td>1.059</td><td>12.0000</td><td>12:0</td><td>10.72</td><td>Peak match -0.0007</td><td></td></tr> <tr><td>1.9137</td><td>94075</td><td>0.009</td><td>1.019</td><td>13.0000</td><td>13:0</td><td>5.36</td><td>Peak match -0.0009</td><td></td></tr> <tr><td>2.2065</td><td>193471</td><td>0.009</td><td>0.987</td><td>14.0000</td><td>14:0</td><td>10.67</td><td>Peak match 0.0002</td><td></td></tr> <tr><td>2.5153</td><td>99706</td><td>0.009</td><td>0.961</td><td>15.0000</td><td>15:0</td><td>----</td><td>Peak match -0.0013</td><td></td></tr> <tr><td>2.5889</td><td>41709</td><td>0.009</td><td>0.956</td><td>15.2321</td><td>14:0 2OH</td><td>2.23</td><td>Peak match 0.0025</td><td></td></tr> <tr><td>2.6789</td><td>21116</td><td>0.009</td><td>0.951</td><td>15.5157</td><td>Sum In Feature 2</td><td>1.12</td><td>Peak match -0.0014</td><td>14:0 3OH/16:1 iso I</td></tr> <tr><td>2.8325</td><td>202536</td><td>0.009</td><td>0.942</td><td>16.0000</td><td>16:0</td><td>10.66</td><td>Peak match -0.0009</td><td></td></tr> <tr><td>3.1498</td><td>103997</td><td>0.009</td><td>0.928</td><td>17.0000</td><td>17:0</td><td>5.39</td><td>Peak match 0.0020</td><td></td></tr> <tr><td>3.2340</td><td>43982</td><td>0.010</td><td>0.924</td><td>17.2669</td><td>16:0 2OH</td><td>2.27</td><td>Peak match -0.0014</td><td></td></tr> <tr><td>3.4652</td><td>209334</td><td>0.009</td><td>0.917</td><td>18.0000</td><td>18:0</td><td>10.73</td><td>Peak match -0.0022</td><td></td></tr> <tr><td>3.7724</td><td>105684</td><td>0.010</td><td>0.910</td><td>19.0000</td><td>19:0</td><td>5.38</td><td>Peak match 0.0013</td><td></td></tr> <tr><td>4.0733</td><td>211227</td><td>0.009</td><td>0.905</td><td>20.0000</td><td>20:0</td><td>10.68</td><td></td><td></td></tr> <tr><td>4.2435</td><td>462</td><td>0.011</td><td>----</td><td>20.5659</td><td></td><td>----</td><td>< min response</td><td></td></tr> <tr><td>----</td><td>21116</td><td>--</td><td>----</td><td>----</td><td>Summed Feature 2</td><td>1.12</td><td>12:0 aldehyde ?</td><td>unknown 10.9525</td></tr> <tr><td>----</td><td>-----</td><td>--</td><td>----</td><td>----</td><td></td><td>----</td><td>16:1 iso I/14:0 3OH</td><td>14:0 3OH/16:1 iso I</td></tr> </tbody> </table>				RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2	0.7228	1.413E+9	0.014	----	6.6801	SOLVENT PEAK	----	< min rt		1.0723	77342	0.012	1.237	9.0000	9:0	5.35			1.2230	164529	0.011	1.167	10.0000	10:0	10.73	Peak match 0.0000		1.4146	86928	0.010	1.108	11.0000	11:0	5.39	Peak match -0.0008		1.4557	36597	0.010	1.099	11.1771	10:0 2OH	2.25	Peak match 0.0008		1.5187	17649	0.010	1.085	11.4480	10:0 3OH	1.07	Peak match -0.0003		1.6470	180972	0.009	1.059	12.0000	12:0	10.72	Peak match -0.0007		1.9137	94075	0.009	1.019	13.0000	13:0	5.36	Peak match -0.0009		2.2065	193471	0.009	0.987	14.0000	14:0	10.67	Peak match 0.0002		2.5153	99706	0.009	0.961	15.0000	15:0	----	Peak match -0.0013		2.5889	41709	0.009	0.956	15.2321	14:0 2OH	2.23	Peak match 0.0025		2.6789	21116	0.009	0.951	15.5157	Sum In Feature 2	1.12	Peak match -0.0014	14:0 3OH/16:1 iso I	2.8325	202536	0.009	0.942	16.0000	16:0	10.66	Peak match -0.0009		3.1498	103997	0.009	0.928	17.0000	17:0	5.39	Peak match 0.0020		3.2340	43982	0.010	0.924	17.2669	16:0 2OH	2.27	Peak match -0.0014		3.4652	209334	0.009	0.917	18.0000	18:0	10.73	Peak match -0.0022		3.7724	105684	0.010	0.910	19.0000	19:0	5.38	Peak match 0.0013		4.0733	211227	0.009	0.905	20.0000	20:0	10.68			4.2435	462	0.011	----	20.5659		----	< min response		----	21116	--	----	----	Summed Feature 2	1.12	12:0 aldehyde ?	unknown 10.9525	----	-----	--	----	----		----	16:1 iso I/14:0 3OH	14:0 3OH/16:1 iso I
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Chapter 5

Sherlock CommandCenter

Overview

To facilitate regenerating reports and data retention, Sherlock stores data from calibration and sample runs into Sherlock data files, which are then stored into data volumes (labeled DATA, DATA1,...DATA7) on the hard disk. Typically, you will collect data from recent batches into the DATA volume and later organize it into other volumes (described in Chapter 6). Sherlock shares the hard disk with the Agilent ChemStation and any other applications that are on the computer. A minimum of one gigabyte of free disk space available for installation and 800 megabytes free for operation is recommended.



The **Sherlock CommandCenter**,  is the heart of the Sherlock software. Instructions on how to use the software for viewing stored sample and calibration data, regenerating reports, and correcting data entry errors is presented in this chapter. The chapter first illustrates how to select and view sample data using either the *Basic Sample* or *Advanced Sample Selector* modes. Then, the chapter describes how to view installed methods and libraries. Chapter 6 describes how to install and update methods and also covers general maintenance functions, such as how to backup and restore data. Chapter 6 also describes the Sherlock disk structures and how to remove old data to maintain the free disk space needed for reliable operation.

Note: The Sherlock CommandCenter is icon intensive and descriptions of all the icons used in the CommandCenter are given in Tables 5-2 through 5-5 at the end of this chapter.

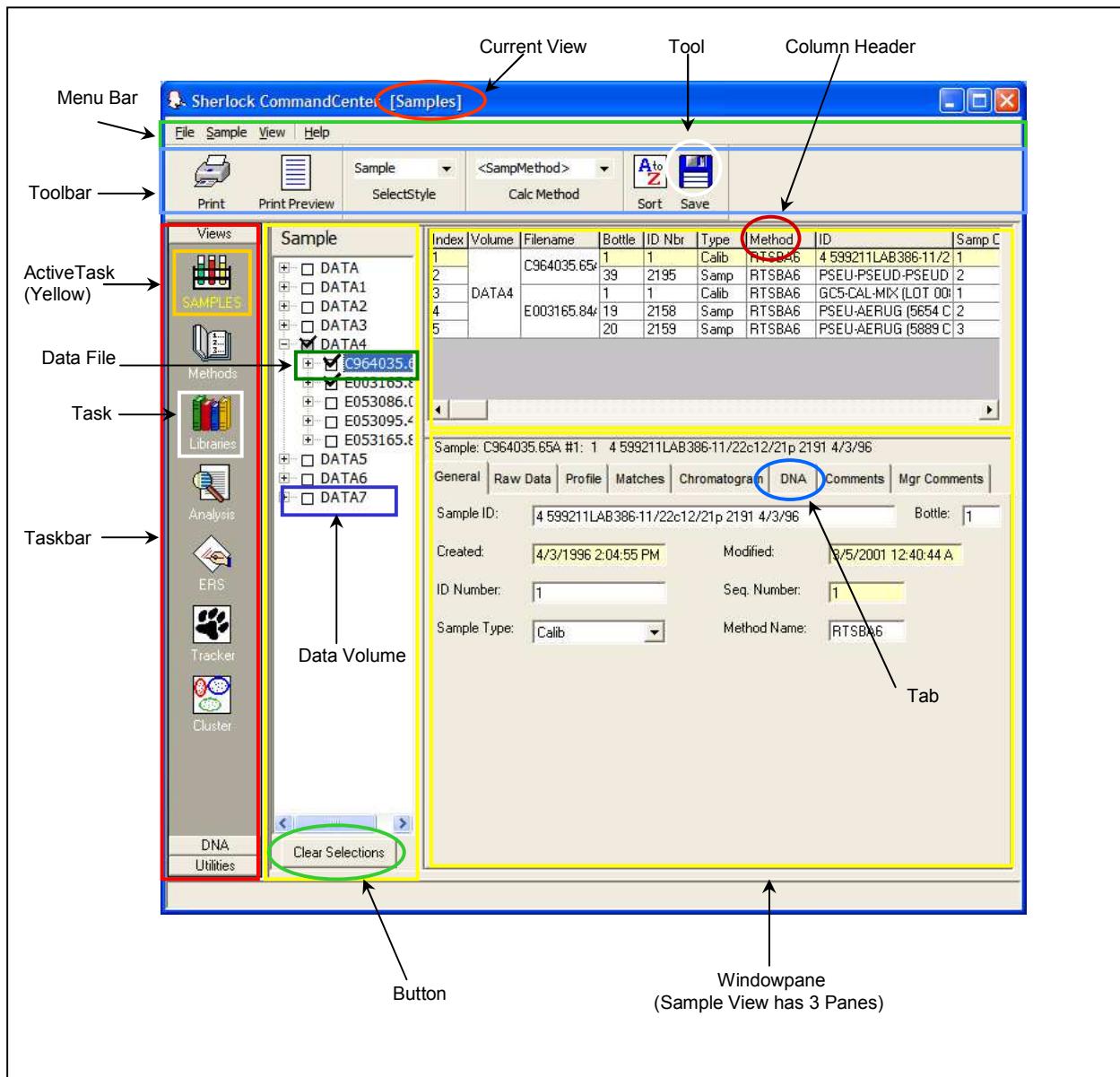
Sherlock Terminology

The Sherlock software uses some terminology that you should be familiar with to better follow the manual text and understand the operation of the software.

Figure 5-1 illustrates some of the commonly used terminology with the Sherlock interface. All of the *Tools* in the *Toolbar* can be accessed through the *Menu Bar*, but the *Tools* provide a shortcut to these features. By clicking on any of the *Column Headers*, you can sort that column in alphabetical or numerical order. Also, when the mouse cursor is placed over any horizontal or vertical resizing

bar within the CommandCenter window, the cursor changes shape to one of the following:  or  or . You can then resize this field. The Taskbar may contain several additional  Tasks, such as  (ERS), or  (Tracker), if these modules have been purchased. Please refer to the manuals that correspond to each of the modules if you need further information.

Figure 5-1
Sherlock CommandCenter Vocabulary



Sample Selection

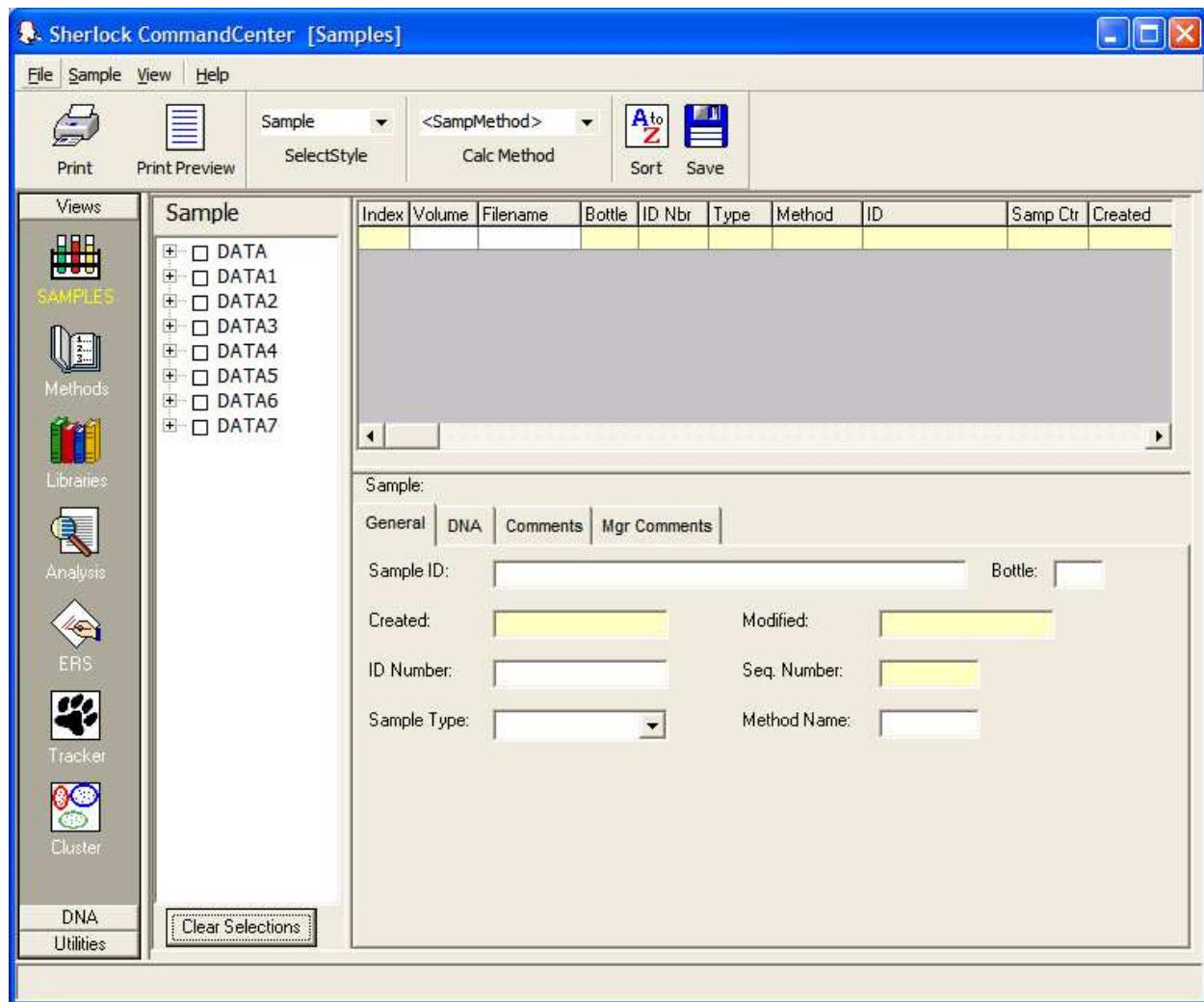
The Sherlock CommandCenter contains tools that allow you to view and print sample and

calibration data stored on the hard disk. Double click the Sherlock *CommandCenter* Icon  on the desktop to start the CommandCenter application. Figure 5-2 shows the CommandCenter main screen. The CommandCenter always calls up the view from the last time it was used. If it is



not in the *Samples View* as shown in Figure 5-2, click the *Samples Task* icon in the Taskbar on the left hand side of the CommandCenter application window. If the *Samples Task* is not visible, click the **Views** button in the Taskbar to make it visible and then click on it.

Figure 5-2
Sherlock CommandCenter Samples View

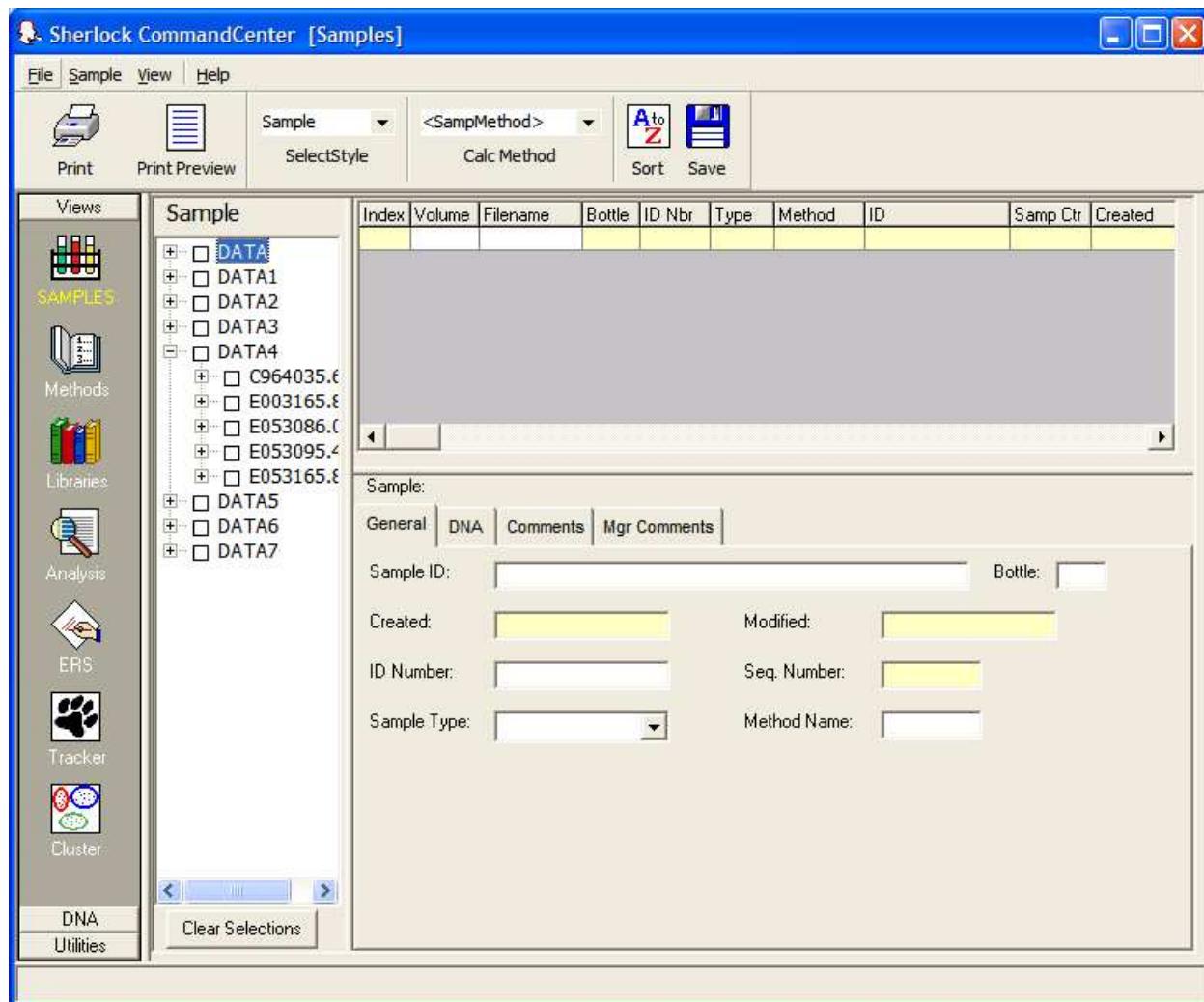


In the *Toolbar*, the *Select Style* dropdown should be in the *Sample* mode (default). This mode is the **Basic Sample Selector** mode, and is recommended for customers new to the Sherlock software. An **Advanced Sample Selector** mode (discussed later in this chapter) is available, and is recommended for customers who are familiar with the Sherlock software. In the bottom right windowpane, (the sample detail area) you can edit the fields in white.

Basic Sample Selector Mode

To better understand the operation of the Sherlock system, an example will be run with real-world data. For this example, the sample data of interest is in DATA4. Clicking on the plus sign next to DATA4 expands it to show the list of Sherlock data files (the plus sign will then change to a minus sign, Figure 5-3).

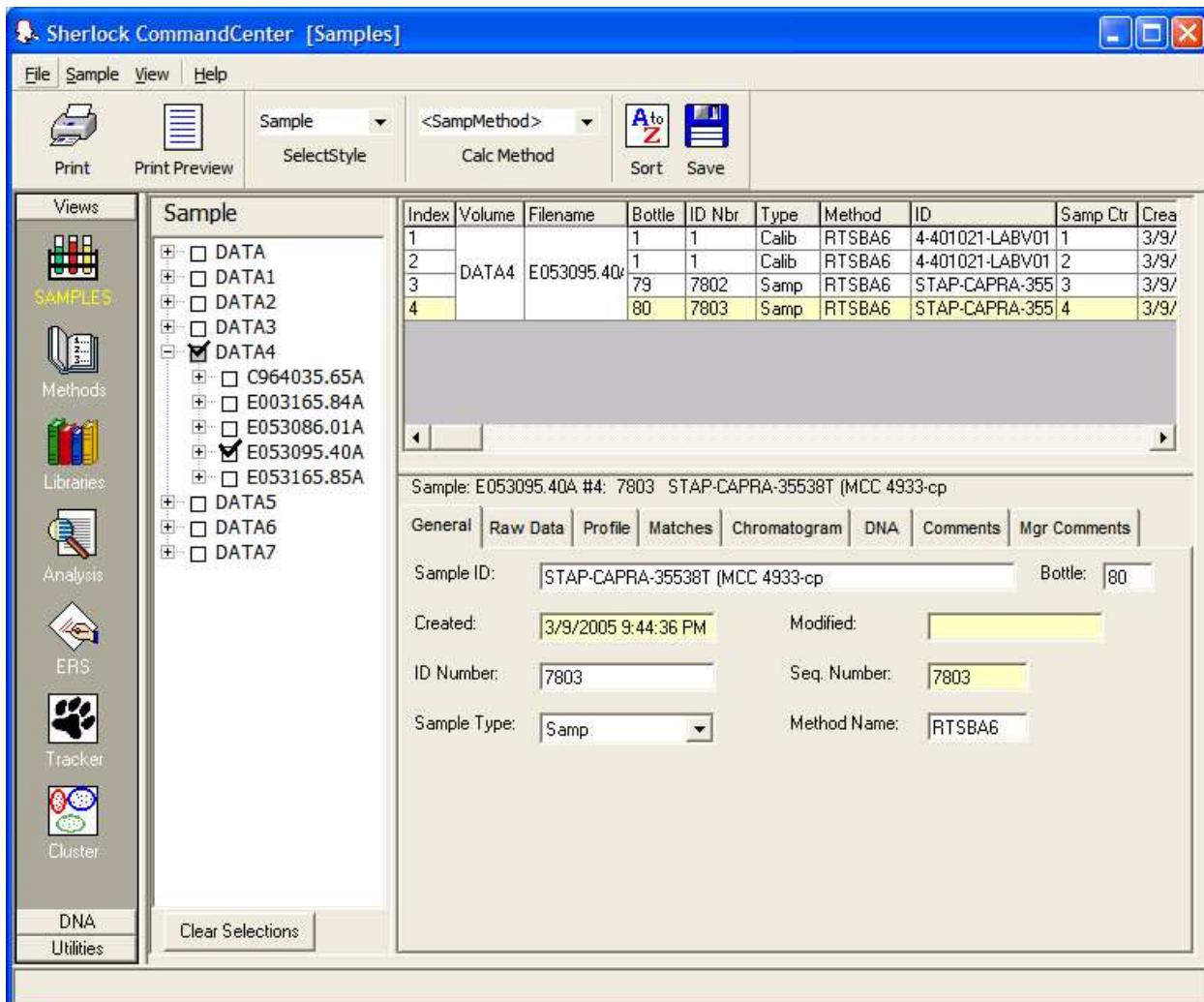
Figure 5-3
Volume DATA4 Expanded to Show Individual Data Files



Each Sherlock data file contains the data from all the sample and calibration runs in that batch. Figure 5-3 shows the expanded list of data files in DATA4. To select the data file(s) of interest, click on the box(es) to the left of the data file name (scroll down if necessary).

In this example, the following data file “E053095.40A” is selected (Figure 5-4). The upper right-hand windowpane then lists the samples in this data file. The 4th sample is selected (highlighted). The details of the 4th sample are shown in the lower right-hand windowpane. The *General* tab shows the Sample ID and its ID Number, along with other tracking information. The Sample ID is the identifying information (e.g. strain designation) that was entered in the Sherlock Sample Processor screen when the sample was entered into the system. The **Advanced Sample Selector** mode could have been used to select this sample data. This is described in the next section.

Figure 5-4
Data File E053095.40A Selected with the 4th Sample Highlighted



Advanced Sample Selector Mode

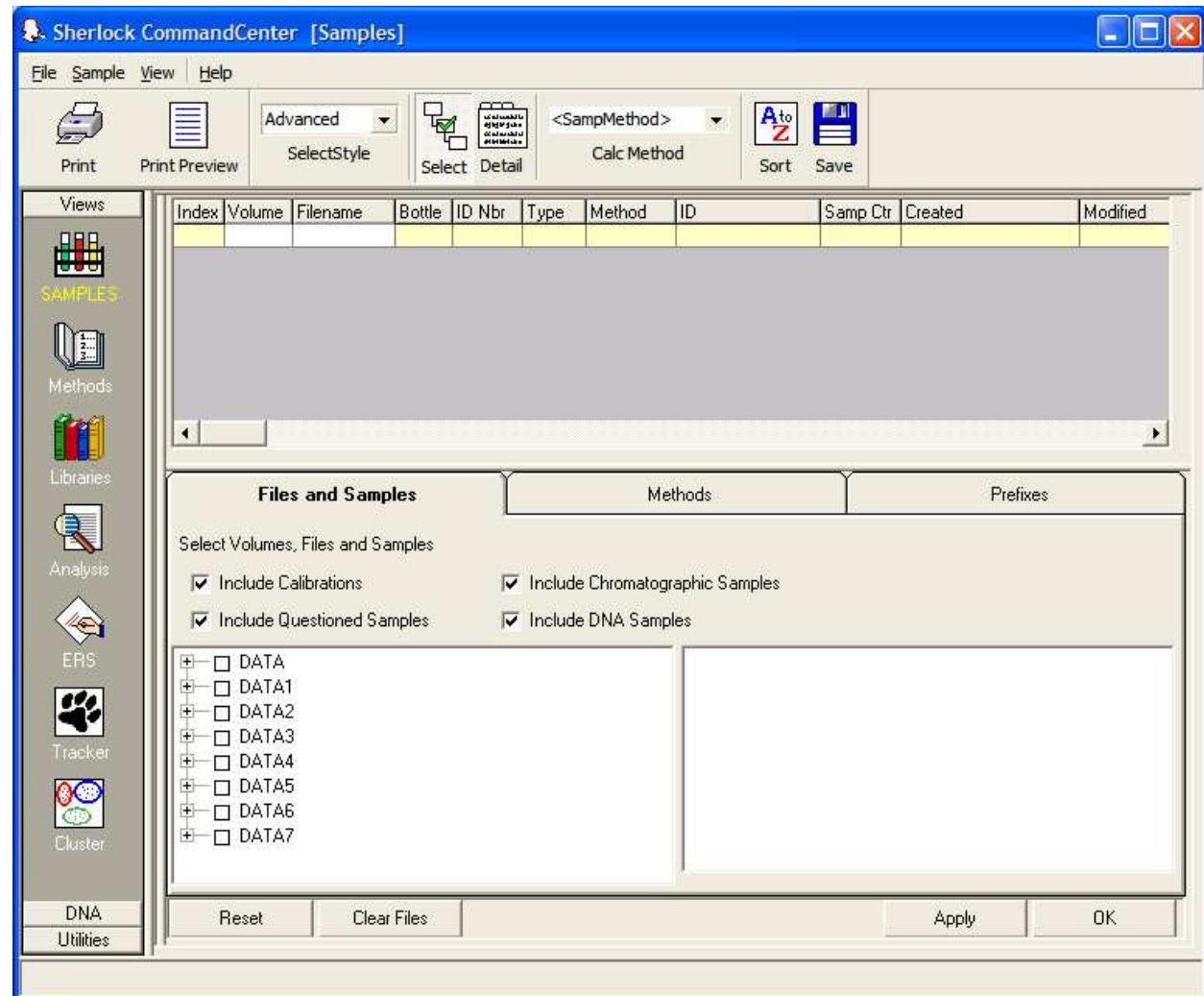
Sherlock CommandCenter also allows you to search for sample data using the **Advanced Sample Selector** mode. In this mode, you can search for data files by **any combination** of the following:

- Data Volume
- Data File Name
- Method
- Sample ID Prefix

To better understand the operation of the advanced sample selector, the previous sample (4th sample in Figure 5-4) will be located using this mode. To get to the Advanced Sample Selector mode from

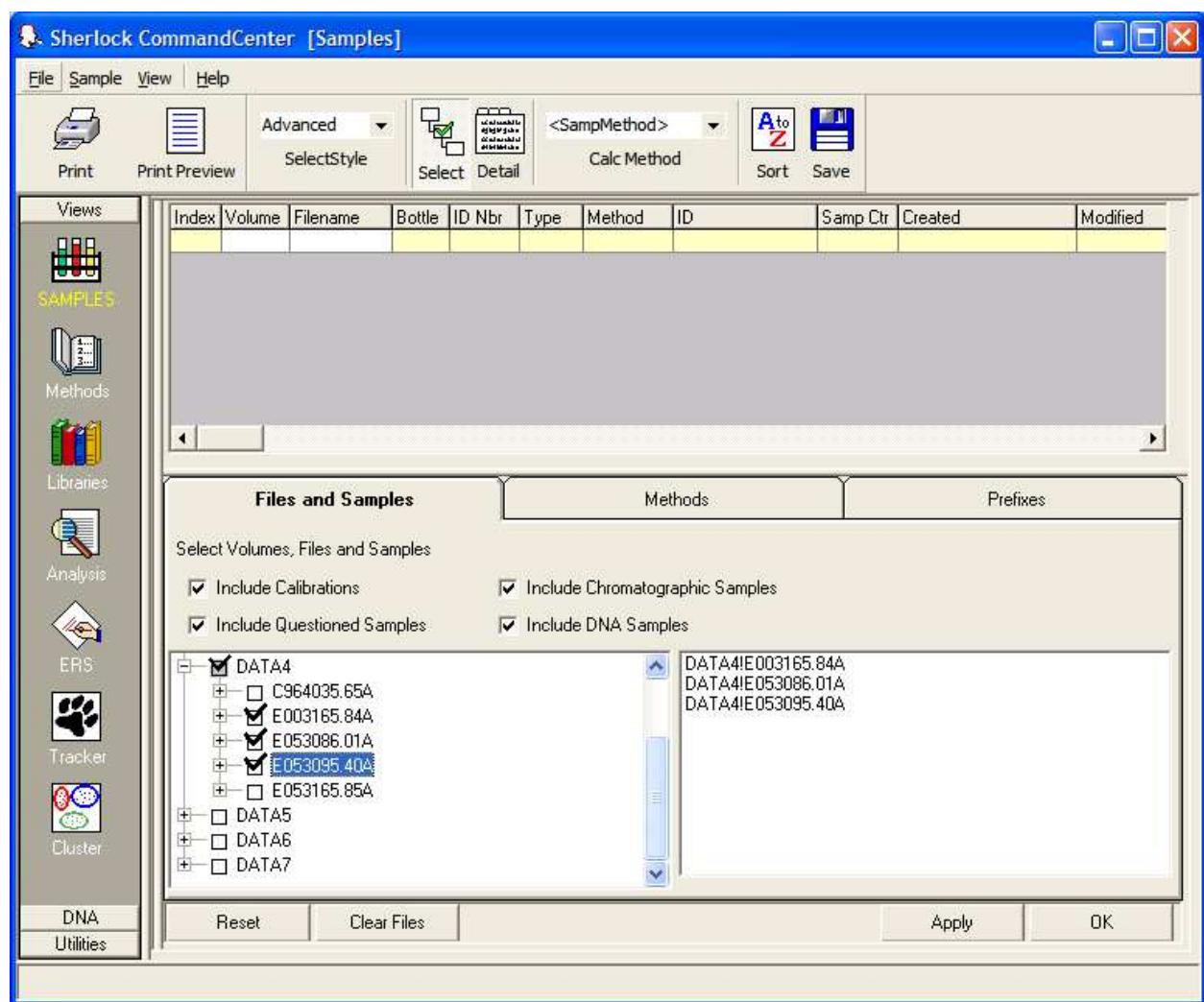
the *Samples View* (Figure 5-2), the down arrow on the *Select Style Tool*,  is pressed and the *Advanced* selected. The screen should then look similar to Figure 5-5.

Figure 5-5
Advanced Sample Selector Mode



In the Advanced Sample Selector, you can first select samples by choosing complete data volumes and/or individual data files. To select a complete data volume, click on the box directly to the left of the data volume name. To expand a data volume, in order to choose individual data files, click on the plus sign to the left of the data volume. The plus sign will change to a minus , and the data files in that data volume will be displayed. In this example, three data files from DATA4 were chosen (Figure 5-6). The names of the individual data files are displayed in the Data File List (bottom right windowpane).

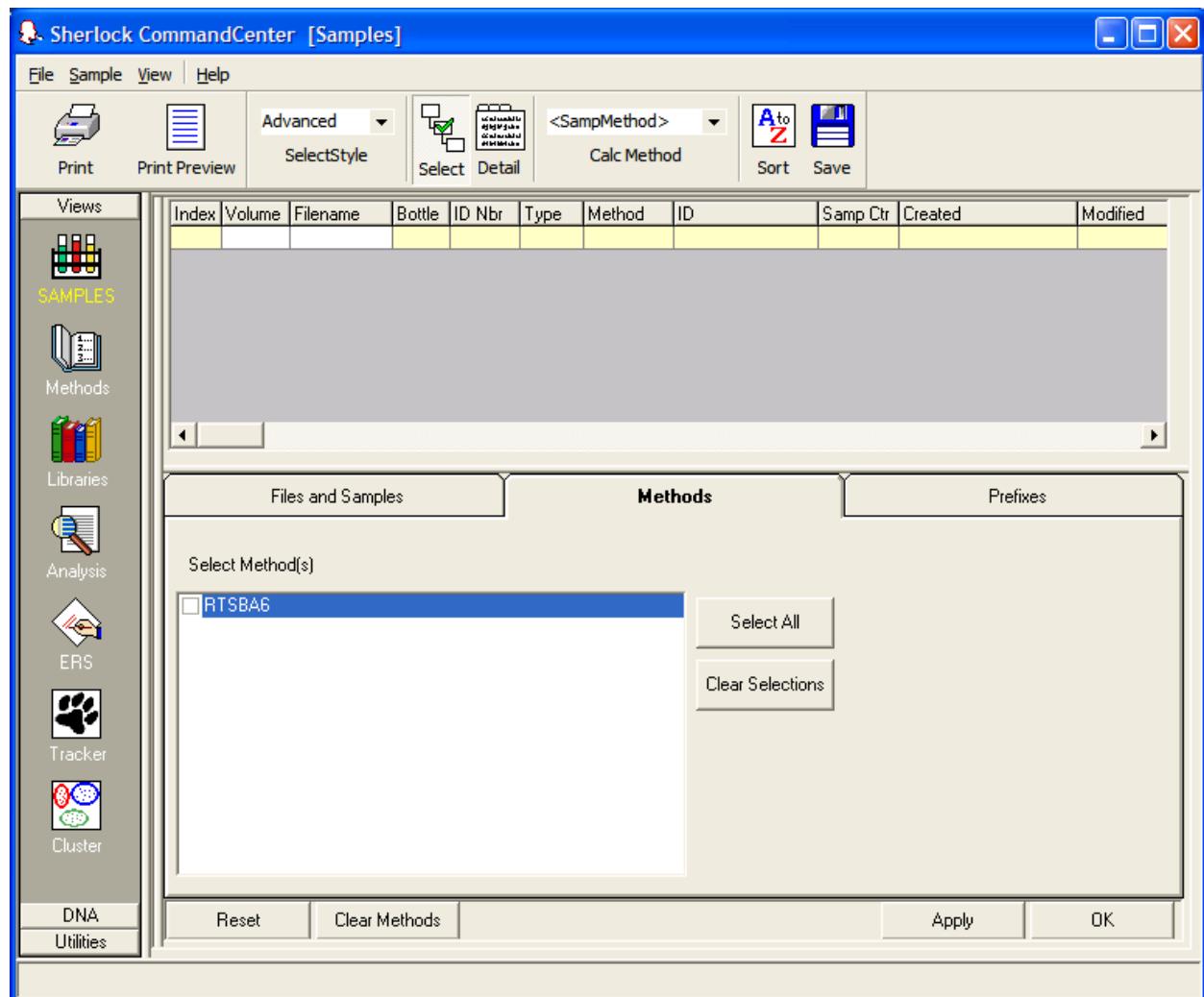
*Figure 5-6
Advanced Sample Selector - Choosing Data Files*



Note: You can click on the **Apply** button in the bottom windowpane at any time in the Advanced Sample Selector to view all the samples in the current selection. These samples will appear in the top windowpane (see Figure 5-8).

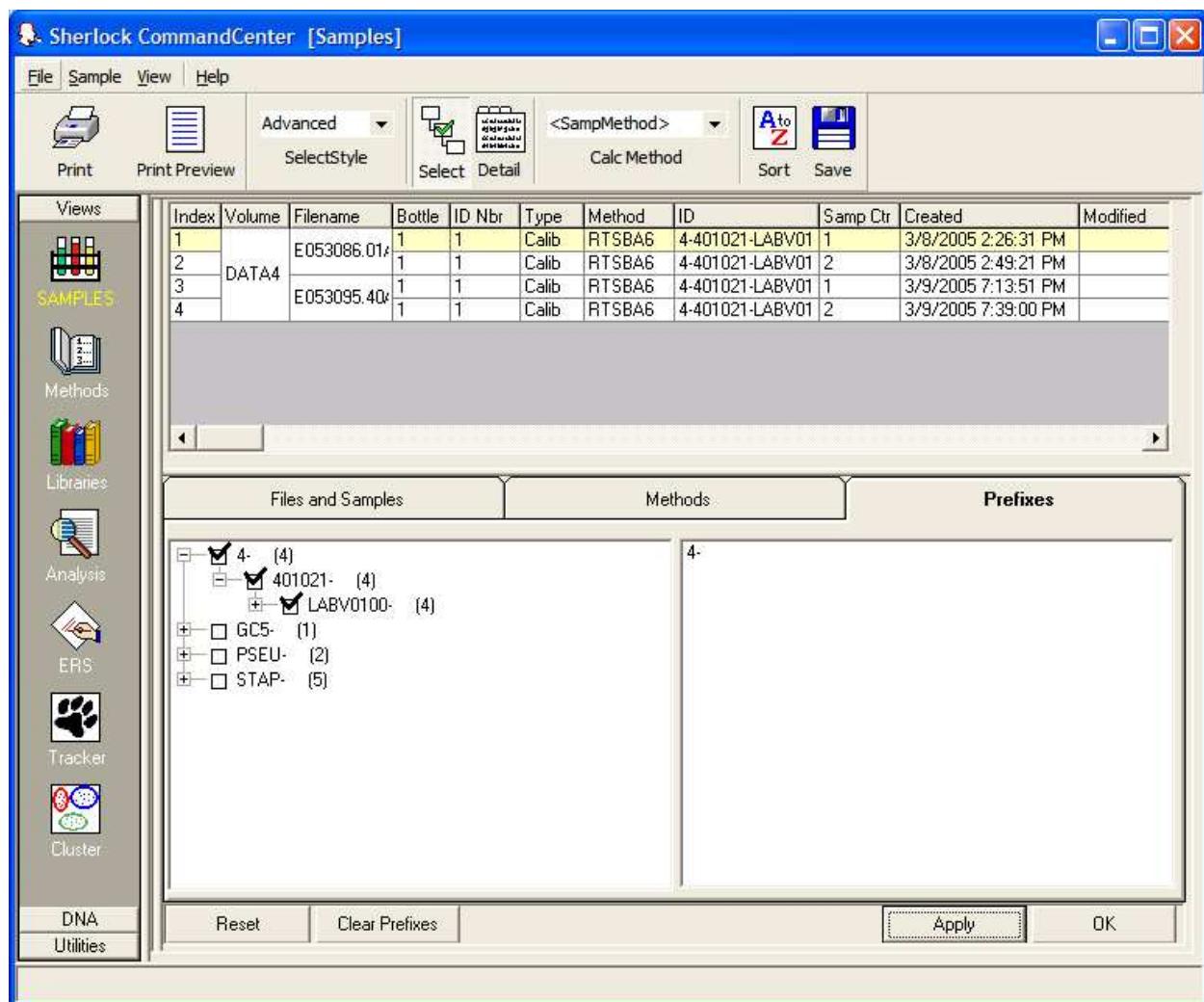
You can then select samples by choosing the method under which the sample was run. To choose a method, click on the *Methods* tab in the bottom windowpane. In this example, the *Methods* tab was pressed and the following method was chosen RTSBA6. This allows for a narrower search of the five data files chosen, by only including samples in the five data files that were run under the RTSBA6 method. If no methods are chosen, this will default to *All*.

Figure 5-7
Advanced Sample Selector-Choosing Methods



You can finally narrow the sample search by selecting specific sample ID field prefixes. To choose a prefix, click on the *Prefixes* tab in the bottom windowpane. The bottom left windowpane of Figure 5-8 shows the sample ID prefixes associated with the selection done in the previous two steps. In this example, samples with prefix “4-401021-LABV0100” were chosen (checked boxes). These prefixes are then displayed in the bottom right windowpane. In this case, the final sample set consists of 4 samples, and pressing the Apply button in the bottom windowpane provides a preview of this data set (the samples in the selection are displayed in the top windowpane). If no prefixes are chosen, this will default to *All*.

Figure 5-8
Advanced Sample Selector-Choosing Prefixes



You can then view the details of the samples from the selection by clicking on the *OK* button on the



bottom windowpane or by clicking the *Detail Tool*  in the Toolbar. Figure 5-9 illustrates what the sample detail screen looks like. If you want to *Select* samples again, click on the *Select*



Tool 

Figure 5-9
Advanced Sample Selector-Viewing Sample Details

The screenshot shows the Sherlock CommandCenter software interface for managing samples. The main window title is "Sherlock CommandCenter [Samples]". The toolbar contains icons for Print, Print Preview, Advanced, SelectStyle, Select, Detail, Calc Method, Sort, and Save. A dropdown menu "Views" is open, showing options like SAMPLES, Methods, Libraries, Analysis, ERS, Tracker, Cluster, DNA, and Utilities. The main content area displays a table of samples:

Index	Volume	Filename	Bottle	ID Nbr	Type	Method	ID	Samp Ctr	Created	Modified
1	DATA4	E053086.01	1	1	Calib	RTSBA6	4-401021-LABV01	1	3/8/2005 2:26:31 PM	
2			1	1	Calib	RTSBA6	4-401021-LABV01	2	3/8/2005 2:49:21 PM	
3			E053095.40	1	1	Calib	RTSBA6	4-401021-LABV01	1	3/9/2005 7:13:51 PM
4			1	1	Calib	RTSBA6	4-401021-LABV01	2	3/9/2005 7:39:00 PM	

Below the table, a sample detail panel is displayed for Sample: E053095.40A #2: 1 4-401021-LABV0100-9/15-11/19-7582-020205. The panel includes tabs for General, Raw Data, Profile, Matches, Chromatogram, DNA, Comments, and Mgr Comments. The General tab is selected, showing fields for Sample ID (4-401021-LABV0100-9/15-11/19-7582-020205), Bottle (1), Created (3/9/2005 7:39:00 PM), Modified (highlighted in yellow), ID Number (1), Seq. Number (1), Sample Type (Calib), and Method Name (RTSBA6).

Note: Figure 5-4 and 5-9 are similar, as both allow you to view sample details. The main difference is that in Figure 5-4, the Basic Sample Selector was used to choose the sample, and in Figure 5-9 the Advanced Sample Selector was used (only the selected samples are displayed). From this point on, the Basic Sample Selector screens (Figure 5-4) will be used to demonstrate the operation of the Sherlock CommandCenter. The Advanced Sample Selector screens are the same, except that they don't show the sample windowpane on the left hand side of Figure 5-4.

Viewing Sample Information

Selecting the *Raw Data* tab in the bottom windowpane allows you to view the raw ChemStation data for the sample of interest (Figure 5-10).

Figure 5-10
Displaying the Raw Data for the 4th Sample

The screenshot shows the Sherlock CommandCenter [Samples] application window. The menu bar includes File, Sample, View, and Help. The toolbar contains icons for Print, Print Preview, SelectStyle, SampMethod (set to A to Z), Calc Method, Sort, and Save. The left sidebar has a tree view under 'SAMPLES' with nodes for DATA, DATA1, DATA2, DATA3, DATA4 (selected), DATA5, DATA6, and DATA7. Below this are sections for Methods, Libraries, Analysis, ERS, Tracker, Cluster, DNA, and Utilities. The main area has tabs for Sample, General, Raw Data (selected), Profile, Matches, Chromatogram, DNA, Comments, and Mgr Comments. The 'Raw Data' tab displays a table of chromatogram data:

Index	Volume	Filename	Bottle	ID Nbr	Type	Method	ID	Samp Ctr	C
1			1	1	Calib	RTSBA6	4-401021-LABV01	1	3
2	DATA4	E053095.40A	1	1	Calib	RTSBA6	4-401021-LABV01	2	3
3			79	7802	Samp	RTSBA6	STAP-CAPRA-355	3	3
4			80	7803	Samp	RTSBA6	STAP-CAPRA-355	4	3

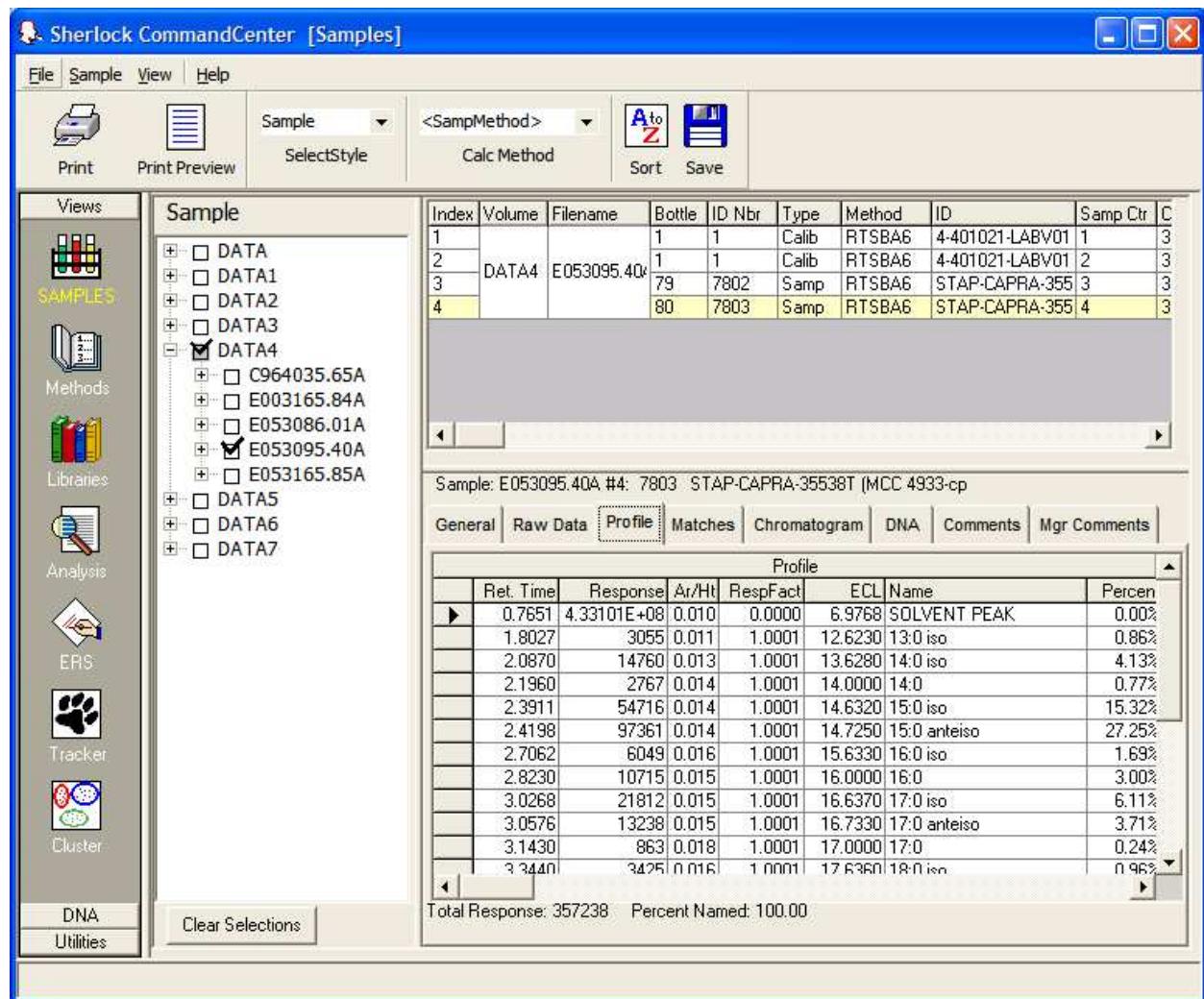
Below the table, it says 'Sample: E053095.40A #4: 7803 STAP-CAPRA-35538T (MCC 4933-cp)'. The 'Raw Data' tab also contains a detailed table of retention times and responses:

Ret. Time	Response	Area/Height
0.7651	4.33101E+08	0.0102
1.8027	3055	0.0113
2.0870	14760	0.0127
2.1960	2767	0.0136
2.3911	54716	0.0136
2.4198	97361	0.0136
2.7062	6049	0.0161
2.8230	10715	0.0153
3.0268	21812	0.0152
3.0576	13238	0.0147
3.1430	863	0.0179
3.3440	3425	0.0157
3.4590	51291	0.0161
3.6574	15548	0.0167
3.6885	3791	0.0158
3.7700	2242	0.0157

Viewing the Sample Profile Information

Selecting the *Profile* tab in the bottom windowpane displays the list of peaks (or compounds) found in the selected sample (Figure 5-11).

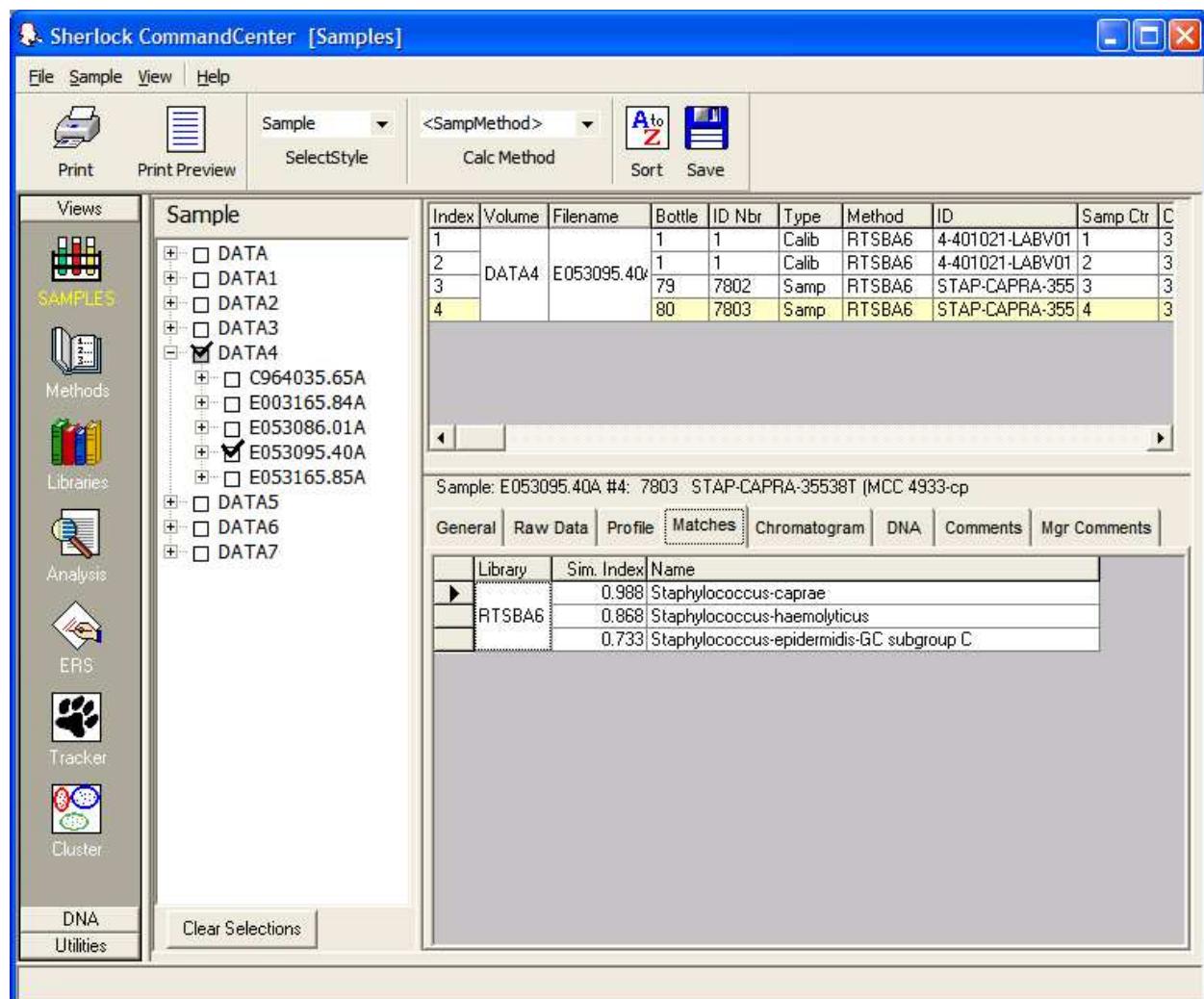
Figure 5-11
Displaying the Fatty Acid Profile for the 4th Sample



Viewing the Library Match Information

Selecting the *Matches* tab displays the sample name as identified from a search of the library (or libraries) specified in the method (see Figure 5-12). In this example, the RTSBA6 library was searched for an organism match. As a result, this sample was matched to the *Staphylococcus caprae* library entry with extremely high confidence (Similarity Index = 0.988).

Figure 5-12
Matches to the RTSBA6 Library

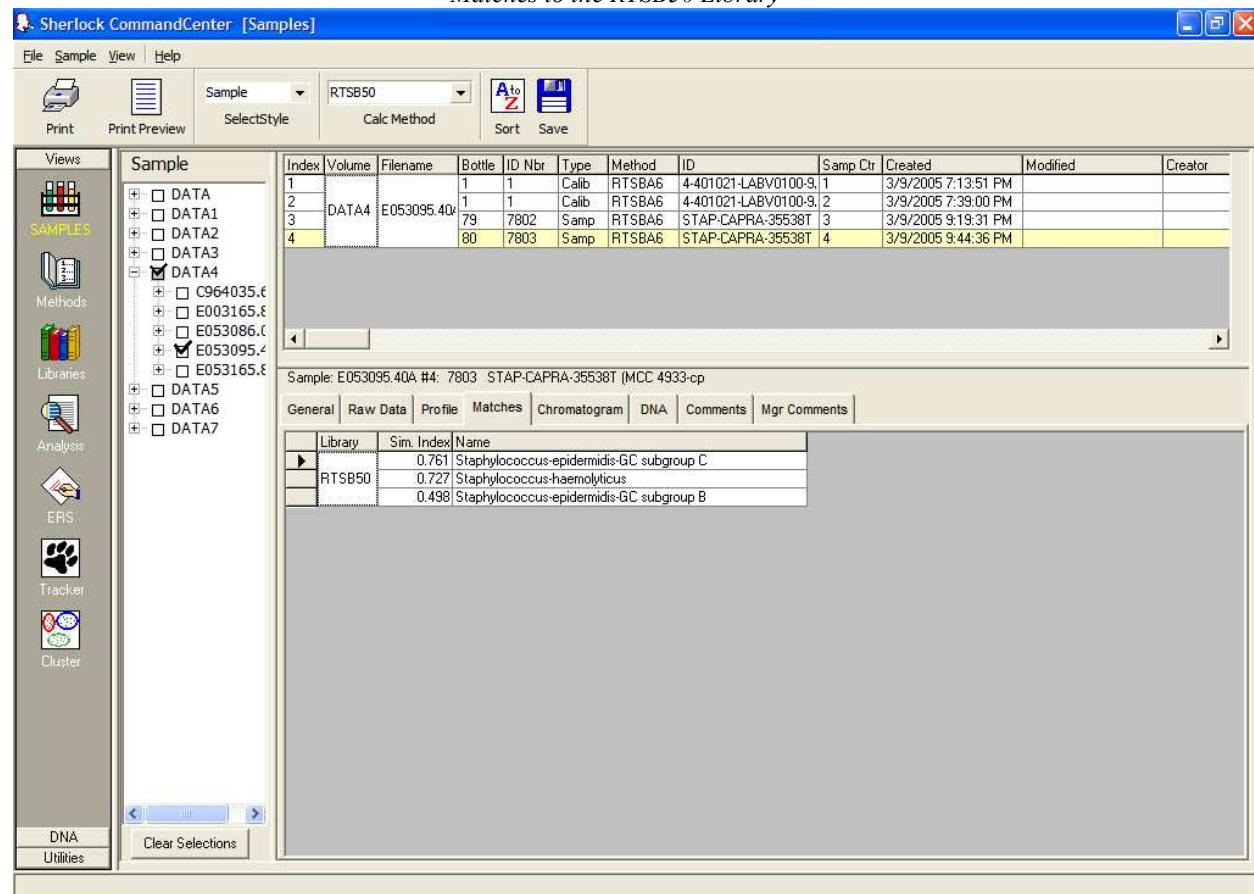


Comparing the Sample using a Different Calculation Method

To see the results of calculating a sample's profile and match using a different method and/or

library, select a different *Calc Method* from the dropdown [Figure 5-12]. In Figure 5-12 the profile of the chosen sample was shown using its collection method (*RTSBA6*) as the calculation method. In Figure 5-13, the calculation method is changed to the older *RTSB50* method. When the sample is compared to *RTSB50*, the sample yielded a match against *Staphylococcus-epidermidis*, because *S. caprae* was not available in that version. The ability to change calculation method helps evaluate the performance of a new method and library versus previous ones.

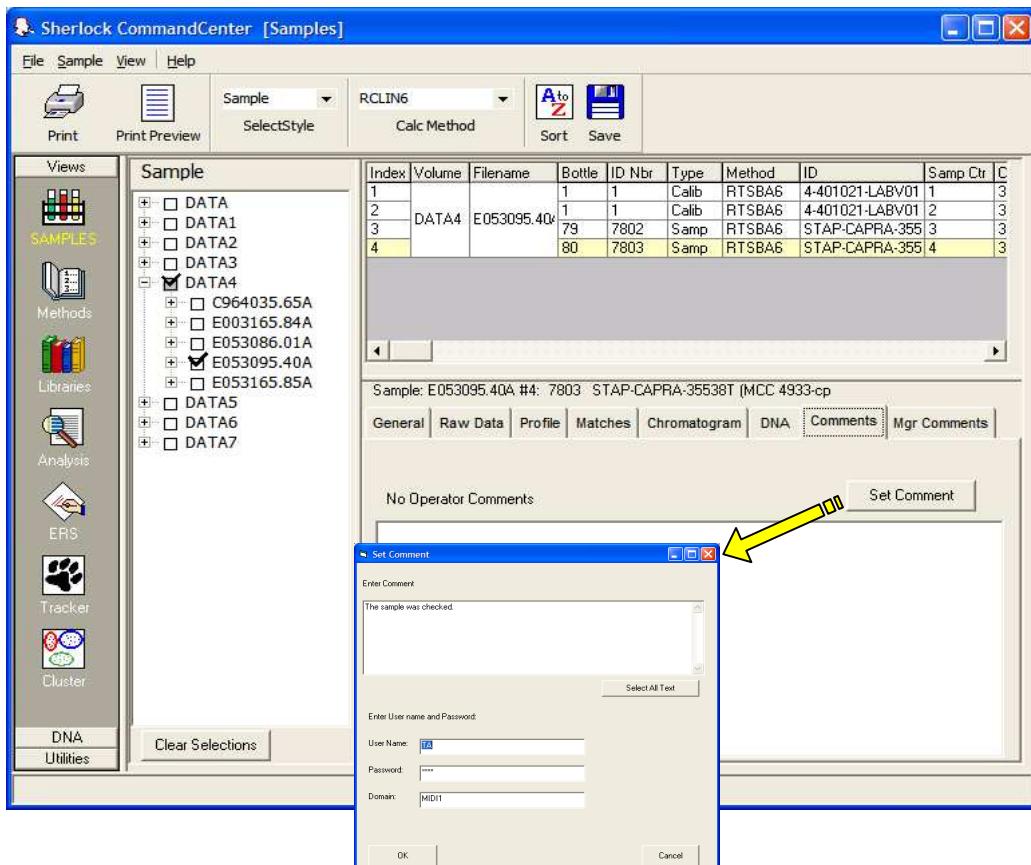
Figure 5-13
Matches to the RTSB50 Library



Viewing and Adding Comments

Any user of the MIDI system can view and add comments to a particular sample. Selecting the *Comments* tab brings up the *Operator Comments* windowpane (see Figure 5-14). If you click on the *Set Comment* button, a dialog box will appear as in Figure 5-14. In this example, a comment is entered, and the appropriate Windows Username and Password are entered.

Figure 5-14
Viewing and Adding Comments to the Sample



Note: The *Set Comment* feature in Figure 5-14 does not require the Sherlock Electronic Records and Signatures (ERS) package. If you have ERS, you must be a Sherlock User or a Sherlock Manager to set the comment.

After adding the comment to the sample, the screen will look like Figure 5-15. To save the



comment, click on the *Save Tool*.

Note: This comment is not protected under the Sherlock Electronic Records and Signatures (ERS) file; for example, no audit log of changes is maintained. Use the ERS view of CommandCenter to include ERS-protected comments.

Figure 5-15
Addition of a Comment to the Sample

The screenshot shows the Sherlock CommandCenter interface. On the left is a vertical toolbar with icons for Samples, Methods, Libraries, Analysis, ERS, Tracker, and Cluster. The main window has a title bar "Sherlock CommandCenter [Samples]" and a menu bar with File, Sample, View, and Help. The central area displays a table of samples with columns: Index, Volume, Filename, Bottle, ID Nbr, Type, Method, ID, Samp Ctr, Created, Modified, and Creator. A red circle highlights the "Comments" tab in the bottom navigation bar. Below the table, a text input field contains the comment "The sample was checked". A red oval encloses this text input field and the "Comments" tab. The status bar at the bottom shows "DNA Utilities".

Index	Volume	Filename	Bottle	ID Nbr	Type	Method	ID	Samp Ctr	Created	Modified	Creator
1			1	1	Calib	RTSBA6	4-401021-LABV0100-9.1		3/9/2005 7:13:51 PM		
2			1	1	Calib	RTSBA6	4-401021-LABV0100-9.2		3/9/2005 7:39:00 PM		
3	DATA4	E053095.40A	79	7802	Samp	RTSBA6	STAP-CAPRA-35538T	3	3/9/2005 9:19:31 PM		
4			80	7803	Samp	RTSBA6	STAP-CAPRA-35538T	4	3/9/2005 9:44:36 PM		

Manager Comments

If there are comments in the sample made by a Sherlock ERS Manager, they would appear in the bottom windowpane, after the *Mgr Comments* tab was pressed.

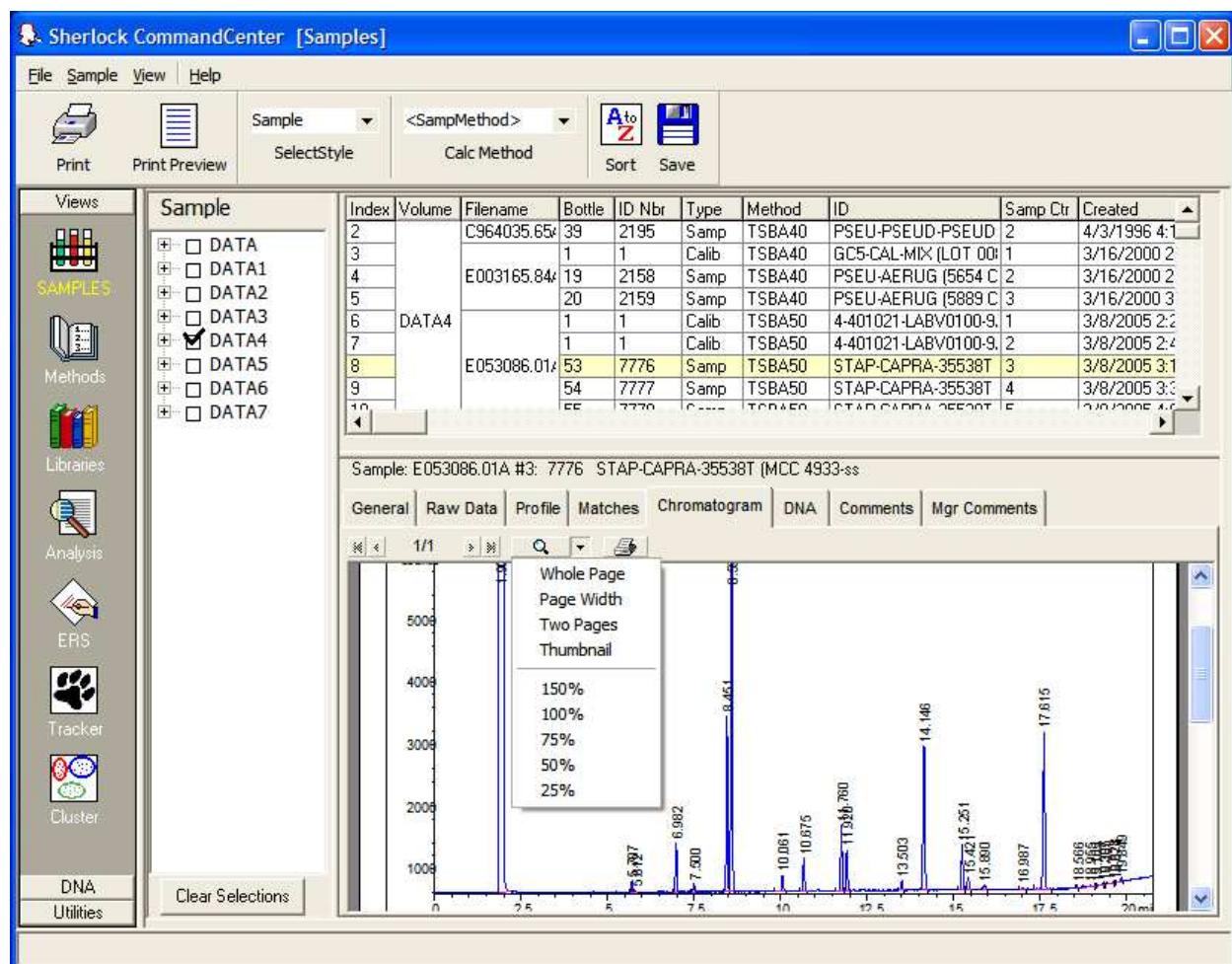
Viewing the Sample Chromatogram

Sherlock has the ability to view the sample chromatogram within the Sherlock *Samples* View. To view the chromatogram, click on the *Chromatogram* tab.

There is a Zoom feature that is available with the chromatogram (see Figure 5-16).

Note: Older versions of Sherlock data files do not show chromatograms. Also, removing ChemStation data files from C:\ Sherlock\ Raw will remove the associated chromatograms. Keep the Raw Data files to ensure access to the chromatograms.

Figure 5-16
Viewing the Sample Chromatogram



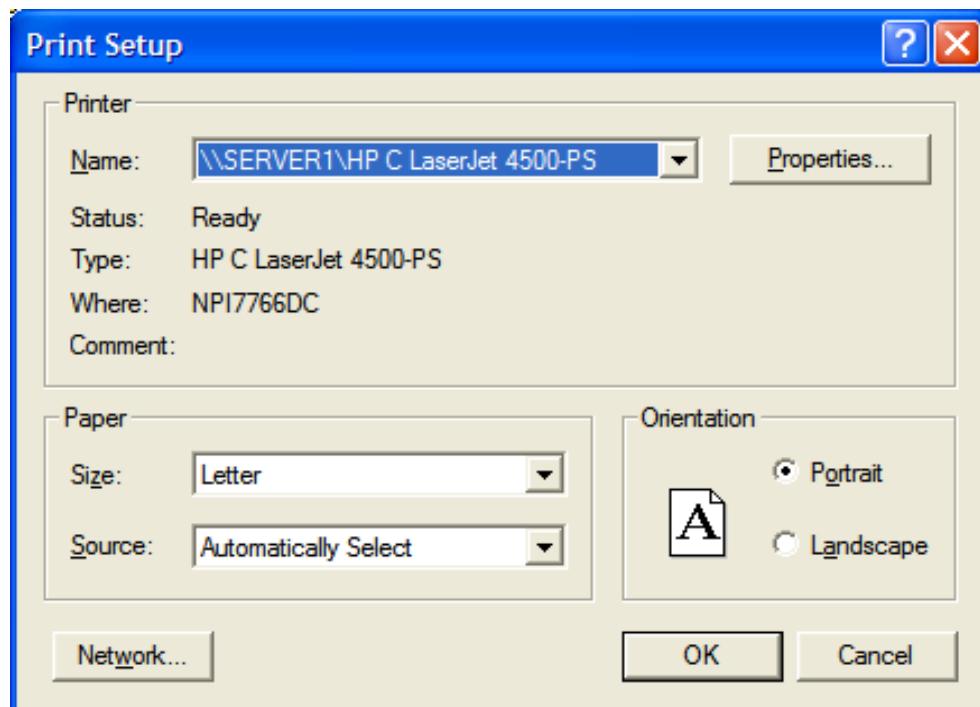
Printing Sample Information

Sherlock allows you to print sample information to a printer, preview sample information on the computer screen and save sample information to an .RTF format (which can be opened in MS Word). In order to print sample information to a printer, you first need to configure the printer that you want this information sent to; otherwise the computer's default printer will be used.

Configuring the Printer

Use the *Setup Printer* tool (on the file menu dropdown) to select and configure the printer for hardcopy output. Clicking this tool displays the standard *Printer Setup* dialog box shown in Figure 5-17. In this example, The HP C LaserJet 4500-PS is selected as the printer. If there is more than one printer installed on the computer, designate which printer will be used in this dialog box. This dialog box can also be used to choose between a Landscape or Portrait mode of printing. Clicking the **Properties** button displays the printer specific configuration dialog. See the documentation and help files supplied by the printer manufacturer for information about the Properties dialog box.

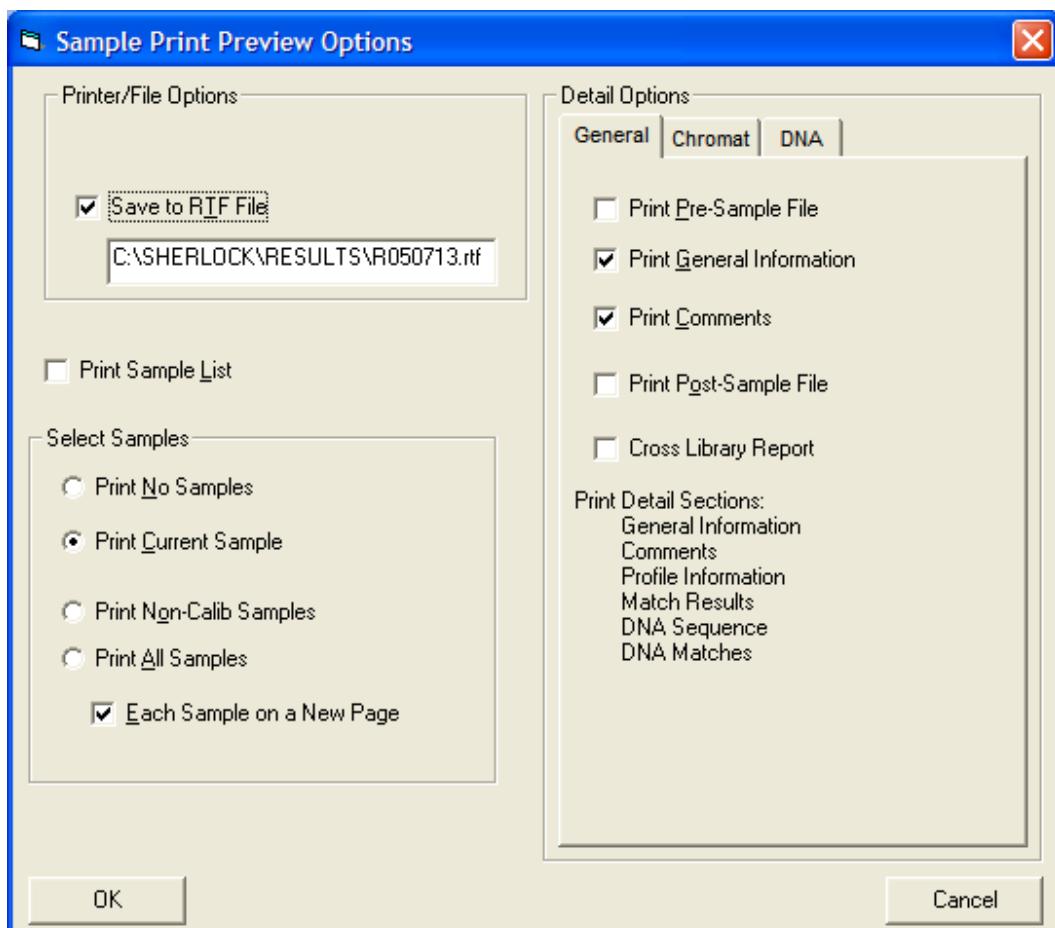
Figure 5-17
The Print Setup Dialog Box





Clicking the *Print Preview Tool* in the Toolbar allows you to preview the results before committing them to paper. Figure 5-18 shows the dialog box for *Sample Print Preview Options*. This dialog box allows you to select how much information is printed. It also allows you to store data as an RTF file, which can be opened and edited in Microsoft Word. In this example, the checked information for the 4th sample in Figure 5-4 will be printed (including one comparison chart). The results are also saved to an RTF file (see Figure 5-18). Clicking the *OK* button in the dialog box of Figure 5-18 results in the *Print Preview* window appearing on the computer screen (Figure 5-19).

Figure 5-18
Viewing the Sample Print Preview Options



Note: Printing all the samples for a large sample list can yield a very large report. Consider just printing the match results and turning off “Each sample on a new page” to minimize paper usage. Or, print preview all the samples, and just print the pages you need.

There are four pages of output in the *Print Preview* screen for this example. Figure 5-19 shows page 4 (the final sample profile). The navigation tools on the left-hand side of Figure 5-19 can be used to navigate the preview pages. You can also set the page range to be printed. For example, pages 1-2 can be printed by typing this range into the *Print Page Range* field. Clicking the *Print* button will then send pages 1-2 to the designated printer. If the *Save to RTF File* box was checked in Figure 5-18, then a *View RTF* button will appear in Figure 5-19. This allows you to view the Microsoft Word™ version of the sample information requested.

Figure 5-19
Viewing the Print Preview Results

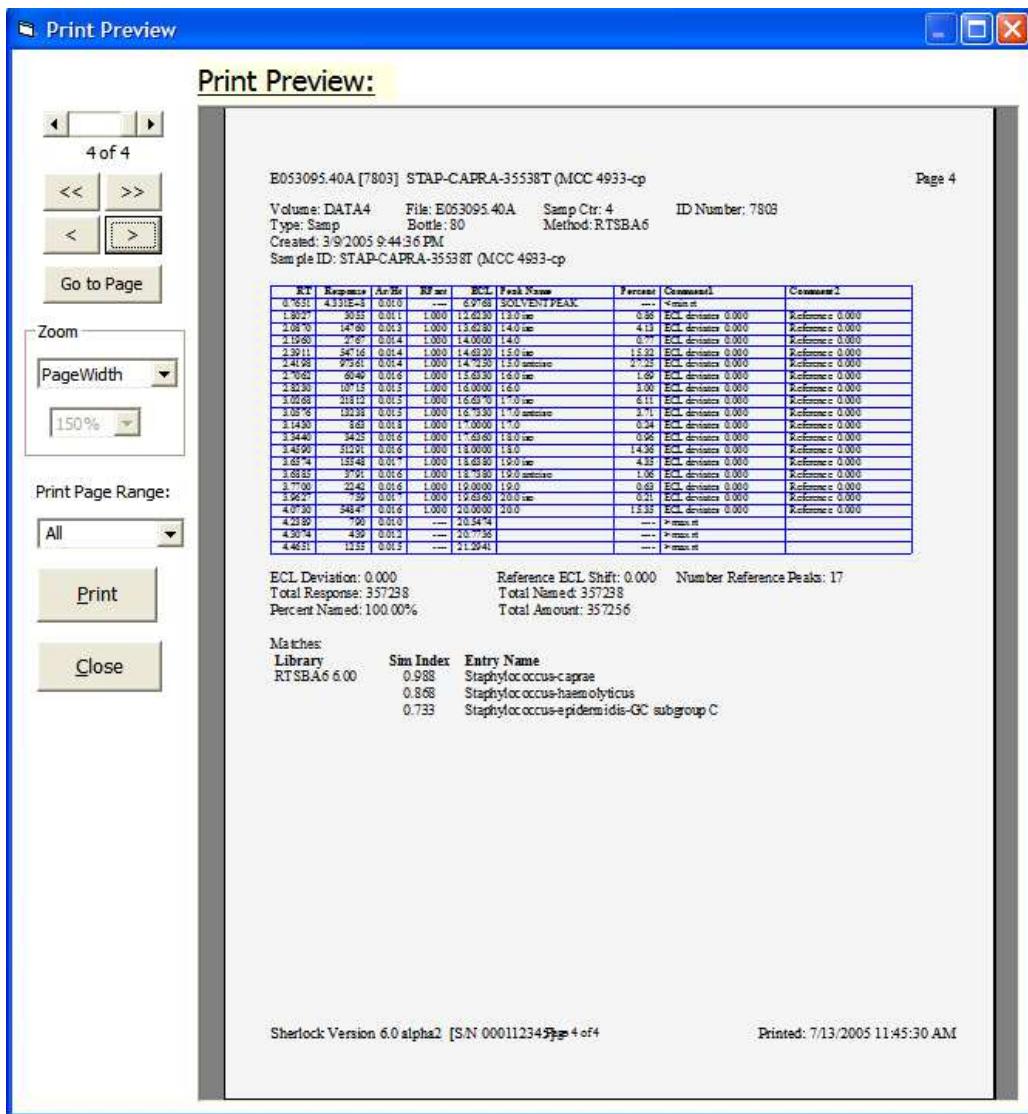
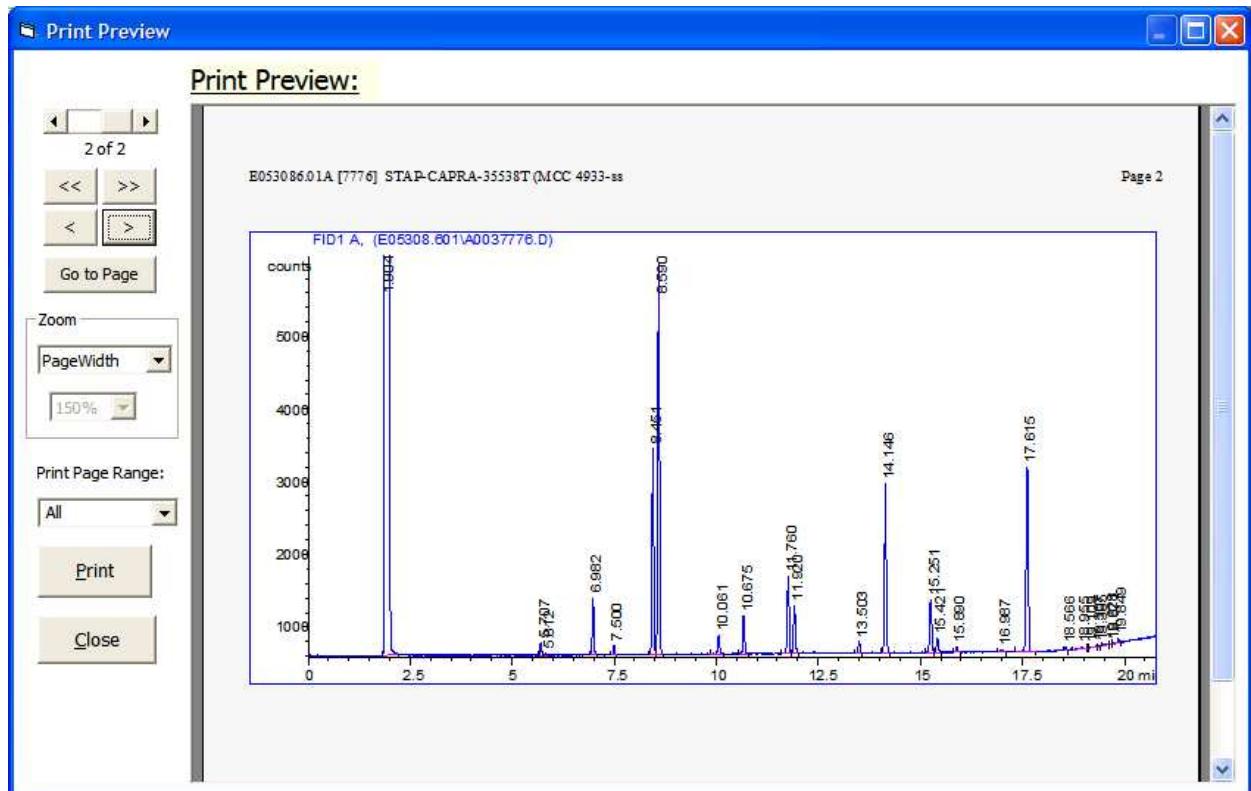


Figure 5-20
Page 2 (Chromatogram) of the Sample Print Preview Data



Correcting Data Entry Errors

Sherlock allows you to correct data entry errors made when samples were logged into the system via the Sherlock sample table. You can correct errors in any of the white fields in the sample detail pane (Figure 5-4, bottom right windowpane or Figure 5-9, bottom windowpane). If any of these fields are edited, the *Modified* field will be set to the date and time of the modification. The data entry for the sample will be permanently marked as modified. Printed reports created from a modified entry will be marked with the date edited.

Viewing Installed Methods

Sherlock contains tools that allow you to view and print method information. The system does not allow you to edit the methods supplied by MIDI, Inc. The ability to view methods is provided mainly for troubleshooting, with the help of MIDI's technical support. Double click the Sherlock



CommandCenter Icon on the desktop to start the Sherlock CommandCenter application. Figure 5-21 shows the *Methods View*. CommandCenter reverts to the view from the last time it



was used. If it is not in the *Methods View* as shown in Figure 5-21, click the *Methods Task* in the Taskbar on the left hand side of the CommandCenter application window. If the *Methods* Task is not visible, click the button in the Taskbar to make it visible and then click on it.

Figure 5-21
Viewing Installed Methods

The screenshot shows the Sherlock CommandCenter application window titled "[Methods]". The menu bar includes File, Method, View, and Help. The toolbar contains icons for Print, Preview, Save, Create, and Delete. A vertical toolbar on the left lists Samples, METHODS (which is selected), Libraries, Analysis, ERS, Tracker, Cluster, DNA, and Utilities. The main area displays a table of installed methods:

Name	Version	Root	Description	Libraries	Created	Modified
ACTINO	3.80	ACTINO	TBSL 4 DAYS SHAKING ACTINOMYCETES PEAK NAMING TABLE	ACTIN1	1/28/1993	2/16/1994 11:04:19 AM
ANAEER1	3.90	ANAEER1	ANAEER1 + unk 19.587 + 18.0 120H MOORE	MOORE	8/10/1995	8/10/1995 12:50:51 PM
ANAEER6	6.00	ANAEER6	BHIBLA & MRSA plates in gas pack	BHIBLA	5/18/2005	5/18/2005 9:21:54 AM
ANAEROBE	3.80	ANAEROBE	BHIBLA & MRSA PLATES IN GAS F	BHIBLA	9/4/1989	2/16/1994 11:04:40 AM
BIOTER	1.00	BIOTER	Use only to test suspected bioterrorists	BIOTER	2/28/2001	2/28/2001 5:07:20 PM
BTR20	2.00	BTR20	Use only to test suspected bioterrorists	CLIN50, BTR2	10/25/2002	10/25/2002 2:53:26 PM
BTR3	3.00	BTR3	Use only to test suspected bioterrorists	BTR3	5/23/2005	5/23/2005 3:33:06 PM
CLIN40	4.00	CLIN40	Blood agar 35C or as noted	CLIN40	11/30/1998	11/30/1998 4:00:00 AM
CLIN50	5.00	CLIN50	Blood agar 35C or as noted	CLIN50	10/25/2002	10/25/2002 2:52:28 PM
CLIN6	6.00	CLIN6	Blood agar 35C or as noted	CLIN6	5/23/2005	5/23/2005 3:32:48 PM
DNAJUST	2.00	"DNAONLY"	16S DNA 500 bp method	D1652	5/24/2005	5/24/2005 10:44:52 AM
EUKARY	3.71	EUKARY	FAMES from Eukaryotes ver 3.71 (31)		12/20/1958	2/26/1997 10:20:19 AM
CLINIC	2.00	CLINIC	CLINIC 200 INCAP DDOU 160 CLINIC		1/14/2004	1/14/2004 5:40:43 PM

Below the table, there are tabs for General, Sample Control, Calib Control, Calib Sequence, and Naming Table. The General tab is selected. The General tab fields include Name: ACTINO, Root Method: ACTINO, Version: 3.80, Created: 1/28/1993, Modified: 2/16/1994 11:04:19 AM, Description: TBSL 4 DAYS SHAKING ACTINOMYCETES PEAK NAMING TABLE, and Libraries: ACTIN1, CLIN50, BTR2, CLIN6, D1652.

Displaying Method Information

The top windowpane lists the methods found on the system. Click on the method row in the top windowpane to select a method. In the current example, the TSBA6 method is selected (highlighted). Details for this selected method are shown in the bottom windowpane. In Figure 5-22, the Peak Naming Table tab is selected to show the TSBA6 peak naming table. Clicking on other tabs, such as *Sample Control* will display other details for the method of interest.

Figure 5-22
Viewing TSBA6 Peak Naming Table

The screenshot shows the Sherlock CommandCenter [Methods] application window. The menu bar includes File, Method, View, and Help. The toolbar contains icons for Print, Preview, Save, Create, and Delete. A vertical toolbar on the left lists Samples, METHODS (selected), Libraries, Analysis, ERS, Tracker, Cluster, DNA, and Utilities. The main area has two panes. The top pane displays a list of methods with columns for Name, Version, Root, Description, Libraries, and Created. The TSBA6 method is selected (highlighted in yellow). The bottom pane shows the Peak Naming Table for TSBA6, with tabs for General, Sample Control, Calib Control, Calib Sequence, and Naming Table (selected). The table has columns for Index, Peak Name, Nom. ECL, Name Window, Nom. RT, Cal / Ind, Index Peak, Ref Peak, Quant Cal, Nom. Amount, Check Mix, Min Amount, and Max Amount. Several peaks are listed, including 9:0, 8:0 3OH, unknown 9.531, 10:0 iso, 10:0, 9:0 3OH, 11:0 iso, 11:0 anteiso, 12:0 aldehyde ?, unknown 10.928, 11:0, and 10:0 2OH.

Name	Version	Root	Description	Libraries	Created	
TSBA50	5.00	TSBA50	TSBA PLATES 28C or as noted	TSBA50	8/28/2002	1
► TSBA6	6.00	TSBA6	TSBA PLATES 28C or as noted	TSBA6	5/23/2005	5
WIDE10	1.01	WIDE10	Wide method for calibration problems		2/10/2004	2
YEAST28	3.80	YEAST28	YEASTS, 28C, 24HR, ON SAB DEX		1/4/1994	1
YST28	3.80	YST28	YEASTS, 28C, 24HR, ON SAB DEX	YST28	2/10/2004	2
YST28N	2.00	YST28N	YEASTS 28C 24HR ON SAB DEX YST28N	YST28N	2/10/2004	2

Peak Naming Table												
General			Sample Control			Calib Control			Calib Sequence			Naming Table
Add Peak			Edit Peak			Delete Peak			<input type="checkbox"/> Use Weights			
Index	Peak Name	Nom. ECL	Name Window	Nom. RT	Cal / Ind	Index Peak	Ref Peak	Quant Cal	Nom. Amount	Check Mix	Min Amount	Max Amount
► 1	9:0	9.000	0.015	2.401	C	True	True	Yes	5.883	Yes	5.049	5.645
2	8:0 3OH	9.392	0.012	2.587	False	False	No			No		
0	unknown 9.531	9.531	0.010	2.653	False	False	No			No		
3	10:0 iso	9.604	0.010	2.688	False	True	No			No		
4	10:0	10.000	0.015	2.876	C	True	True	Yes	11.765	Yes	10.109	11.248
5	9:0 3OH	10.408	0.012	3.145	False	False	No			No		
6	11:0 iso	10.606	0.015	3.275	False	True	No			No		
7	11:0 anteiso	10.695	0.015	3.334	False	True	No			No		
-2	12:0 aldehyde ?	10.914	0.010	3.478	False	False	No			No		
-2	unknown 10.928	10.928	0.010	3.488	False	False	No			No		
8	11:0	11.000	0.015	3.535	C	True	True	Yes	5.883	Yes	5.049	5.645
9	10:0 2OH	11.153	0.012	3.669	I	False	False	No		Yes	1.866	2.643

Printing Method Parameters

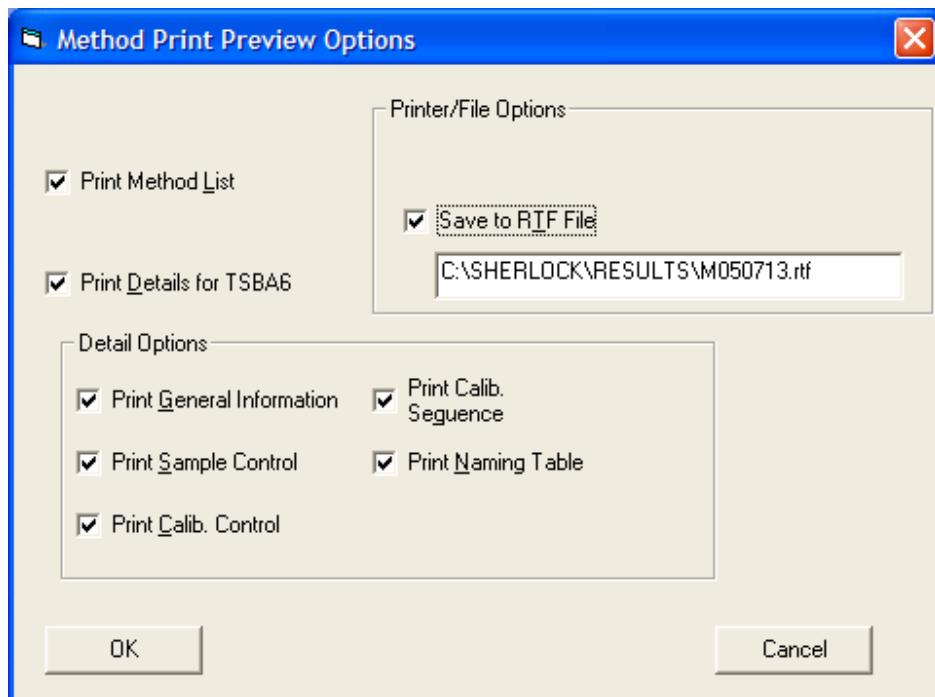


To print the method parameters, click the *Print Tool* in the Toolbar. The *Method Print Options* dialog box will then appear (Figure 5-23). You can then choose the portions of the method to print by checking the appropriate boxes and clicking the *OK* button. The method information can also be saved to an RTF file, which can be opened in Microsoft Word. The *Print Preview Tool*



in the Toolbar can also be used to view the same method information on the computer screen.

Figure 5-23
Printing Method Parameters



Viewing Installed Libraries (Databases)

Sherlock contains tools that allow you to view and print library information. The system does not allow you to edit the libraries supplied by MIDI, Inc. The ability to view libraries allows you to determine the list of species a particular library can recognize. Deviations from standard growth conditions are also described for individual entries. If the library does not contain a particular species, then the system will not be able to name that species accurately. Double click the Sherlock

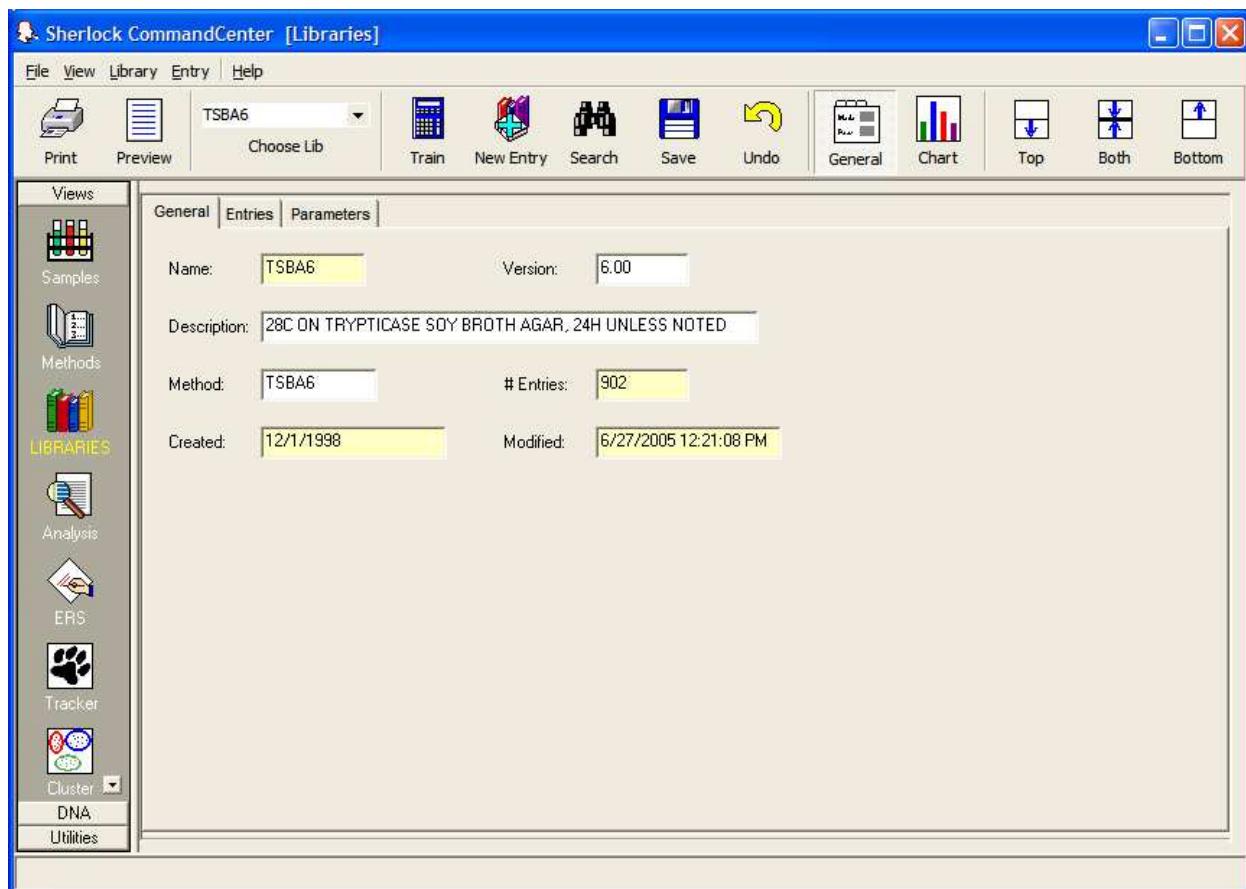


CommandCenter Icon on the desktop to start the Sherlock CommandCenter application. Figure 5-24 is the *Libraries View*. The CommandCenter application remembers the view from the last time you used it. If it is not in the *Libraries View* as shown in Figure 5-24, click the *Libraries*

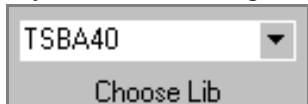


Task in the Taskbar on the left hand side of the CommandCenter application window. If the *Libraries* Task is not visible, click the *Views* button in the Taskbar so that it is visible and click on it.

Figure 5-24
Viewing Installed Libraries (TSBA6 Selected)



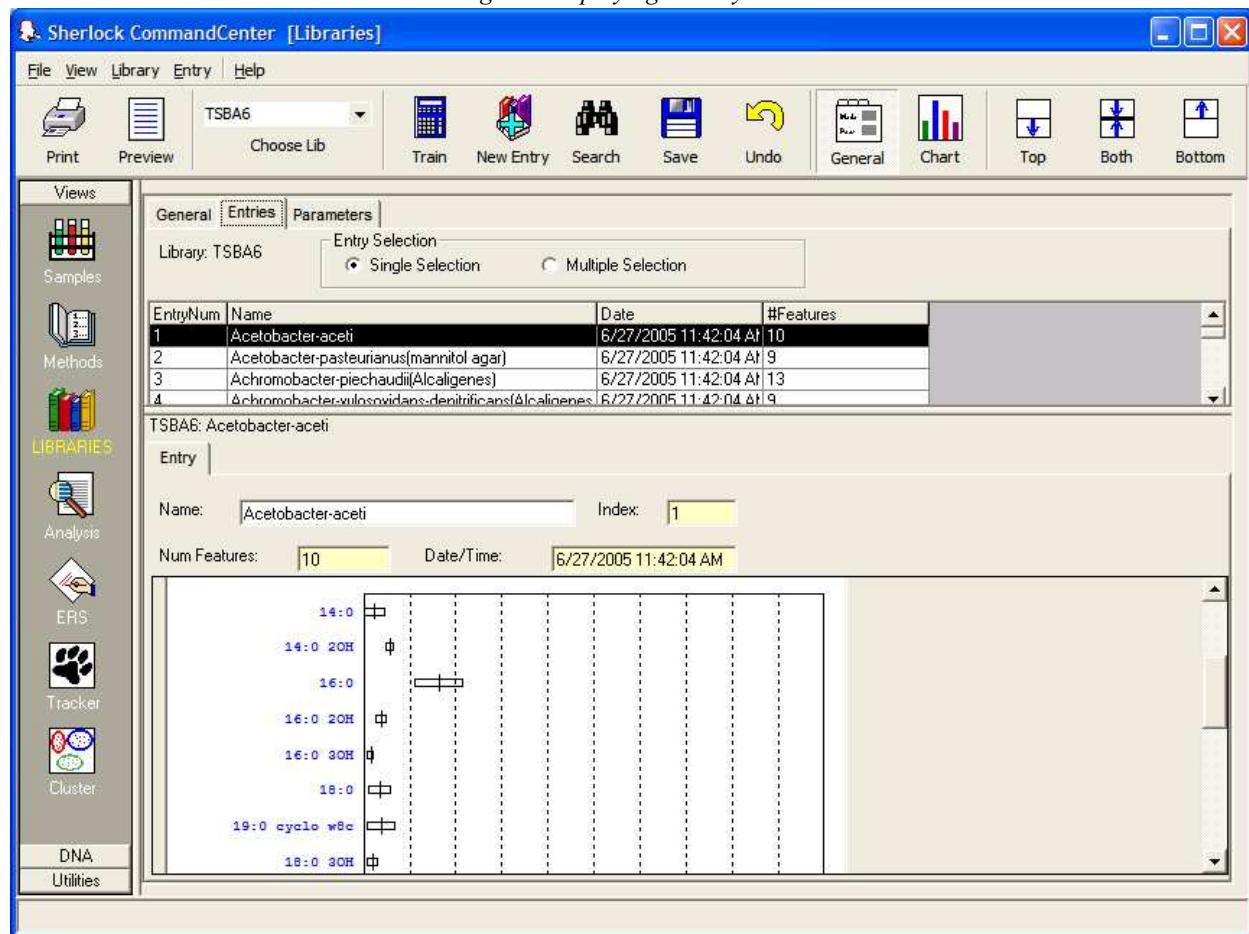
If you want to change which library you are viewing, click the down arrow on the *Choose Lib* Tool



and dropdown to the library of interest. Figure 5-24 shows the *TSBA6* library selected. Initially, just the general library information is shown in the top windowpane. To view the list of library entries, click the *Entries* tab in the top windowpane as shown in Figure 5-25. The list of entries for that library is displayed.

The bottom windowpane displays information for the entry selected in the top windowpane. The graphic in the bottom windowpane in Figure 5-25 gives the typical percentages and ranges for each feature (fatty acid or summed feature) in the selected library entry. The boxes show a ± 2 -sigma range for each feature in the data used to build the library entry. The vertical line through the middle is the mean for the library entry data.

Figure 5-25
Selecting and Displaying Library Entries



Searching Libraries

Users can search the libraries for entries that contain a particular percentage of up to three features.



To access this search feature, click on the *Search Tool*. In the example in Figure 5-26, three different fatty acids were used with the associated ranges of percentages. Only one library genus, *Staphylococcus*, has species that match these criteria.

Figure 5-26: The Search Library Tool

Search Library

Feature:	15:0 iso	15:0 anteiso	18:0
Low:	10	20	10
High:	20	30	20

Find Matches Matches Found: 14

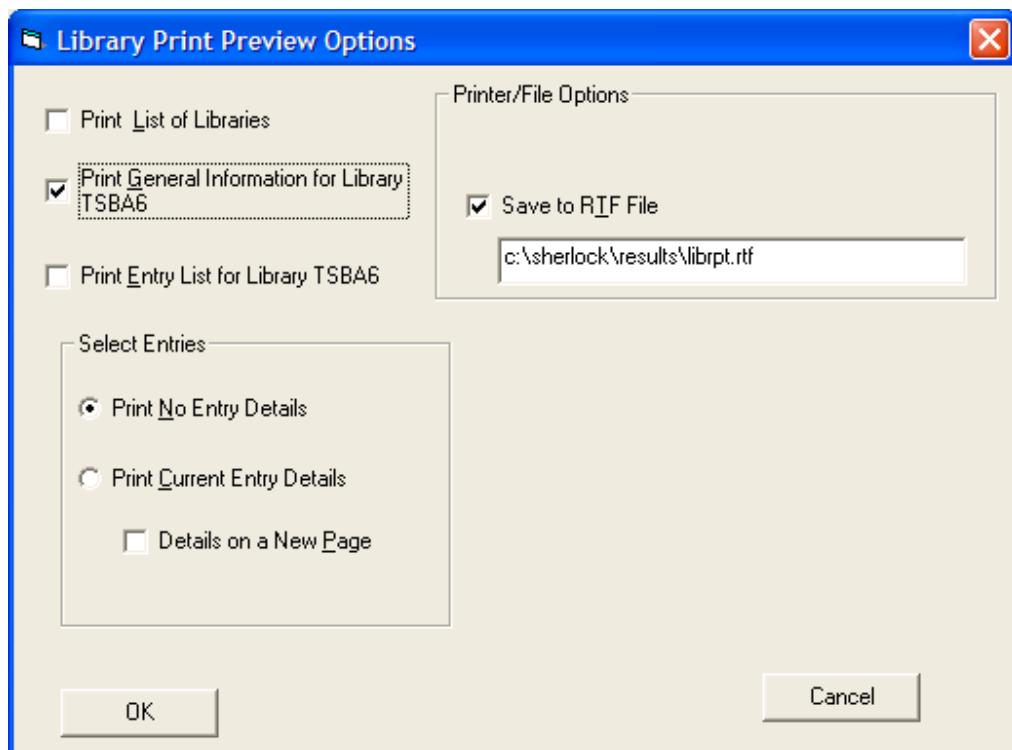
Library Entry	Feature 1	Feature 2	Feature 3
► <i>Staphylococcus-aureus</i> -GC subgroup	11.3 (5.7 - 17.0)	25.7 (21.1 - 30.2)	14.1 (10.9 - 17.2)
<i>Staphylococcus-aureus</i> -GC subgroup	12.1 (8.2 - 15.9)	17.7 (13.8 - 21.7)	17.0 (12.3 - 21.7)
<i>Staphylococcus-aureus</i> -GC subgroup	13.2 (10.8 - 15.5)	22.0 (18.8 - 25.3)	13.8 (9.4 - 18.2)
<i>Staphylococcus-auricularis</i> -GC subg	8.3 (4.4 - 12.2)	22.6 (12.7 - 32.5)	8.6 (4.2 - 12.9)
<i>Staphylococcus-capitis</i> -capitis	7.9 (3.4 - 12.4)	25.1 (20.5 - 29.8)	14.4 (9.6 - 19.2)
<i>Staphylococcus-caprae</i>	15.5 (13.8 - 17.2)	27.7 (25.6 - 29.8)	13.8 (12.4 - 15.2)
<i>Staphylococcus-chromogenes</i>	30.4 (18.2 - 42.7)	25.5 (20.0 - 31.0)	7.1 (3.0 - 11.3)
<i>Staphylococcus-epidermidis</i> -GC sub	9.8 (6.7 - 12.9)	26.9 (19.6 - 34.3)	12.0 (8.9 - 15.1)
<i>Staphylococcus-epidermidis</i> -GC sub	13.6 (9.8 - 17.4)	25.9 (21.3 - 30.6)	12.8 (9.6 - 15.9)
<i>Staphylococcus-epidermidis</i> -GC sub	11.8 (7.1 - 16.4)	32.9 (24.5 - 41.3)	11.8 (8.2 - 15.4)
<i>Staphylococcus-haemolyticus</i>	16.0 (11.4 - 20.5)	27.8 (22.9 - 32.8)	15.8 (12.4 - 19.2)
<i>Staphylococcus-pasteuri</i>	9.9 (9.5 - 10.2)	23.4 (22.6 - 24.2)	12.7 (12.0 - 13.4)
<i>Staphylococcus-simulans</i> -GC subgra	15.1 (13.1 - 17.0)	27.5 (20.7 - 34.4)	19.8 (15.8 - 23.8)
<i>Staphylococcus-warneri</i>	8.6 (5.3 - 11.9)	25.3 (17.0 - 33.6)	11.0 (8.3 - 13.7)

OK

Printing Library Information

Selected library information can be sent to the printer or viewed on the screen the same as was done for methods. Clicking on the *Print* Tool will bring up a dialog box similar to Figure 5-27. You can then choose the portions of the library to print by checking the appropriate boxes and clicking the *OK* button. The library information can also be saved to an RTF file, which can be opened in Microsoft Word. The *Print Preview* Tool can also be used to view the same library information on the computer screen.

Figure 5-27
Printing Library Information



Sherlock Methods and Associated Libraries

Table 5-1 summarizes all the current methods and libraries that may be shipped with Sherlock, and the calibration mixture used for each. See Table 1-1 for a complete description.

*Table 5-1
Standard Methods with Associated Libraries and Calibration Mixtures*

Type	Method	Library	Calibration Mix
Rapid	RTSBA6	RTSBA6	Rapid (P/N 1300-AA)
Rapid	RCLIN6	RCLIN6	Rapid (P/N 1300-AA)
Rapid	RBTR3	RBTR3	Rapid (P/N 1300-AA)
Standard	TSBA6	TSBA6	Standard (P/N 1200-A)
Standard	CLIN6	CLIN6	Standard (P/N 1200-A)
Standard	BTR3	BTR3	Standard (P/N 1200-A)
Sensitive	SMYCO6	M17H10	Rapid (P/N 1300-AA)
Standard	MYCO6	M17H10	Standard (P/N 1200-A)
Sensitive	SMOORE6	MOORE6	Rapid (P/N 1300-AA)
Sensitive	SANAER6	BHIBLA	Rapid (P/N 1300-AA)
Standard	MOORE6	MOORE6	Standard (P/N 1200-A)
Standard	ANAER6	BHIBLA	Standard (P/N 1200-A)
Sensitive	SFUNGI6	FUNGI	Rapid (P/N 1300-AA)
Sensitive	SYSTCLN6	YSTCLN	Rapid (P/N 1300-AA)
Sensitive	SYEAST6	YST28	Rapid (P/N 1300-AA)
Standard	FUNGI6	FUNGI	Standard (P/N 1200-A)
Standard	YSTCLN6	YSTCLN	Standard (P/N 1200-A)
Standard	YEAST6	YST28	Standard (P/N 1200-A)
<i>Instant FAME*</i>	IBA1	IBA1	Rapid (P/N 1300-AA)
<i>Instant FAME*</i>	IMYC1	IMYC1	Rapid (P/N 1300-AA)
<i>Instant FAME*</i>	IR2A1	IR2A1	Rapid (P/N 1300-AA)
<i>Instant FAME*</i>	ITSA1	ITSA1	Rapid (P/N 1300-AA)
<i>Instant FAME*</i>	ITY1	ITY1	Rapid (P/N 1300-AA)
Special	EUKARY	<none>	Eukary (P/N 1201-A)

* See the *Instant FAME™ User's Guide* for details.

CommandCenter Tools and Tasks (Icons)

The icons associated with the **CommandCenter** are described in Tables 5-2 through 5-5. All of the *Tools* in the *Toolbar (Tools)* can be accessed through the *Menu Bar*, but the *Tools* provide a shortcut to these features.

*Table 5-2
Samples View- Basic Selector*

Name	Icon	Description
Sherlock Samples View		Clicking this icon will open up the Sherlock Samples view, where the user can view and edit sample data.
Print Sample Information		Allows the user to print sample information for the number of samples desired to the printer or to a Rich Text Format (RTF) file.
Print Preview		Allows the user to preview sample information directly on the computer screen.
Sort		The user can sort the sample table by any of the headers in the sample table.
Save		Any changes made to the sample table (changes are made in the bottom right window pane) can be saved.

*Table 5-3
Samples View- Advanced Selector*

Name	Icon	Description
Select Samples		In the Advanced Selector View, allows the user to toggle back to the sample selection mode from the detail mode.
Detail		In the Advanced Selector View, allows the user to view the details for the samples in the current selection.

Table 5-4
Methods View

Name	Icon	Description
Sherlock Methods View		Clicking this icon will open up the Sherlock Methods view, where the user can view the entry information from each method.
Print Method Information		Allows the user to print information pertaining to each method to the designated printer or to a Rich Text Format (RTF) file.
Print Preview		Allows the user to preview method information directly on the computer screen.
Save		Any changes made to a custom method (changes can only be made with the Library Generation Software upgrade installed) can be saved.
Create Method		Allows the user to create a custom method based on a previously installed method.
Delete Method		The user can delete an installed method.

Table 5-5
Libraries View

Name	Icon	Description
Sherlock Libraries View		Clicking this icon will open up the Sherlock Libraries view, where the user can view the entry information from each library.
Print Library Information		Allows the user to print information pertaining to each library to the designated printer or to a Rich Text Format (RTF) file.
Print Preview		Allows the user to preview library information directly on the computer screen.
Train		Allows the user to update a custom library after any changes are made to the entries (only available with the Library Generation Software upgrade installed).
New Entry		Enables the user to add an entry to any custom library (only available with the Library Generation Software upgrade installed).
Search Library		The user can locate specific entries based on up to three features (specific fatty acid or summed feature) and the values of those features.
Save		Any changes made to a custom library (changes can only be made with the Library Generation Software upgrade installed) can be saved.
Undo		The user can undo the last change made.
General		Allows the user to view general library information.
Chart		When creating a custom library, these tools allow the user to view the quality of the entries (only available with the Library Generation Software upgrade installed).
Top View		Enables the user to view the top windowpane of library information.
Both View		Allows the user to view both windowpanes of library information.
Bottom View		Allows the user to view the bottom windowpane of library information.

Chapter 6

Routine Maintenance

Overview

Routine maintenance of your Sherlock MIS comprises:

- Data backup, removal and storage.

To prevent loss of data in the event of a computer hard drive crash, it is important to regularly back up data to a more permanent storage system. Sherlock provides a Windows-based program to copy Sherlock data files out of the Sherlock DATA directories.

- Instrument Maintenance

The gas chromatograph operates at high sensitivity and with great retention time precision. To maintain quality performance, the gas distribution system must be maintained leak tight and free of contamination. In this chapter, we will focus on this maintenance objective and discuss the replacement of consumable parts for your GC hardware. This includes:

- Changing the Septum.
- Changing the Injection Port Liner.
- Changing the Gas Cylinders.
- Changing the Capillary Column.
- Calibrating the Capillary Column.

Data Storage

When a Sherlock batch is run, data is stored in two directories. The raw ChemStation chromatogram and integration results are stored in the Sherlock/RAW directory while the Sherlock data file used to create profiles is stored in the selected Sherlock/DATA directory.

All the information needed to manipulate Sherlock data files in the future is in the Sherlock/DATA directory. Users should routinely copy data files from this directory and store them on a data storage device. The only reason to save or store files from the Sherlock/RAW directory is if it will be necessary to regenerate a ChemStation chromatogram in the future. ChemStation files will accumulate on the hard drive and may need to be copied or removed from the Sherlock/RAW

directory using Windows Explorer. These files take up a modest amount of disk space on the hard drive and may cause shortage of disk space if the system has a small disk drive.

It is also possible to backup Sherlock/DATA files using Windows Explorer, the details of which will not be discussed here. Sherlock provides Backup and Restore functions that allow the user to copy files between Sherlock DATA directories and backup storage, which is the preferred method for backing up Sherlock data.

Note: Removing ChemStation data files will cause Chromatograms to no longer be displayed or printed for the associated Sherlock data files.

Sherlock Utilities

Data Backup



Double click the *Sherlock CommandCenter* Icon on the desktop to start the CommandCenter application. CommandCenter reverts to the view from the last time it was used.

If it is not in the *Backup* view as shown in Figure 6-1, click the Utilities button at the bottom of



the Taskbar, then click the *Backup* Task in the Taskbar, so that [Backup] appears in the Current View. In addition, the *Backup* Task will be highlighted in a capitalized yellow font.

The *Source Volume* label is displayed in the middle windowpane (the default is DATA). The Sherlock data files in this volume will be displayed in the bottom windowpane.

To access other *Source Volumes*, select a data volume from the drop-down menu (click on the down arrow). Data files can be selected for backup in three ways:



- All data files in the data volume can be selected by clicking on the *Select All* Tool in the Toolbar.
- Individual data files may be chosen by clicking the small box to the left of the data file name.



- The *Invert Selection* Tool will reverse which data files that have been selected.
- All data files in the data volume can be unselected by clicking the *Clear Selections*

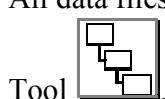
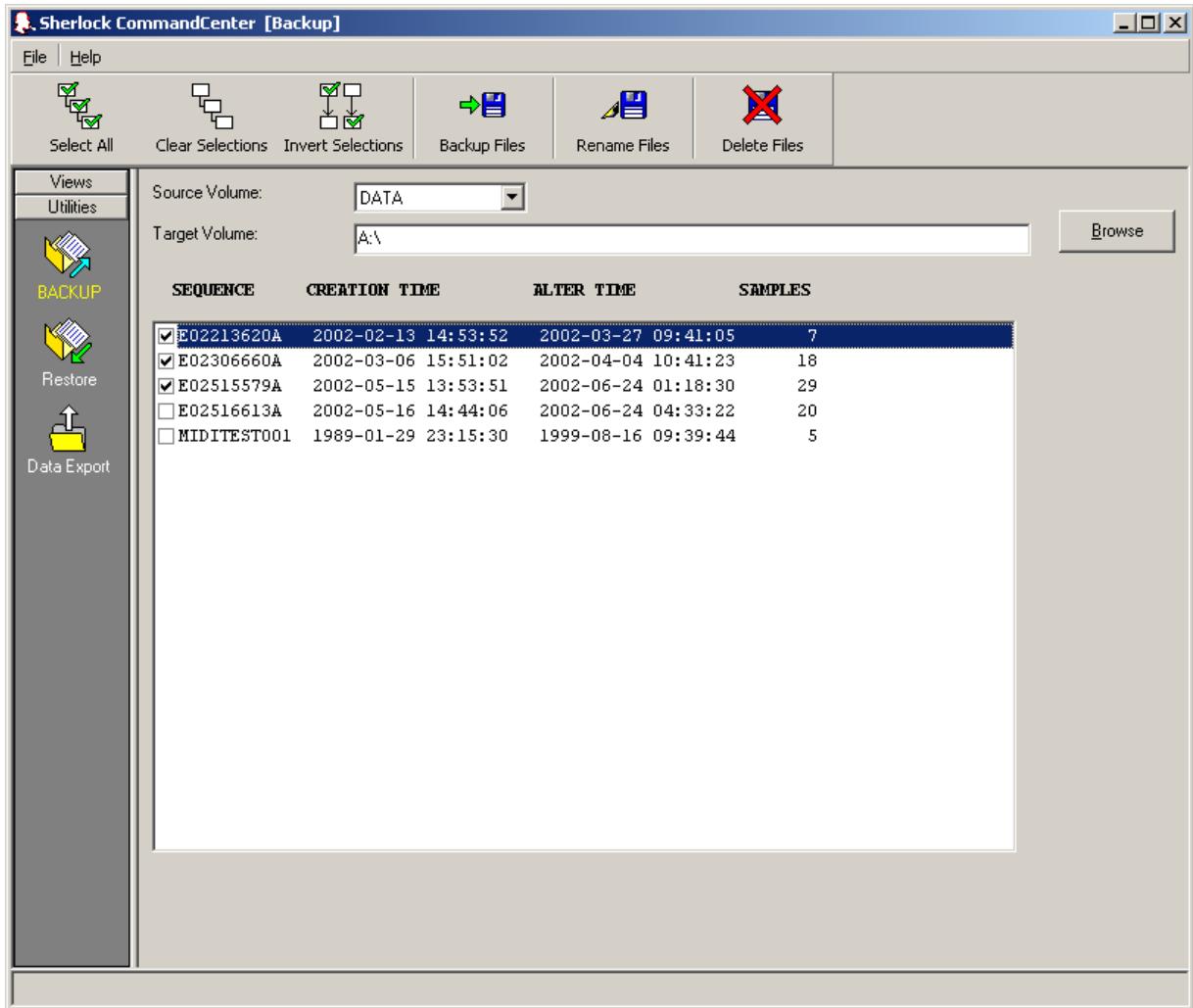


Figure 6-1
Data Backup View



Once the data files are selected, insert a formatted floppy disk into drive A: and click on the *Backup*

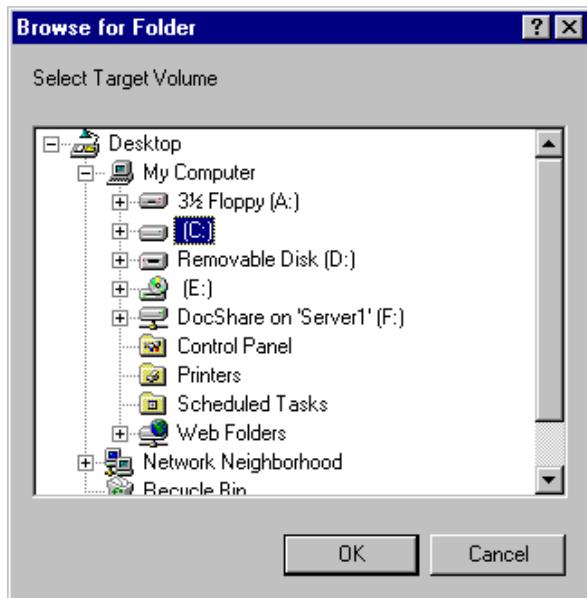


Files Tool. The user can also designate another directory, other than the A: in the *Target Volume* field to backup data files to the hard disk, a network share, a zip disk, etc.

Figure 6-2
Browse for Folder

To choose a *Target Volume* other than the A:, click the  button. A screen like Figure 6-2 will appear, and you can then select a new location for the *Target Volume*. After selecting a new location, press the *OK* button and this new target will appear in the *Target Volume* field.

As the files are copied onto the floppy disk or selected directory, the selection box(s) will clear. If backing up onto a floppy, and the floppy becomes full during this backup process, Sherlock will prompt the user to insert another floppy disk into drive A. The program will prompt the user with an “**overwrite**” confirmation box if the selected files already exist on the floppy or directory. Any files that were not copied will remain checked.



The user can rename Sherlock data files in the Backup View by selecting the data file of interest



(one at a time) and then pressing the **Rename Files** Tool .



To delete any data file(s), choose 

the data file(s) of interest and press the **Delete Files** Tool .

*Note: Under the File menu, select the **Export** (different than Data Export) button to backup data files for use on older versions of Sherlock (Sherlock 3.1 and older).*

Also under the *File* menu, you can select *Exit* to exit the Sherlock software.

To go to another CommandCenter view, click the  button and select the view of interest.

Tip: If you have a CD/DVD writer, external USB drive, or large-capacity memory stick, you can save a complete backup by simply copying the entire C:\SHERLOCK folder to the backup device. You can restore individual files and folders from ..\SHERLOCK\DATA and ..\SHERLOCK\RAW. Do not attempt to restore the complete C:\SHERLOCK folder without contacting MIDI Tech Support.

Data Restore



Double click the *Sherlock CommandCenter* Icon on the desktop to start CommandCenter application. CommandCenter reverts to the view from the last time it was used. If it is not in the *Restore* view as shown in Figure 6-3, click **Utilities** button at the bottom of the Taskbar, then click

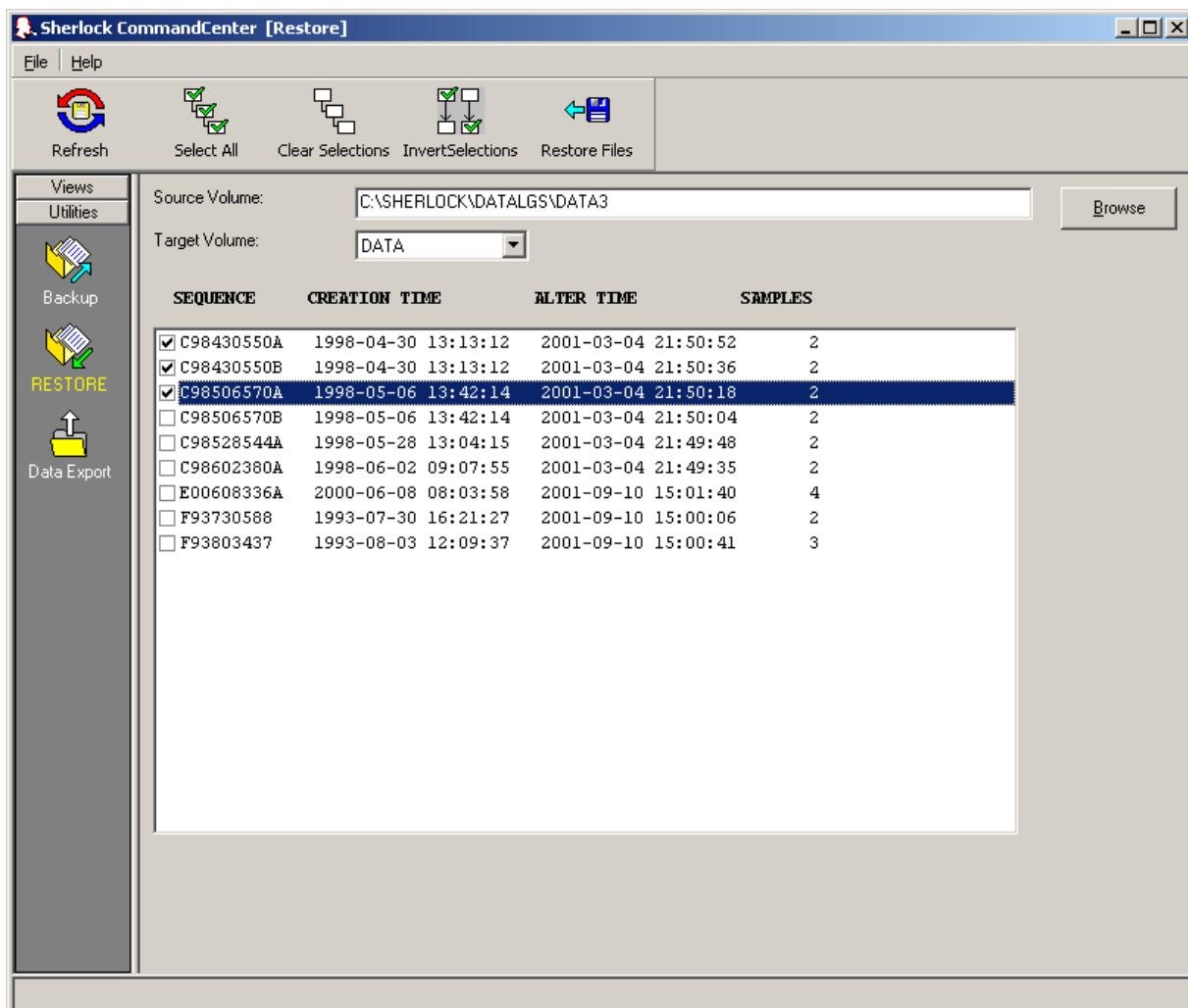


the *Restore* Task in the Taskbar, so that [Restore] appears in the Current View. In addition, the *Restore* Task will be highlighted in a capitalized yellow font.

The *Source Volume* label is displayed in the middle windowpane (the default is A:\). To change

this, click the **Browse** button or anywhere in the *Source Volume* field and choose a directory as was done in Figure 6-2. In the example below (Figure 6-3), the Source Data came from C:\SHERLOCK\DATA\LS\DATA3 (the default A:\ was changed to this directory) because the data of interest was in this directory.

Figure 6-3
Data Restore View

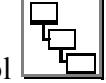
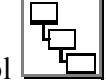


The Sherlock data files contained on the disk or directory will appear in the bottom windowpane.

The default *Target Volume* location is DATA, but other *Target Volumes* can be chosen with the down arrow. Data files can be selected in several ways:

- All data files in the bottom windowpane can be selected by clicking on the  *Select All Tool* in the Toolbar.
- Individual data files can be chosen by clicking the small box to the left of the data file name.



- The *Invert Selection Tool*  will reverse the data files that have been selected.
- All data files in the data volume can be unselected by clicking the *Clear Selections*  Tool .



Once the data files are selected, click on the *Restore Files Tool* . As the files are copied onto the hard drive, the selection box(es) will clear. The program will prompt the user with an “**overwrite**” confirmation box if the selected files already exist on the Target Volume. Any files



that were not copied will remain checked. In the *Data Restore View*, the *Refresh Tool*  allows the user to update the directory if a new floppy is inserted.

Under the *File* menu, you can select *Exit* to exit the Sherlock software, or to go to another CommandCenter view, click the  *Views* button and select the view of interest. The icons associated with various *Tools* in Sherlock Utilities are summarized in Table 6-1.

Table 6-1
Utility- Associated Tools

Select All		In the Backup or Restore View, allows the user to choose all the data files.
Clear Selections		In the Backup or Restore View, allows the user to deselect all the data files.
Invert Selections		In the Backup or Restore View, allows the user to invert the current selections.
Backup Files		In the Backup View, pressing this icon enables the user to backup the selected data files to the target volume.
Rename Files		Allows the user to rename a selected data files in the Backup View.
Delete Files		In the Backup View, this allows the user to delete any of the selected data files.
Refresh Directory		In the Restore View, this feature is used when the user changes floppy disks and wants to refresh the directory.

Sherlock Toolbox

The Sherlock Toolbox contains ancillary tools that are used to manage the Sherlock system. The following tools are described in more detail in this chapter: **Install**, **Offload**, **Set Pressures** and **Configure Methods**.

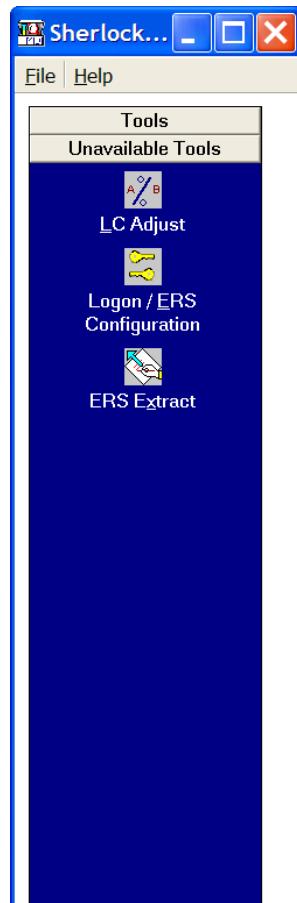


To access the Toolbox, double click on the *Toolbox Icon* on the desktop. A window will appear similar to Figure 6-4. There are several tools, which may or may not be available (Figure 6-5), depending on your system configuration, and these are described as well in Table 6-2.

Figure 6-4
Sherlock Toolbox



Figure 6-5
Unavailable Tools



All of the tools associated with the Sherlock Toolbox are described in Table 6-2.

Table 6-2: Toolbox Associated Tools

Name	Icon	Description
Sample Processor Configuration		Allows for adjustment of the instrument configuration and sample processor output parameters.
Activate Methods and Libraries		Installs MIDI Inc.'s factory copies of the methods and libraries ordered by the customer.
Set Pressures		When using the Agilent ChemStation, allows one to set the pressure of the GC to adjust peak retention times. Must be run before a method is used the first time.
Configure Methods		Similar to Set Pressures, but for Agilent series 7800 Gas Chromatographs. Must be run before a method is used the first time.
Logon / ERS Configuration		Set User/Manager logon groups, as well as control Electronic Record and Signature (ERS) parameters.
ERS Extract		Runs the CommandCenter using data from a selected ERS file.
Install		Allows the user to install methods and/or libraries from floppy or zip disks as well as network stores.
Offload		Allows the user to copy current methods and/or libraries to floppy or zip disks as well as network stores.
LC Adjust		Adjustment program used only in the HPLC system.

Offload Methods

The user will occasionally want to offload methods (make a copy of a method). To offload a



method, double click on the *Toolbox Icon* on the desktop and then click on the *Offload Tool*



in the Toolbox. The **Offload Methods** screen should look like Figure 6-6. Make sure that the *Offload Methods* tab is selected. Next, Click on the *Browse* button to search for a destination directory to copy the method(s). The destination path can be on the hard disk, removable media or Local Area Network (LAN). In this example, the first four methods in Figure 6-6 are copied to the C:\NewMethods directory (Figure 6-7). This is done by checking the methods to offload



(Figure 6-6) and then clicking on the *Offload Tool*

Figure 6-6
Offload Methods-Step A

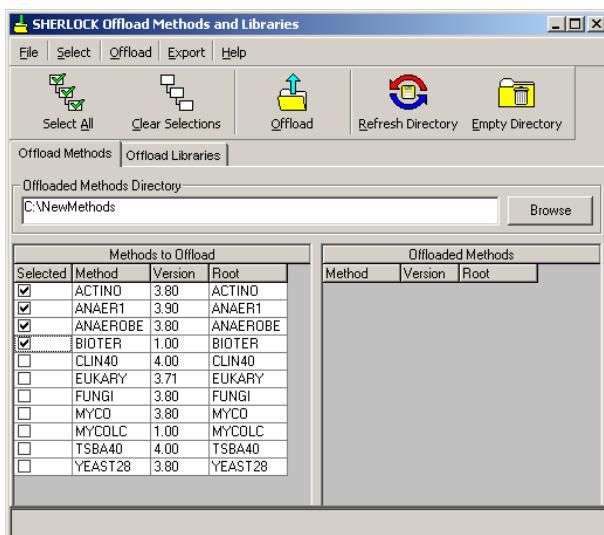
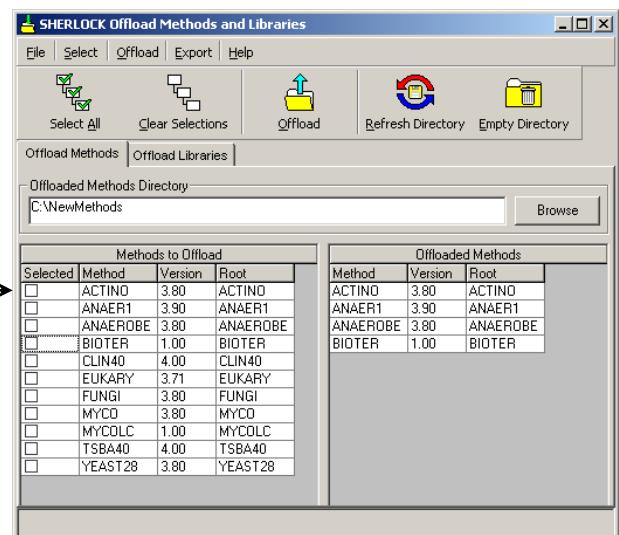


Figure 6-7
Offload Methods-Step B



The *Empty Directory Tool* will delete all the methods in the destination path.

Offload Libraries

The user will occasionally want to offload libraries (make a copy of a library). To offload a library,



double click on the *Toolbox Icon* on the desktop and then click on the *Offload Tool* in the Toolbox. The **Offload Libraries** screen should look like Figure 6-8. Make sure that the *Offload Libraries* tab is selected. Next, Click on the *Browse* button to search for a destination directory to copy the library (or libraries).

The destination path can be on the hard disk, removable media or Local Area Network (LAN). In this example, the first four libraries are selected in Figure 6-8 and offloaded to the C:\NewLibraries directory (Figure 6-9). This is done by checking the libraries to offload (Figure



6-8) and then clicking on the *Offload Tool*

Figure 6-8
Offload Libraries-Step A

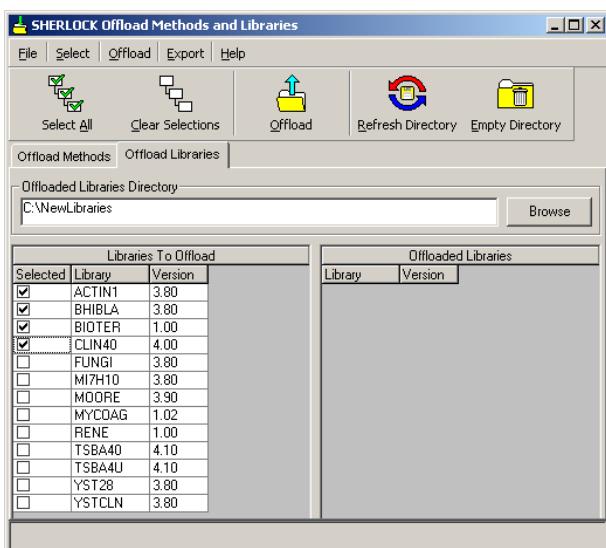
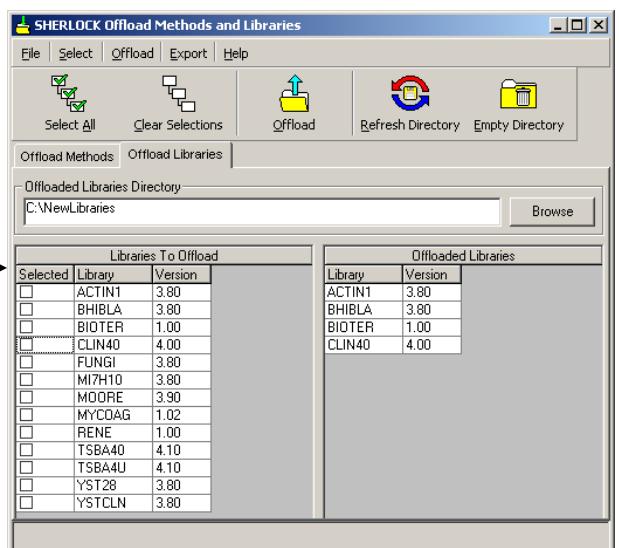


Figure 6-9
Offload Libraries-Step B



The *Empty Directory Tool* will erase all the libraries in the destination path.

Install Methods

The user will occasionally need to install methods. To install methods, double click on the *Toolbox*

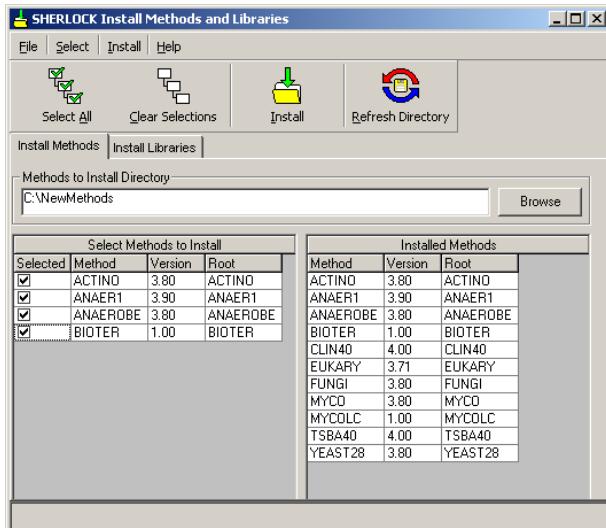


Icon on the desktop and then click on the *Install Tool* icon in the Toolbox. The **Install Methods** screen should look like Figure 6-10. Make sure that the *Install Methods* tab is selected. Next, click on the *Browse* button to search for the methods to install. These methods can be on the hard disk, removable media or Local Area Network (LAN). In this example, four methods from the C:\NewMethods directory are added to the Sherlock system (Figure 6-11). This is done by

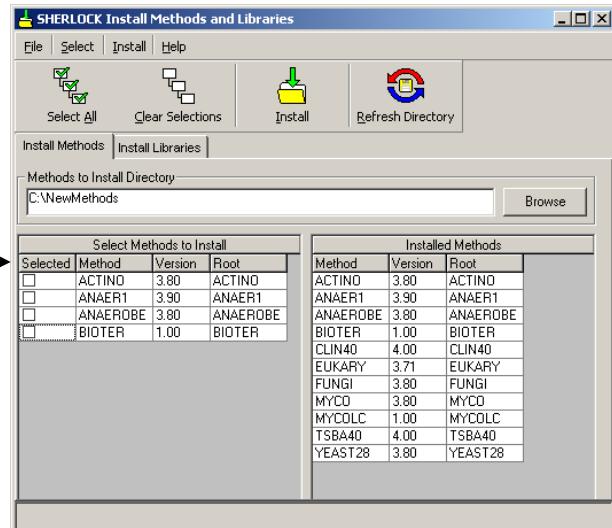


checking the methods to install (Figure 6-10) and then clicking on the *Install Tool* icon. If the methods already exist on the Sherlock System, as in Figure 6-10, a message box will ask if you would like to overwrite the existing methods.

*Figure 6-10
Install Methods-Step A*



*Figure 6-11
Install Methods-Step B*



Install Libraries

The user will occasionally also need to install libraries. To install libraries, double click on the



Toolbox Icon on the desktop and then click on the *Install Tool* in the Toolbox. The **Install Libraries** screen should look like Figure 6-12. Make sure that the *Install Libraries* tab is selected. Next, click on the *Browse* button to search for the libraries to install. These libraries can be on the hard disk, removable media or Local Area Network (LAN). In this example, four libraries from the C:\NewLibraries directory are added to the Sherlock System. This is done by



checking the libraries to install (Figure 6-12) and then clicking on the *Install Tool*. If the libraries already exist on the Sherlock System, as in Figure 6-12, a message box will ask if you would like to overwrite the existing libraries.

Figure 6-12
Install Libraries-Step A

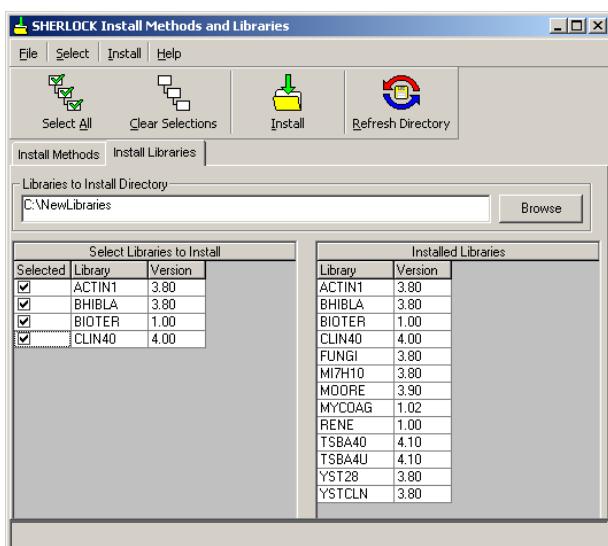
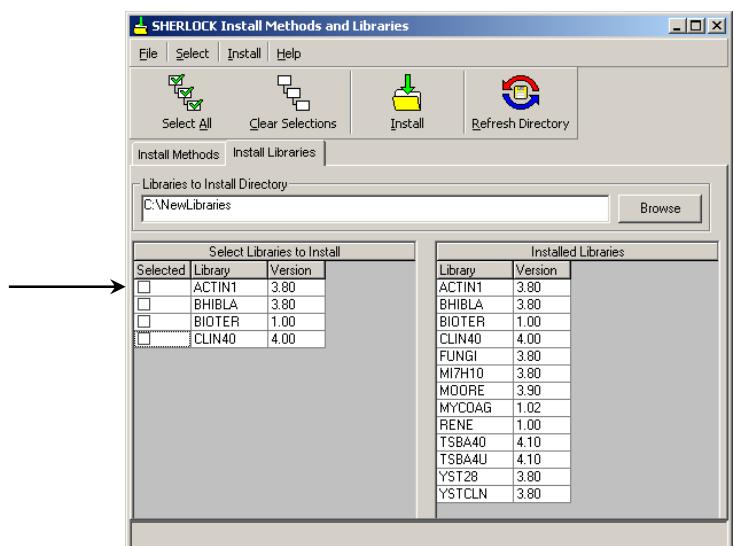


Figure 6-13
Install Libraries-Step B



Setting Pressures (Agilent 5800/6800 series GCs)

The Sherlock user may need to reset the gas pressures. This screen is accessed by double clicking



on the *Toolbox* Icon on the desktop and then clicking on the *Set Pressures Tool* in the Toolbox. Figure 6-14 shows what the Set Pressures screen looks like. Select the methods to update, or see page 6-29 for further information.

The GC oven temperature **must be at 170°C** before setting pressures for Rapid or *Instant FAME* methods. Failure to do this will result in incorrect flow settings, which will cause calibration failures and/or incorrect fatty acid identifications.

NOTE: Set Pressures must be run before a method is used for the first time on your system.

Figure 6-14
Set Pressures Screen

The screenshot shows a Windows application window titled "Set Pressures S/N: 123456 - [6890GC]". The window has three main sections: a left panel with a table of methods and their values, a central "Pressure Update Status" area, and a bottom section for unavailable methods.

Method	Col A	Update
ACTIN6	9.0	<input type="checkbox"/>
ANAER6	9.0	<input type="checkbox"/>
BTR3	9.0	<input type="checkbox"/>
CLIN6	9.0	<input type="checkbox"/>
EUKARY	9.0	<input type="checkbox"/>
FUNGI6	9.0	<input type="checkbox"/>
MOORE6	9.0	<input type="checkbox"/>
MYC06	9.0	<input type="checkbox"/>
RBTR3	20.0	<input checked="" type="checkbox"/>
RCLIN6	20.0	<input checked="" type="checkbox"/>
RTSBA6	20.0	<input checked="" type="checkbox"/>
SACTIN6	9.0	<input type="checkbox"/>
SANAER6	9.0	<input type="checkbox"/>
SFungi6	9.0	<input type="checkbox"/>
SMOORE6	9.0	<input type="checkbox"/>
SMYC06	9.0	<input type="checkbox"/>
SYEAST6	9.0	<input type="checkbox"/>
TSBA6	9.0	<input checked="" type="checkbox"/>
YEAST6	9.0	<input type="checkbox"/>
YSTCLN6	9.0	<input type="checkbox"/>

Pressure Update Status

Unavailable Methods

DNAJUST
MDNAJUST

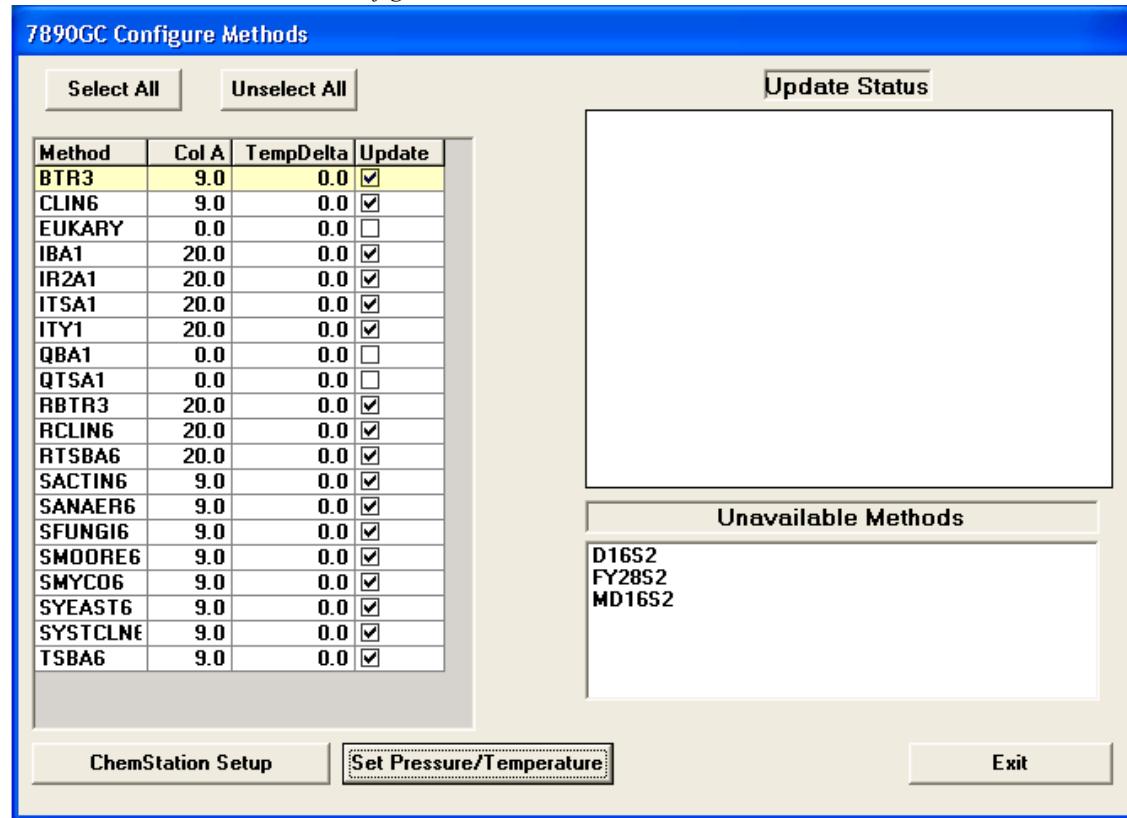
Set Pressures **Exit**

Configuring Methods (Agilent 7800 series GCs)

The process for configuring methods on the 7800 series is slightly different from the 5800/6800 series. The tool *Configure Methods* is found in the toolbox instead of *Set Pressures*. This tool is

accessed by double clicking on the *Toolbox Icon*  on the desktop and then clicking on the *Configure Methods Tool*  in the Toolbox. Figure 6-14B shows what the Configure Methods screen looks like.

Figure 6-14B
Configure Methods Screen



NOTE: Configure Method's ChemStation Setup must be run before a method is used for the first time on your system.

When first installing the system, or if a significant change occurs to the instrument (e.g. a new tower), use the *ChemStation Setup* button to run the ChemStation's Method Resolution on each method that you plan to use. This process will work much like *Set Pressures*, starting the ChemStation and making configuration corrections specific to your instrument.

Configure Methods allows setting both the pressure and the temperature offset for each method. This approach simplifies configuring the system to calibrate Sherlock methods.

Note that the *PSI* column is an absolute pressure to set; on the other hand, the *TempDelta* column is a **change** to the current temperature offset.

Select the methods to update, or see page 6-29 for further information.

GC Routine Maintenance

Septum

The septum and injection port liner are the most often changed components of the hardware. The septum is the rubber disk that is pierced by the needle to introduce the sample into the GC. After repeated injections, the septum will begin to leak, yielding unstable retention times, loss of response, and/or loss of column head pressure. For reliable operation, the septum should be changed at least every 100 injections. The septum is located in the injection port on the top left-hand side of the GC directly below the injector.

The **Merlin Microseal™** is an extended life (7,000 injections) replacement for the gray septum. It has a duck-billed design that is pushed apart by the needle, rather than being repeatedly pierced. It can be purchased from Merlin Instrument Company or through Agilent Technologies. A Merlin Microseal is supplied with the purchase of the Sherlock system.

Injection Port Split Liner

The injection port vaporizes liquid samples and mixes the vapors to produce a homogeneous sample. The Sherlock method uses a split inlet, meaning that only a small percentage of the sample actually enters the capillary column while the rest is vented out the split vent. The vaporization and mixing occur in a heated glass tube inside the injection port below the septum. This tube, called the split liner (the terms “split liner” and “injection port liner” are used interchangeably in this chapter), contains silanized glass wool packing, to ensure vaporization and adequate mixing of the sample before it enters column. The inside of this tube must be clean and free of particulates such as small pieces of septum broken off by repeated injections.

The liner is maintained at 250°C, and carbonaceous residue from sample extracts tends to build up with time. If proper care is not taken, small amounts of sodium hydroxide (NaOH) from the sample preparation base wash may be carried over into the GC extract, reducing the recovery of the hydroxy fatty acids. To avoid interaction between sample and liner, replace the liner periodically. One replacement liner is furnished with the system, and reordering information is in Appendix A. MIDI recommends keeping a minimum stock of six liners.

Glass liners may also be cleaned and repacked with fresh silanized glass wool. To minimize liner-introduced failures, this practice is not recommended. MIDI supplied liners are manufactured to meet the Sherlock requirements. This cleaning and repacking procedure is discussed later in this section.

Replace the split liner at least every 100 samples for the Standard Methods and every 200 samples for the Instant FAME, Rapid and Sensitive Methods. Consult the PQ tables in Chapter 7. If you are not using the Merlin Microseal, always change the septum first while changing the split liner.

Preparing to Replace the Septum and/or Injection Port Liner

Note: The column will be damaged, if it is hot (above 50 °C) and air enters. Always cool the column to near ambient temperature before changing the septum or liner.

To avoid potential column damage, decrease the GC oven temperature to near ambient:

- For the 6890 and 7890 GCs, press the keys [OVEN], [0], [ENTER] on the front.
- For the 6850 GC, press the *Settings* softkey and then the *Oven* softkey on the hand-held keypad. Use the left and right arrows to move to the oven temperature field. Type [0] in the oven temperature field and press [Enter].

It is only necessary to wait until the oven temperature drops below 50°C. However, 30°C will provide for an extra margin of protection for the column.

To avoid a safety shutdown, the column head pressure must be set to zero:

Note: Wait for the oven to cool below 50°C before setting pressure to zero.

- **6890 and 7890 GCs:**
 - Press the key for the desired inlet, [FRONT INLET] or [BACK INLET].
 - Record the current pressure.
 - Use the up and down arrows to scroll to the Pressure value.
 - Press [0] and [ENTER] to set it to zero.
- **6850 GC:**
 - Press the *Settings* softkey and then the *Inlet* softkey on the hand-held keypad.
 - Record the inlet pressure and flow.
 - Use the left and right arrows to move to the inlet pressure or inlet flow field (the pressure field is not available when using constant flow methods like the Rapid methods).
 - Type [0] and press [ENTER] to set the value to zero.

Remove the solvent and waste vials from the turret.

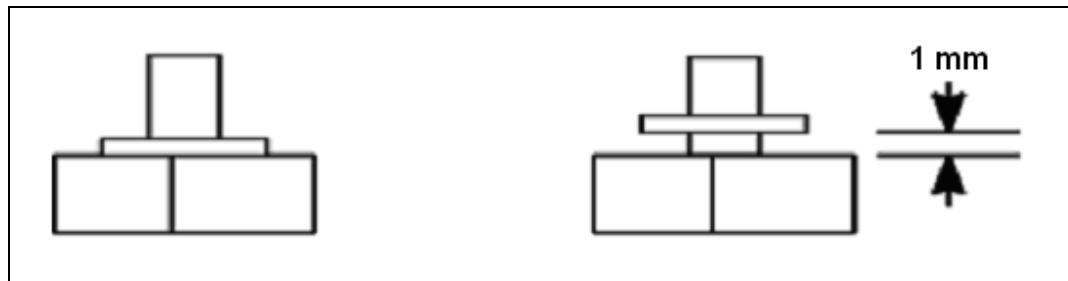
Lift the injector tower from the GC to expose the inlet and retainer nut (Figures 6-15 and 6-16). Older model injectors must be rotated counterclockwise and then lifted.

Replacing the Gray Septum:

- Follow the instructions in the section “Preparing to Replace the Septum and/or Injection Port Liner.”
- Loosen and remove the 5/8-inch septum retainer nut using the wrench provided with the GC.
Caution: *The septum retainer nut is HOT (250 °C). Exercise extreme care in its removal. Use cotton gloves or handle with pliers.*
- Remove the septum with a pair of tweezers or forceps. The septum may be stuck inside the retainer nut or in the septum seat.
- Clean septum debris from the seat and retainer nut to ensure a leak free seal.
- Press the new septum into the septum seat.
- The septum retainer nut must be tightened enough to obtain a good seal, but not so much as to compress the septum and make it difficult to push a syringe needle through it. The standard septum retainer nut contains a spring that applies pressure to the septum.

Replace the septum retainer nut and tighten finger tight until there is a gap between the locking washer and the top surface of the nut. Refer to Figure 6-15. Continue to tighten until the gap does not increase (and no further), and then back off roughly one-quarter turn until the gap just starts to decrease. The C-ring should lift about 1 mm above the top surface.

*Figure 6-15
Tightening the Septum Retainer Nut*



- Restore the inlet pressure (or column flow) to the previous value. If the inlet pressure does not immediately return to the previous value, adjust the retainer nut slightly. Do not over tighten the retainer nut.

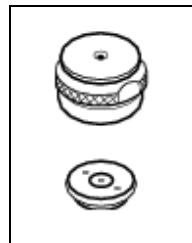
Remount the automatic injector back onto the GC.

If not replacing the injection port liner, remont the automatic injector. Otherwise, proceed with the “Steps to Change the Split Liner.”

Replacing the Merlin Microseal:

- Follow the instructions in the section “Preparing to Replace the Septum and/or Injection Port Liner.”
- Loosen the Microseal retainer.
Caution: The septum retainer nut is HOT (250 °C). Exercise extreme care in its removal. Use cotton gloves or handle with pliers.
- Remove the Microseal with a pair of tweezers.
- Clean any debris from the septum seat and retainer nut to ensure a leak free seal.
- Press the new Microseal into the septum seat.
- Barely tighten the retainer nut with your fingers.
- Restore the gas pressure/flow settings to the previous value.
- Slowly tighten the retainer nut until gas pressure begins to increase.
- Tighten an additional 30° (one clock mark on the nut). The pressure/flow should hold steady at the original value. . The Microseal retainer nut must be tightened just enough to obtain a good seal. Over tightening will cause leaks.

*Figure 6-16
Merlin Microseal™ and Retainer Nut*



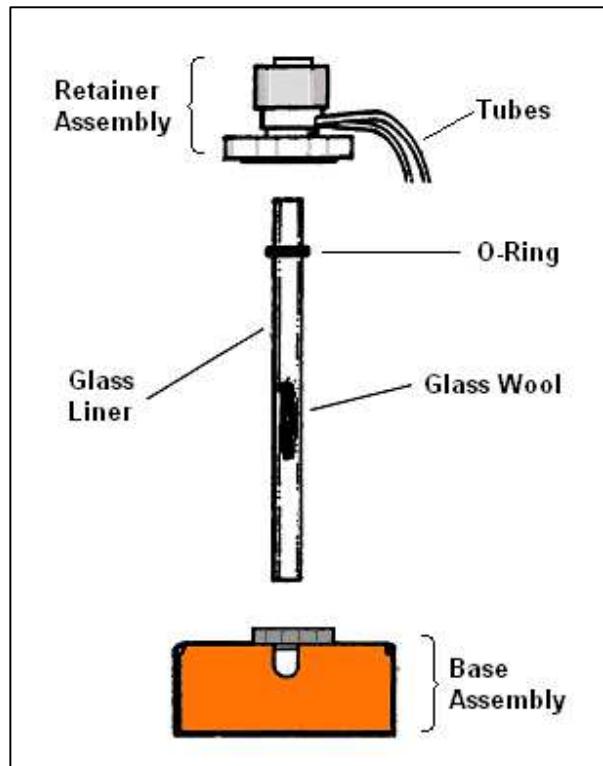
Remount the automatic injector back onto the GC.

If not replacing the injection port liner, remont the automatic injector. Otherwise, proceed with the “Steps to Change the Split Liner.”

Changing the Split Liner

- Follow the instructions in the section “Preparing to Replace the Septum and/or Injection Port Liner.”
- Figure 6-17 shows a schematic representation the injection port liner assembly. The exact appearance, retainer assembly to base assembly locking mechanism, and O-ring size depends on the GC model and options.
 - Older 6890 and 6850 Sherlock Systems have 1-inch locking nuts.
**(Requires MIDI Part # 1221
injection port liner and O-ring.)**
 - A quick release Flip Top Inlet Sealing System (Agilent Part # 5188-2717) was included with newer 6890 and 6850 Sherlock Systems. Older 6890 and 6850 GCs can be upgraded.
**(Requires MIDI Part # 1221-F
injection port liner and O-ring.)**
 - The standard 7890 split/splitless inlet has a quick release retainer assembly.
**(Requires MIDI Part # 1221
injection port liner and O-ring.)**

Figure 6-17
Injection Port Liner Assembly



To prevent leaks, use the MIDI injection port part number that is specified for your instrument and inlet configuration.

- Prepare a new MIDI port liner and O-ring by positioning the O-ring about 20 mm from the top of the glass tube. The position is not critical. Using the correct MIDI part number to obtain the correct O-ring is critical.

Note: Do not touch the glass liner with bare hands. Cotton gloves should be worn.

- Unlock the retainer assembly to base assembly connection:
 - On older 6890 and 6850 models, loosen the 1-inch liner retainer nut, located below the septum retainer nut, until the retainer assembly can be pulled free of the base. Use the wrench supplied with the GC.
 - On 6890 and 6850 models equipped with the Flip-Top system, lift the latch to release.
 - On 7890 models, slide/rotate the yellow locking tab counterclockwise.

- Raise the entire retainer assembly straight up along with its attached carrier gas and septum purge tubes. Lifting it at an angle will break the top edge of the glass liner. The used split liner should not lift up with the retainer assembly. If it does, hold it down with forceps.
- Carefully pull out the old split liner with forceps. The old O-ring should come out with it.
- Install the new split liner into the injection port body with the O-ring end at the top. Be sure to use the MIDI liner specified for your GC and inlet configuration.
- Press the split liner down firmly against the bottom of the injection port chamber forcing the O-ring into the correct position.
- Replace the retainer assembly and lock in place. On systems with the 1-inch locking nut, avoid over tightening.
- Restore the inlet pressure (or column flow) to the previous value.
- Be sure that the column head pressure (the inlet pressure) rises to the previous set point. If the correct pressure cannot be obtained check that the retainer assembly is correctly seated.
- Remount the automatic injector.

Bake-out of the new liner:

- Be sure that the flame is still lit. On the front panel of the 6890 and 7890 GCs, press the key for the desired detector, [**FRONT DET**] or [**BACK DET**]. Scroll to “Output”. On a 6850 press the *Settings* softkey, then the *Detector* softkey and note the “Output” value. The background current of the flame should be greater than ~2 pA.
- Bake out the system by entering [**OVEN TEMP**] [290] [**ENTER**] on the appropriate keyboard and wait at least 30 minutes. The system should be ready to start within 30-45 minutes.

Starting a batch in the Sample Processor will automatically load the correct set points into the GC. If a batch is NOT going to be started, set the oven temperature back to the initial resting value of 170°C.

Cleaning the Glass Liner Tube

MIDI recommends that new liners and O-ring kits be obtained from MIDI. After the glass liner tube has been used several times, deposits on the inside of the tube will start to affect performance. The tubes can be cleaned and put back into service.

- Remove the old glass wool plug.
- Stand the liners in a beaker with a magnetic stirrer.
- Cover the liners with deionized or distilled water. Add two drops of a mild glass-cleaning detergent. We recommend Joy® brand detergent.

- Place on a hot plate and allow to stir and heat to approximately 90°C (almost boiling). Hold at this temperature for about one hour, adding water as necessary to keep covered.
- Drain and rinse with deionized or distilled water. Optionally rinse with ethanol or Reagent 3.

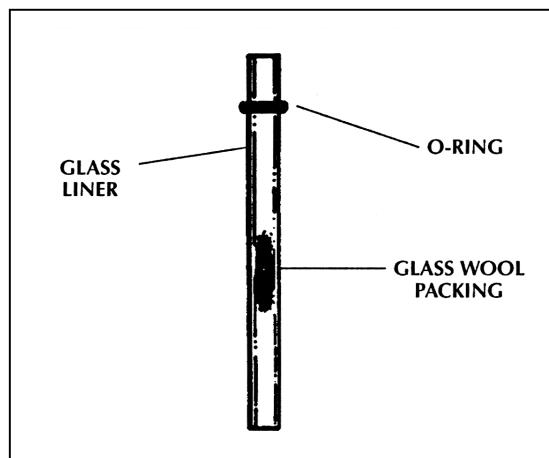
Allow to air dry and store in a screw-top tube until needed. From this point forward, only handle the liners while wearing cotton gloves.

Rewrapping a Split Liner

MIDI recommends that new liners and O-ring kits be obtained from MIDI. The aging of the glass wool in the split liner causes degraded hydroxy fatty acid recovery. New glass wool can be placed in the old glass liner tube if the tube is clean. It is recommended that you follow the following procedure under a hood:

- Using a small diameter stick (e.g. the stick from a Q-Tip®), wind a small plug of fresh silanized glass wool (See Appendix A). The new plug should be about the size of the old plug. Avoid touching the glass wool with your fingers. Use cotton gloves.
- Insert the glass wool plug into the top of the glass liner. Push the plug down in the tube approximately 15 mm.
- Rotate the stick in the opposite direction from winding the plug and withdraw, leaving the glass wool plug in the tube and some leftover glass wool hanging out.
- Using scissors cut the glass wool flush with the top of the liner.
- Use the stick to push the glass wool plug to the center of the tube. If the plug slides too easily, it may not stay in position during operation (push out and start over, using more material). Do not overwork the glass wool plug; if it breaks into smaller pieces, the silanization process used to deactivate the glass wool surface area is defeated.
- Store the repacked liner in a clean tube (use a screw top tube from the sample prep procedure).

*Figure 6-18
Glass Liner Tube with Glass Wool
Positioned in Center*



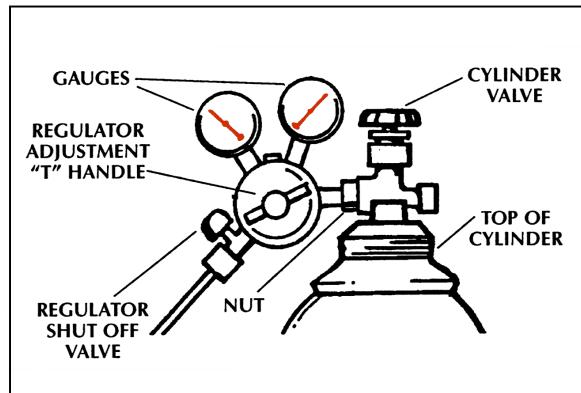
The Gas Supply

Changing the Gas Cylinders

Replace the gas cylinder before the pressure drops below 200 psi. Never replace cylinders with lower grades of gas.

- Decrease the GC oven temperature to near ambient. See instructions in the section “Preparing to Replace the Septum and/or Injection Port Liner.”
- Record the current inlet pressure and reduce it to zero. See instructions in the section “Preparing to Replace the Septum and/or Injection Port Liner.”

Figure 6-19
Cylinder Valve and Regulator



To turn OFF the flame for the 6890 and 7890:

- Press the [FRONT DET] and scroll to “Flame” and press [OFF]. Repeat for the back detector if it is installed,

To turn OFF the flame for the 6850:

- Press the *Settings* softkey, then the *Detector* softkey. Press the *Flame* softkey to turn the flame OFF.

For the 6890, 6850 and 7890:

- Turn off the valve on top of the cylinder and allow the system pressure to drop to atmospheric pressure.
- Loosen the large nut holding the regulator to the gas cylinder (nuts with a groove are left handed) and remove the regulator. Be very careful not to kink the tubing connecting the regulator to the GC. Find a safe place to lay the regulator; be careful not to drop it, or loosen the copper tubing.

Caution: Dropped or damaged regulators are dangerous and should be replaced.

- Replace the cylinder cap to cover the valve, and remove the used cylinder to the proper storage area.
- Obtain a new cylinder and secure it in place.

- Remove the cylinder cap and replace the regulator. Tighten it firmly. Do not use any sealing compound or Teflon tape on this fitting; it is designed to be a metal-to-metal seal.
- Slowly open the valve on top of the cylinder.
- Check for leaks around the regulator/cylinder connection with a commercial leak testing liquid, and tighten further, if required, to stop leaks. Also, check the stem of the valve for leaks.
- Open the cylinder valve completely when it is leak tight.
- Restore the inlet pressure to the previous setting. The small amount of air that enters the regulator during cylinder changes is quickly flushed from the system, and analyses can commence in 10 minutes.
- Reset the GC oven temperature by entering the key-strokes [OVEN TEMP] [170] [ENTER] on the appropriate keyboard.

To turn ON the flame for the 6890 and 7890:

- Press the [**FRONT DET**] and scroll to “Flame” and press [**ON**]. The signal output should return to its previous value.

To turn ON the flame for the 6850:

- Press the *Settings* softkey, then the *Detector* softkey. Press the *Flame* softkey to turn the flame ON. The signal output should return to its previous value.

Gas Supply Traps

Copper tubing, fittings, valves, and sometimes gas cylinders contain trace organics. Water can also be present at trace levels. These contaminants can result in high or noisy baselines on the detector output, produce ghost peaks, and even plug up gas distribution components. To protect against these problems, molecular sieve traps are recommended to trap out heavy organic compounds and water. Ordering information is included in *Appendix A*.

Installing the Capillary Column

The standard column that should be used with the Sherlock GC System is a 25 m X 0.2 mm crosslinked 5% phenyl methyl silicone fused silica capillary column (Agilent 19091B-102, or -102E for the 6850GC). Under normal use, this column should last for at least 20,000 injections. The main cause of column failure is due to inadequate care during sample preparation. Great care must be taken to avoid picking up any of the lower (aqueous) phase when transferring the organic extract to the GC sample vial. For that reason it is recommended that only **two-thirds** of the organic phase should be transferred.

Column failure results in tailing peaks. Again, as with contaminated injection port liners, the hydroxy acids are the first peaks to indicate damage. This situation is most often due to dirty or etched injection port liners that should be replaced before attempting to change the column. If the problem persists, the column should be replaced. Contact MIDI Technical Support for assistance.

Typically, the degraded portion of the column is within a few inches of the end installed in the injection port. It may be possible to restore the column by removing that end of the column from the injection port and cutting off a length equal to one turn on the basket. Reinstall the same slightly shortened column. To avoid significant changes in retention times and resolution, this should be done only a few times.

Preparing a Column

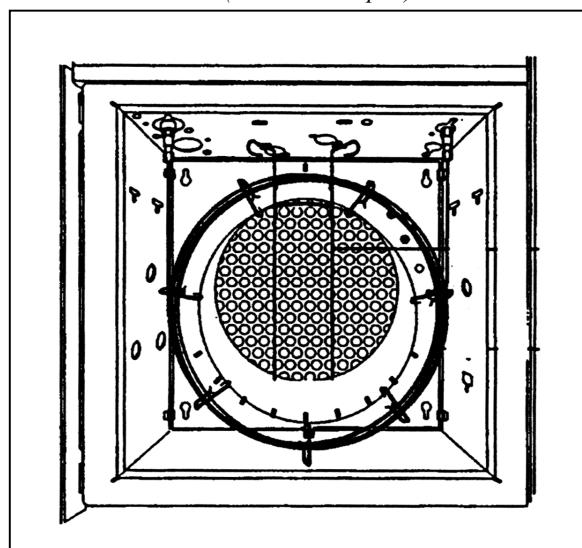
Note: For additional information, consult the Agilent column installation guide.

Fused silica columns are inherently straight, so no straightening procedures are necessary. It is important, however, to have fresh ends of the column free of burrs, jagged edges, and/or loose particles of column, or material from graphite ferrules. Always install a freshly cut end.

Installing Column in the Injection Port

- Decrease the GC oven temperature to near ambient. See instructions in the section “Preparing to Replace the Septum and/or Injection Port Liner.” Turn off the power to the GC and let the system cool down.
- Open the oven door.

Figure 6-20
Capillary Column Fittings and Bracket in a 6890 (Oven Door open)



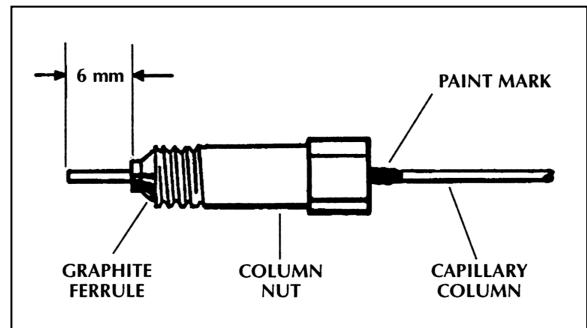
- Remove the old column by loosening the column nut and remove the column and column nut together.
- Pull the nut off the column. The nut and ferrule can be reused, but first enlarge the hole with a pointed object slightly larger than the column.
- Slide a column nut over the end of the new column.
- If the ferrule cannot be reused, slide a new 0.5 mm ID graphite ferrule over the column end so that ferrule and nut are several inches from the end of the column.
- Use a suitable glass inscribing tool to first score the column at the point about 2 cm (1 inch) from the end.
- Applying tension and side pressure with your fingers, break the column at the scribe mark. If the break is not clean, repeat the procedure.
- Slide the column nut and ferrule toward the end until 6 mm of column extends beyond the ferrule.
- With a suitable marking pen, mark the column at the bottom of the column nut (away from the cut end).
- Insert the column end, ferrule, and column nut into the injection port base. While maintaining the mark on the column so it is even with the bottom of the column nut, tighten the nut to finger tightness. Continue tightening until the ferrule starts to seal against the column (this point can be felt as the column is moved up and down during tightening), and then turn one-quarter turn more using a wrench.
- MIDI Technical Support always recommends removing and cleaning the gold Inlet Split Seal before reinstalling a column.

Installing Column at the Detector

To install the column at the detector use the following procedure:

- Install a column nut (and new graphite ferrule if necessary) over the column in the same manner used at the injection port end.
- Score and break off the end of the column in the same way.

*Figure 6-21
Injection Port Column Fitting*

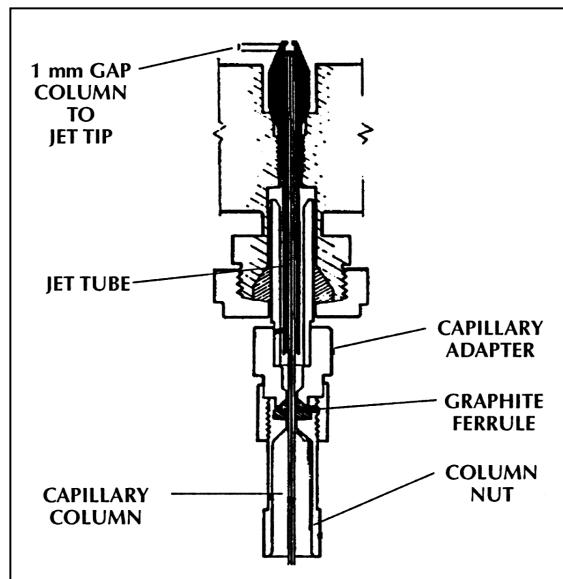


- Gently insert the column into the detector until it hits the end of the jet. The jet tube is usually visible. **DO NOT** attempt to force it further.

If the column will not go in the correct distance or if it appears to be binding rather than hitting a hard stop (jet tip), remove completely and insert again. Guide the column straight up in the center to insert in the hole of the jet tube.

- Slide the ferrule and column nut up to the base of the detector while holding the column in place.
- Tighten the nut until the ferrule begins to seal against the column. This point can be found by moving the column up and down a small amount until resistance is felt.
- Withdraw the column approximately 1 mm, and then tighten the nut an additional quarter turn with a small wrench.
- Turn the power back on to the GC.
- Be sure that the column pressure has returned to its normal setting.
- Purge the column with carrier gas for at least 15 minutes at ambient temperature. Be sure that the detector gases are turned on. Ignite the flame. If the flame is ignited, the display should indicate greater than two counts (~2 pA). On the front panel of the 6890 and 7890 GCs, press the key for the desired detector, **[FRONT DET]** or **[BACK DET]**. Scroll to “Output”. On a 6850 press the *Settings* softkey, then the *Detector* softkey and note the “Output” value.
- Bake out the GC system by entering **[OVEN TEMP][290][ENTER]** on the appropriate GC keypad and wait at least one hour for the column to condition. The background current will rise to a high level and then slowly drop to below 30 pA. At that time, the system is ready to run samples.

*Figure 6-22
Flame Detector Column Fitting*



Calibrating the Capillary Column (Standard Methods)

The head pressure on the column and the oven calibration temperature can be adjusted to compensate for the small variation between new columns and for long-term drift. Using the procedure outlined below will calibrate a system so that the ECLs of the polar hydroxy fatty acids will be centered on the expected ECLs listed in the peak-naming table. This will make the system appear identical to those that generated the libraries and will allow for the maximum drift while still correctly naming the peaks.

The ECL of the 2OH fatty acids varies +0.0012 per +1°C change in the elution temperature of the peak. The first hydroxy peak in the run (10:0 2OH) is most influenced by the starting temperature and can be adjusted by the oven temperature calibration. The late eluting hydroxy peak (16:0 2OH) is most influenced by the linear velocity (flow) of the carrier gas and can be adjusted by the column head pressure. The two adjustments are somewhat interrelated. The procedure and the Table 6-3 describe the simultaneous adjustment of both the pressure and temperature to calibrate the column correctly.

If a new column is being installed, set the oven temperature CALIB = 0.00 and set the inlet pressure (9 ± 1 psi) to obtain a solvent peak time of about 1.60 minutes. The solvent time (first large peak) is inversely proportional to the inlet pressure and can be used to track and correct long-term drift of the pressure control after the system is in operation. Make a few calibration runs and then calibrate the column, using the procedure below.

If during normal operation of the system the average ECL of the 10:0 2OH or the 16:0 2OH drifts more than ± 0.004 from the target values, make the minor adjustments needed to bring them back to the target values. In calculating the ECLs, there is approximately ± 0.001 noise in the measurements from the 0.001-minute peak time resolution and math errors. If large corrections must be made, it may be necessary to repeat the procedure a few times to converge on the target values.

Find ECLs for 10:0 2OH and 16:0 2OH

From a few recent calibration runs, calculate the average ECL for the 10:0 2OH and the 16:0 2OH fatty acids and round off the results to the nearest 0.001 ECL. Use only the calibration run just before the first unknown sample and any calibration runs in the middle of a batch. Do not use the first calibration run in a batch, or any that are immediately repeated, since these runs are not used by the system for ECL calculations.

Determine the Temperature and Pressure Adjustment

Find the column in Table 6-3 corresponding to the average ECL of the 10:0 2OH and the row corresponding to the average ECL of the 16:0 2OH. The intersection of the column and the row contains the temperature and pressure adjustments necessary to bring the system towards the target values. The ECL target values for the hydroxy compounds are 0.001 below the peak-naming table values because the large straight chain reference peaks in the calibration standard are slightly overloaded, causing the hydroxys to elute 0.001 ECL early.

Adjust the Temperature

The temperature offset in the table is added to the present oven temperature value. The values in the table, as well as the instrument, are degrees Celsius.

Example: If Calibration value or Correction value = -1.50 and the table indicates a change of +0.50, the new value to be entered should be the following:

$$[(-1.50) + (+0.50)] = -1.00$$

For a 6890:

- Enter the following keystrokes on the Keypad: **[OPTIONS]**.
- Use the arrows to scroll to Calibration. Press **[ENTER]**.
- The < should be at OVEN. Press **[ENTER]**.
- Use the arrows to scroll to CORRECTION. Type the value in and press **[ENTER]**.

For a 6850:

- From the main screen of the hand-held keypad, press the Service softkey.
- Select the Calibration softkey and then the Oven Cal softkey.
- Type the new value in the Correction box and press the OK softkey.

For a 6890:

Simply enter the number directly in the *TempDelta* column of the *Configure Methods* tool.

Adjust the Pressure (Standard Methods)

The pressure adjustment from the table is added (with the sign) to the present column head pressure setting. The values in the table, as well as the instrument, are in psi.



- Double Click on the Sherlock Toolbox Icon and select *Set Pressures* (or *Configure Methods* for the 7800 series), .
- Calculate the pressure adjustment required. Round that value to the nearest tenths. For example: If the original pressure is 9.0 psi and Table 6-3 indicates an adjustment of -1.34, the resulting pressure would be: **9.0 + (-1.34) = 7.66 (7.7 psi)**.

- Select the checkbox after each installed method. Type the new adjusted pressure value in all of the installed methods in box for the appropriate Column. If you have a single tower system, use “Column A”.
- Click on the Set Pressures button at the bottom of the box. The pressure update status box will say “Writing Pressure Files, Starting Agilent ChemStation”. ChemStation will load automatically.
- A “METHOD RESOLUTION FOR CHANGED CONFIGURATION” window may appear. Click on OK for each option to accept all defaults during this Method Resolution. You will need to do this for all of the affected methods.
- When all checked methods have been resolved, the following message will appear in the Pressure Update Status box:

“Stopping HP ChemStation”
“Done Updating Pressures”
- Click on Exit.

Table 6-3
Temperature and Pressure Adjustment Table

ECL	<u>10:0</u> <u>2OH</u>	11.152	11.153	11.154	11.155	11.156 target	11.157	11.158	11.159	11.160
<u>16:0 2OH</u>										
17.230	Temp	-1.74	-0.83	+0.08	+0.99	+1.90	+2.81	+3.72	+4.63	+5.54
	Press	-0.64	-0.69	-0.74	-0.79	-0.84	-0.89	-0.94	-0.99	-1.04
17.231	Temp	-2.21	-1.30	-0.39	+0.52	+1.43	+2.34	+3.25	+4.16	+5.07
	Press	-0.43	-0.48	-0.53	-0.58	-0.63	-0.68	-0.73	-0.78	-0.83
17.232	Temp	-2.69	-1.78	-0.87	+0.04	+0.95	+1.86	+2.77	+3.68	+4.59
	Press	-0.22	-0.27	-0.32	-0.37	-0.42	-0.47	-0.52	-0.57	-0.62
17.233	Temp	-3.16	-2.25	-1.34	-0.43	+0.48	+1.39	+2.30	+3.21	+4.12
	Press	-0.01	-0.06	-0.11	-0.16	-0.21	-0.26	-0.31	-0.36	-0.41
17.234	Temp	-3.64	-2.73	-1.82	-0.91	0.00	+0.91	+1.82	+2.73	+3.64
target	Press	+0.20	+0.15	+0.10	+0.05	0.00	-0.05	-0.10	-0.15	-0.20
17.235	Temp	-4.12	-3.21	-2.30	-1.39	-0.48	+0.43	+1.34	+2.25	+3.16
	Press	+0.41	+0.36	+0.31	+0.26	+0.21	+0.16	+0.11	+0.06	+0.01
17.236	Temp	-4.59	-3.68	-2.77	-1.86	-0.95	-0.04	+0.87	+1.78	+2.69
	Press	+0.62	+0.57	+0.52	+0.47	+0.42	+0.37	+0.32	+0.27	+0.22
17.237	Temp	-5.07	-4.16	-3.25	-2.34	-1.43	-0.52	+0.39	+1.30	+2.21
	Press	+0.83	+0.78	+0.73	+0.68	+0.63	+0.58	+0.53	+0.48	+0.43
17.238	Temp	-5.54	-4.63	-3.72	-2.81	-1.90	-0.99	-0.08	+0.83	+1.74
	Press	+1.04	+0.99	+0.94	+0.89	+0.84	+0.79	+0.74	+0.69	+0.64

Calibrating the Capillary Column (Rapid Method)

The procedure for calibrating the capillary column using the *Instant* FAME and Rapid methods is similar to that for the Standard Methods, but the table used is different. If the standard methods are used along with either the *Instant* FAME or Rapid methods, first calibrate for the standard methods, following that procedure. After calibrating any standard methods, calibrate the *Instant* FAME and Rapid methods using the procedure described below for a fine-tuning of the system.

Note: Before adjusting *Instant* FAME or Rapid method pressures make sure the oven temperature is stabilized at 170°C. Failure to have the oven at 170°C will result in an incorrect flow setting for these methods that operate in constant flow mode.

Calibrate the *Instant* FAME and Rapid methods using the following procedure:

1. If you have adjusted the pressure for Standard Methods, use the following formula to get an initial pressure for an *Instant* FAME or Rapid method:

$$R = S * 20/9$$

That is, the pressure for these methods is 20/9 times the standard pressure. If you have not adjusted for standard methods, start with the default pressure setting for the *Instant* FAME and Rapid methods (the nominal default is 20 PSI).

2. Run a test calibration and note the retention time for the solvent peak. If the solvent peak (largest peak) retention time is not shown, increase the pressure by one PSI and repeat the test run.
3. Adjust the pressure based on the solvent peak retention time. If the retention time is less than 0.700 minutes, decrease the pressure. If it is greater than 0.780 minutes, increase the pressure. Changing the pressure by 0.25 PSI changes the retention time by approximately 0.01 minutes.
4. Repeat steps 2 and 3 until the solvent peak retention time is within the 0.740 ± 0.040 .
5. Check the 9:0 and 20:0 retention times against Table 6-4. If either needs significant (> 1 PSI, or $> 1^\circ C$) adjustment, then make the pressure / oven calibration temperature adjustments based on this table.
6. Run another test calibration.
7. Repeat steps 5 and 6 if necessary.
8. After adjusting one *Instant* FAME or Rapid method by this procedure, all other *Instant* FAME and Rapid methods can be set to the same pressure value. Rapid methods always start with "R" and Instant FAME methods always start with "I". All other methods should follow the procedure above for standard methods. See Table 5-1 for a method list.

Table 6-4: Temperature / Pressure Adjustment Table for Instant FAME and Rapid Methods

9:0 RT → ↓ 20:0RT ↓	0.967	0.987	1.007	1.027	1.047	1.067	1.087	1.107	1.127	1.147	1.167
3.913	0.46 -1.5	1.28 -1.2	1.60 -0.9	1.84 -0.2	2.05 0.4	2.22 1.0	2.38 1.3	2.53 1.5	2.66 1.7	2.78 1.9	2.90 2.0
3.933	-0.46 -1.5	0.92 -1.3	1.40 -1.0	1.69 -0.4	1.91 0.2	2.11 0.9	2.28 1.2	2.43 1.5	2.57 1.7	2.70 1.8	2.82 2.0
3.953	-1.14 -1.5	0.00 -1.3	1.14 -1.1	1.50 -0.5	1.77 0.1	1.98 0.7	2.17 1.2	2.33 1.4	2.48 1.6	2.62 1.8	2.74 2.0
3.973	-1.40 -1.6	-0.92 -1.4	0.46 -1.1	1.28 -0.6	1.60 0.0	1.84 0.6	2.05 1.1	2.22 1.4	2.38 1.6	2.53 1.8	2.66 1.9
3.993	-1.60 -1.6	-1.28 -1.4	-0.46 -1.2	0.92 -0.7	1.40 -0.1	1.69 0.5	1.91 1.1	2.11 1.3	2.28 1.5	2.43 1.7	2.57 1.9
4.013	-1.77 -1.7	-1.50 -1.5	-1.14 -1.2	0.00 -0.9	1.14 -0.2	1.50 0.4	1.77 1.0	1.98 1.3	2.17 1.5	2.33 1.7	2.48 1.9
4.033	-1.91 -1.7	-1.69 -1.5	-1.40 -1.3	-0.92 -1.0	0.46 -0.4	1.28 0.2	1.60 0.9	1.84 1.2	2.05 1.5	2.22 1.7	2.38 1.8
4.053	-2.05 -1.7	-1.84 -1.5	-1.60 -1.3	-1.28 -1.1	-0.46 -0.5	0.92 0.1	1.40 0.7	1.69 1.2	1.91 1.4	2.11 1.6	2.28 1.8
4.073	-2.17 -1.8	-1.98 -1.6	-1.77 -1.4	-1.50 -1.1	-1.14 -0.6	0.00 0.0	1.14 0.6	1.50 1.1	1.77 1.4	1.98 1.6	2.17 1.8
4.093	-2.28 -1.8	-2.11 -1.6	-1.91 -1.4	-1.69 -1.2	-1.40 -0.7	-0.92 -0.1	0.46 0.5	1.28 1.1	1.60 1.3	1.84 1.5	2.05 1.7
4.113	-2.38 -1.8	-2.22 -1.7	-2.05 -1.5	-1.84 -1.2	-1.60 -0.9	-1.28 -0.2	-0.46 0.4	0.92 1.0	1.40 1.3	1.69 1.5	1.91 1.7
4.133	-2.48 -1.9	-2.33 -1.7	-2.17 -1.5	-1.98 -1.3	-1.77 -1.0	-1.50 -0.4	-1.14 0.2	0.00 0.9	1.14 1.2	1.50 1.5	1.77 1.7
4.153	-2.57 -1.9	-2.43 -1.7	-2.28 -1.5	-2.11 -1.3	-1.91 -1.1	-1.69 -0.5	-1.40 0.1	-0.92 0.7	0.46 1.2	1.28 1.4	1.60 1.6
4.173	-2.66 -1.9	-2.53 -1.8	-2.38 -1.6	-2.22 -1.4	-2.05 -1.1	-1.84 -0.6	-1.60 0.0	-1.28 0.6	-0.46 1.1	0.92 1.4	1.40 1.6
4.193	-2.74 -2.0	-2.62 -1.8	-2.48 -1.6	-2.33 -1.4	-2.17 -1.2	-1.98 -0.7	-1.77 -0.1	-1.50 0.5	-1.14 1.1	0.00 1.3	1.14 1.5
4.213	-2.82 -2.0	-2.70 -1.8	-2.57 -1.7	-2.43 -1.5	-2.28 -1.2	-2.11 -0.9	-1.91 -0.2	-1.69 0.4	-1.40 1.0	-0.92 1.3	0.46 1.5

The left column indicates the 20:0 Retention Time; the top row indicates the 9:0 Retention Time.
The top number in the box is the Oven Calibration temperature adjustment in degrees Celsius.
The bottom number in the box is the pressure adjustment in PSI.

Chapter 7

Troubleshooting

Overview

Sherlock is an easy-to-use system. Except for the time required to carry out routine maintenance procedures (Chapter 6), Sherlock should operate with little “downtime”. The software prints messages to alert you when the system is unable to function properly. These messages may appear in a message box on the computer screen or on the final composition report.

The easiest way to ensure dependable system functioning is to be certain to follow the sample preparation instructions in Chapter 2 very carefully. If reagent preparation, sample extraction procedures and routine maintenance protocols are followed, instrument downtime can be kept to a minimum and reproducible sample identifications will be obtained.

A Performance Qualification table is a valuable record of normal operating parameters and should be filled out daily. A sample PQ Table for the Standard and Sensitive methods is shown in Figure 7-1a; the table for the Rapid methods is shown in Figure 7-1b. See the **Instant FAME™ User’s Guide** for its recommended PQ Table.

This chapter discusses many of the most common sources of problems. Reading this chapter before problems arise will result in prevention and easy troubleshooting of problems, should complication occur. As always, if you encounter difficulty, the MIDI Technical Support staff is available to assist you. When contacting MIDI for technical support, it is helpful if you have the following information ready:

- Hardware model number (7890, 6890 or 6850).
- ChemStation version number.
- Sherlock version number.
- Record of the exact text of all software error messages including the dialog box title.

You can fax a copy of your Calibration Chromatogram and sample Composition Report to MIDI for evaluation and assistance.

For MIDI Technical Support:

Tel: (302) 737-4297
Monday-Friday 8 AM - 5 PM EST
Fax: (302) 737-7781
Email: support@midi-inc.com

Figure 7-1a

* If using a combination of Standard and Sensitive methods, this number can be increased. If running only Sensitive methods, 200 ± 10 is the target value.

** The total named will be somewhat dependent on your lab procedures. You should determine an acceptable range for your lab. It should be close to those recommended by MIDI, Inc.

Figure 7-1b **PQ Table for Rapid Methods**

* If using a combination of Standard and Rapid methods, this number should be reduced. If running only standard methods, 100 ± 10 is the target value.

** The total named will be somewhat dependent on your lab procedures. You should determine an acceptable range for your lab. It should be close to those recommended by MIDI, Inc.

Installation Problems

This section discusses typical problems encountered with the software installation process.

Cannot Install Software

If you are having trouble loading software on your system:

- Make sure you are following the Installation Instructions. These instructions are located on the Sherlock CD and can be printed out.

Problems Attempting to Run First Set of Samples or Accessing Sherlock CommandCenter

Invalid Security module or module not in place

Consult your Installation Qualification. The security module, which was included with your software purchase, must be installed for the Sherlock software to run.

No Samples to Run in Sample Processor

This usually indicates a problem with the Sample Table entries. Confirm that samples were added



to the Sample Table properly using the *Add Samples* tool. Confirm that all samples and Calibrations have a Sample ID number and are Queued. Verify that there is a Calibration (Calib) for each Method of Queued samples. Refer to the examples in *Chapter 3*.

Cannot Connect to ChemStation

This indicates that the Sherlock Sample Processor and the ChemStation are not communicating. Several things may cause this:

- Confirm that the Installation Instructions were followed.
- You should have been able to run a sample using only ChemStation using the Checkout method that was supplied.
- Confirm that ChemStation is installed on the C: drive.
- Confirm that ChemStation is closed before starting a Sherlock Sample Processor.
- Confirm that the correct instrument selections have been made in the Sherlock Sample Processor Configuration. Specifically, if the GC is a dual tower, make sure that *GC Type* is set to *Dual* even if *Tower* is set to *Front*.

Flat chromatogram or no Output reading on GC

If the plot of the chromatogram is a flat baseline without any peaks during a run, or if you press the **[SIGNAL 1]** key on the 6890GC and the signal level reading displayed is less than ~2pA there are two possible problems:

- The Signal 1 is turned OFF. Turn it on by pressing the following keys on the GC keyboard: **[SIGNAL 1] [ON]**.
- The FID detector flame is not lit. Check for an ignited detector by using the “condensation” test. Hold a cold (room temperature) wrench or beaker over the FID. If water condensation appears, the flame is lit; otherwise, it is not lit.

To view the signal on a 6850 GC, press *Settings*, then *Detector* on the handheld keypad. The signal is referred to as “Output.”

First Calibration run has no hydroxy compounds

The Calibration Standard contains five diagnostic hydroxy compounds. These are significant, but not full scale, on the chromatogram and should name in the Calibration Report. They should be recovered quantitatively. Exposed reactive sites on the injection port liner will reduce their recovery.

- There could be graphite from the ferrule stuck in the column at the injector end. Clip 2-3 inches from the injector end and reinstall. See section titled “The Capillary Column” in *Chapter 6 - Routine Maintenance*.

The solvent peak elutes much quicker than nominal

This is what would occur if the Agilent check out column has been used with the Calibration Standard. Verify that the proper column was installed. Follow the Installation Qualification carefully.

- Install the proper column (Agilent Technologies Ultra 2 5% phenyl methyl siloxane capillary column, Part No. 19091B-102 or 102E) following the instructions in this manual.

Chromatographic Problems

If your Sherlock fails to calibrate, or fails to name peaks, there are a myriad of potential causes, but most are predictable. In this section, we discuss how chromatographic problems manifest themselves in Sherlock Composition Reports.

Sherlock has been designed to accept and search fatty acid methyl ester extracts only if specific chromatographic requirements are met. Since the system relies on the qualitative and quantitative results of a single analysis for microbial identifications, several thresholds are set to assure the quality of the analyses. If results are outside the acceptable tolerance windows, error messages are printed in the Composition Report to assist you in troubleshooting the system. Sherlock usually identifies these problems during the initial calibration runs.

Good Calibration

*Figure 7-2
Very Good Recovery*

Volume: DATA4	File: E051135.29B	Samp Ctr: 21	ID Number: 1
Type: Calib	Bottle: 2	Method: RTSBA6	
Created: 1/13/2005 3:40:52 PM			
Sample ID: 6-402140(RTSBA6 Calibration Mix			
RT	Response	Ar/Ht	RFact
0.7746	1.258E+9	0.013	----
1.0932	56201	0.013	1.257
1.2004	406	0.010	----
1.2322	119256	0.012	1.186
1.4104	62467	0.010	1.125
1.4486	26662	0.010	1.116
1.5073	12685	0.010	1.101
1.6287	131761	0.009	1.074
1.8818	68724	0.009	1.030
2.1622	142095	0.009	0.994
2.4605	72962	0.009	0.964
2.5312	31122	0.009	0.958
2.6179	15622	0.009	0.952
2.7684	149925	0.008	0.941
3.0781	76444	0.009	0.922
3.1589	33118	0.009	0.918
3.3865	156018	0.009	0.908
3.6888	78708	0.009	0.897
3.9848	158883	0.010	0.889
4.2141	820	0.013	----
----	15622	---	----
----	-----	---	-----
Total Response: 1320097	Total Named: 1319690		
Percent Named: 99.97%	Total Amount: 1390029		
Profile Comment: Good peak matching. Peak position matching error (RMS) is 0.0022.			
Matches:			
Library	Sim Index	Entry Name	
RTSBA6 6.00	0.999	MIDI Calibration Mix 1	

Shown above in Figure 7-2 is a calibration report from a system with a new column and a new injection port liner. The hydroxy recovery is very good with the following:

$$10:0\text{ 2OH} = 2.25\%, 14:0\text{ 3OH} = 1.13\% \text{ and } 16:0\text{ 2OH} = 2.30\%$$

As the system is used, the hydroxy recoveries will drop. Changing the injection port liner will improve the hydroxy recovery again. Over a longer period of time, column damage will affect the maximum recovery obtained.

Marginal Calibration

*Figure 7-3
Calibration Report- Marginal Recovery*

Volume: DATA4	File: E051203.74A	Samp Ctr: 7	ID Number: 1					
Type: Calib	Bottle: 1	Method: RTSBA6						
Created: 1/20/2005 11:27:25 AM								
Sample ID: 6-402140(RTSBA6 Calibration Mix								
RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
0.7358	1.33E+9	0.014	----	6.7092	SOLVENT PEAK	----	< min rt	
1.0719	61587	0.013	1.217	9.0000	9:0	5.38		
1.1844	638	0.012	----	9.7670		----	< min response	
1.2186	129933	0.012	1.153	10.0000	10:0	10.75	Peak match 0.0006	
1.4057	70016	0.011	1.099	11.0000	11:0	5.52	Peak match -0.0003	
1.4460	28698	0.011	1.091	11.1771	10:0 2OH	2.25	Peak match -0.0023	
1.5069	13067	0.010	1.078	11.4448	10:0 3OH	1.01	Peak match 0.0037	
1.5341	412	0.011	----	11.5643		----	< min response	
1.6332	141590	0.010	1.055	12.0000	12:0	10.72	Peak match -0.0012	
1.8954	73271	0.009	1.020	13.0000	13:0	5.36	Peak match 0.0006	
2.1849	150890	0.008	0.992	14.0000	14:0	10.74	Peak match 0.0001	
2.4900	77672	0.009	0.969	15.0000	15:0	----	Peak match -0.0009	
2.5629	30571	0.009	0.965	15.2315	14:0 2OH	2.12	Peak match 0.0008	
2.6514	13120	0.009	0.959	15.5125	Sum In Feature 2	0.90	Peak match 0.0022	14:0 3OH/16:1 iso I
2.8050	157919	0.009	0.951	16.0000	16:0	10.78	Peak match -0.0021	
3.1206	80412	0.009	0.937	17.0000	17:0	5.41	Peak match -0.0004	
3.2031	30383	0.009	0.933	17.2634	16:0 2OH	2.03	Peak match 0.0021	
3.4340	164022	0.009	0.924	18.0000	18:0	10.88	Peak match -0.0019	
3.7409	82591	0.009	0.912	19.0000	19:0	5.41	Peak match 0.0007	
4.0408	166414	0.010	0.900	20.0000	20:0	10.75		
----	13120	---	----	----	Summed Feature 2	0.90	12:0 aldehyde ?	unknown 10.9525
----	-----	---	----	----		----	16:1 iso I/14:0 3OH	14:0 3OH/16:1 iso I

Total Response: 1395533 Total Named: 1394483
 Percent Named: 99.92% Total Amount: 1468721
 Profile Comment: Good peak matching. Peak position matching error (RMS) is 0.0016.

Matches:

Library	Sim Index	Entry Name
RTSBA6 6.00	0.995	MIDI Calibration Mix 1

Shown in Figure 7-3 is a calibration report where the hydroxy recovery has dropped to a point where corrective action should be taken. Even though the RMS is still very good and the match is quite acceptable, the 14:0 3OH has dropped to 0.90% and the 16:0 2OH has dropped to 2.03%. Notice also the appearance of a peak at ECL 11.5643. This is likely a break down product of 14:0 3OH. Changing the injection port liner will usually improve the hydroxy recovery.

Rejected Calibration

Figure 7-4
Calibration Report Rejected With Messages- Very Poor Recovery

Volume: DATA4	File: E052235.79B	Samp Ctr: 20	ID Number: 1					
Type: Calib	Bottle: 2	Method: RTSBA6						
Created: 2/23/2005 4:42:28 PM								
Sample ID: 6-402140(RTSBA6 Calibration Mix								
RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
0.7734	1.326E+9	0.013	----	6.6969	SOLVENT PEAK	----	< min rt	
1.0925	59052	0.012	1.293	9.0000	9:0	5.42		
1.1992	450	0.010	----	9.7700		----	< min response	
1.2310	126515	0.011	1.201	10.0000	10:0	10.79	Peak match 0.0004	
1.4094	70450	0.010	1.126	11.0000	11:0	5.64	Peak match -0.0009	
1.4479	28089	0.009	1.115	11.1767	10:0 2OH	2.22	Peak match -0.0013	
1.5063	12582	0.009	1.098	11.4449	10:0 3OH	0.98	Peak match 0.0033	
1.6273	142294	0.009	1.066	12.0000	12:0	10.78	Peak match -0.0008	
1.8803	74241	0.009	1.019	13.0000	13:0	5.38	Peak match -0.0003	
2.1609	154890	0.008	0.983	14.0000	14:0	10.82	Peak match 0.0002	
2.3151	666	0.009	----	14.5176		----	< min response	
2.4588	79452	0.009	0.956	15.0000	15:0	----	Peak match -0.0026	
2.5294	28335	0.009	0.951	15.2294	14:0 2OH	1.91	Peak match 0.0029	
2.6159	11584	0.008	0.945	15.5102	14:0 3OH/16:1 iso	0.78	Peak match 0.0035	Q-check < 0.89
2.7667	163654	0.009	0.936	16.0000	16:0	10.89	Peak match -0.0019	
3.0765	83402	0.009	0.921	17.0000	17:0	5.46	Peak match -0.0031	
3.1571	25483	0.009	0.917	17.2615	16:0 2OH	1.66	Peak match 0.0040	Q-check < 1.87
3.3847	170194	0.009	0.909	18.0000	18:0	10.99	Peak match -0.0005	
3.6871	85568	0.009	0.897	19.0000	19:0	5.45	Peak match -0.0001	
3.9829	172337	0.009	0.884	20.0000	20:0	10.83		

Total Response: 1409786 Total Named: 1408670
Percent Named: 99.92% Total Amount: 1483525
Profile Comment: QUANTITATION(Q-check) OUT OF BOUNDS FOR PEAKS. Review report comments
*** Library match not attempted

The hydroxy recovery is below the limits set in the Peak Naming Table in the example report shown above in Figure 7-4. The quantitation check error messages (Q-check) are printed. The system will re-inject the Calibration Standard and then stop the batch if the recovery does not improve.

Calibration Messages

Sherlock is trained to recognize the Calibration Standard. The calibration analyses require a specific number of calibration peaks with a set pattern (retention times) and quantitation (Response). With each calibration analysis, the following messages may be printed:

Good Peak Matching: Peak Position Matching Error (RMS) is 0.XXXX.

The normal deviation from the best-fit line is in the range of 0.0010 to 0.0030 for the Rapid Methods. This message indicates that the values were within allowable tolerance. Large deviations from the expected calibration values result in one of the error messages that follow.

BAD PEAK MATCHING: PEAK POSITION MATCHING ERROR (RMS) IS 0.XXXX.

The system will reject a calibration regardless of the resulting similarity index if the RMS is above the expected value. Consult MIDI Technical Support for more information.

NOT ENOUGH GOOD PEAKS TO MATCH EXPECTED CALIBRATION STANDARD.

- One or more peaks were rejected or are missing.
- Verify that the proper Calibration Standard bottle was in the correct tray position. This message may also occur if an inadequate amount of calibration was injected.
- Repeated use of a Calibration bottle may have allowed for evaporation of the solvent. Discard the vial, place a new bottle in position and try again.
- Check the chromatogram. If there are no peaks except solvent, the level of Calibration Standard may be too low to be picked up by the syringe. Discard the vial, place a new one in position and begin the batch again.
- This error can also occur when there is a septum leak in the injection port. A tailing solvent peak with a flat top characterizes this situation. Install a new septum and injection port liner.
- The syringe may not have injected an adequate volume of Calibration Standard. Check to see if the syringe is partially plugged, or if the set screw holding the syringe plunger has loosened or come off. This is most likely the cause when the solvent peak is very small.

CANNOT ALIGN PEAKS TO EXPECTED RELATIVE POSITIONS IN CALIBRATION STANDARD.

Compare with past calibration analyses for excessive retention time drift, and check column head pressure.

Verify that the solvent peak is eluting within the expected timeframe.

QUANTITATION CHECK (Q-Check) OUT OF BOUNDS FOR ONE OR MORE PEAKS. Review report comments.

- The hydroxy peak quantitation is most indicative of problems with the injection port septum and the injection port liner. There are preset lower limits of recovery of these compounds. If you are near the injection limit for the same injection port liner, regularly inject samples containing mycolic acids, or run “dirty” samples, it is most likely that you need to install a fresh injection port liner and septum.

Sample Run Messages

Composition Reports for sample runs also include performance diagnostics.

Total response less than 50,000. Concentrate And re-run.

Extracts that are too dilute will not result in valid searches since the smaller fatty acid peaks will not be detected. Possibly the isolate has very small cells and/or is a very slow grower. In the future, use the entire plate if necessary to get approximately 40 mg (20 mg for Rapid and Sensitive methods). Alternatively, streak two or three plates and harvest from all. Also, expect this message when running the reagent blank (negative control).

To rerun the extract, remove the cap and allow the solvent to partially evaporate using a stream of clean nitrogen. A reduction of the solvent by half will double the area count. Transfer to a tapered, low volume insert, re-queue and run. Be sure to edit the name field to reflect the CONCENTRATION step.

If many samples give this error, there may be a problem with the GC or the extraction.

Possible Solutions:

- Check to see if the syringe is partially plugged, or if the nut holding the syringe plunger has loosened or come off.
- Check the split flow and check the septum.

- Compare analysis with last calibration to determine if excessive retention time drift has occurred.
- Run fresh Calibration Standard and verify that it has a Total Area of between $1.0\text{-}1.6 \times 10^6$.
- Verify that the reagents were prepared properly and the temperature of the 80° water bath was within specifications.
- Verify with pH paper that acidic conditions are present during the methylation step.
- Verify that basic conditions are present during the base wash step.

SOLVENT PEAK LESS THAN 15,000,000. CHECK SAMPLE LEVEL AND INJECTOR NEEDLE.

This indicates that not enough sample was injected onto the column.

Possible Solutions:

- The Auto Injector requires that sample vials be filled with at least 0.3 ml of sample. Smaller volume samples can be analyzed if transferred to a tapered, small-volume sample vial insert.
- Verify that the set screw for the plunger is secured.
- If the proper volume is in the sample vial, but the solvent peak remains too small, remove the syringe and, by manually pumping wash solvent, verify that it is not plugged. Install a new syringe if unable to draw solvent easily into the barrel.

Column Overload: A peak's response is greater than 400,000. Dilute and re-run.

Overloaded peaks may have altered retention times and could lead to peak misidentification. They usually result from too many cells being harvested.

Possible Solution:

- Dilute the sample with a small volume of Reagent 3 and rerun.

Percent Area Named Is Less Than 85.

This error usually occurs when there are many unnamed, contaminating peaks. Properly prepared, clean, bacterial FAME samples should have a high percentage of identified peaks. If the sample

does not contain contaminating peaks, it may contain extra peaks with very large area to height ratios.

Possible Solutions:

- Verify that the 80°C water bath temperature is 80°±1°.
- Verify the timed steps in the extraction procedure.
- Verify that the peaks are being properly named by comparing with the last calibration analysis.
- Repeat the analysis. Report consistently unnamed large peaks to MIDI.
- Check the reagent blank (negative control) for contamination peaks.
- Check for retention time drift as above.
- Check the media. MRS media will add area counts.
- Check the column calibration by noting the ECLs of the 10:0 2OH and 16:0 2OH. See “Calibrating the Capillary Column” in Chapter 6.

Review report comments.

This message will occur if a named peak is flagged.

Possible Solutions:

- If there is just one obviously large, wide peak, re-run the sample. There may have just been a random “hiccup” of the system. This should not happen on a regular basis.
- Check for retention time drift of the solvent peak as above.
- Check the column calibration by verifying the ECLs of the 10:0 2OH and 16:0 2OH. See “Calibrating the Capillary Column” in Chapter 6.
- Replace the injection port liner if peaks are rejected due to excessive area/height ratios.
- Replace the capillary column if the problem persists. This is a last resort. Contact MIDI Technical Support to confirm.

ECL SHIFT OR DEVIATION EXCEEDS X.XXX. System will recalibrate.

This indicates that substantial peak drift has occurred since the last calibration run.

Possible Solutions:

- The system will attempt to correct itself by recalibrating.
- If ECL shifts occur frequently, the instrument is not reliably reproducing retention times. Contact MIDI Technical Support

Chromatographic Errors

The following problems may or may not cause the Calibration to fail. These are some situations that may cause you to question sample runs although the Calibration passed.

Problem: Wandering baseline.

The baseline of the chromatogram should be flat with a slight rise toward the end of the analysis due to the high oven temperature. Wave-like humps in the baseline may cause low similarity indices or misidentifications due to poor peak integration.

Possible Solutions:

- Check the y-scale on a blank run. The scale is expanded on a blank run which magnifies the baseline. What looks atrocious on a blank run may be fine on a normally scaled calibration run.
- Check your maintenance records. Did the baseline problem occur after a maintenance procedure? Confirm that the procedure was done correctly.
- Confirm that the carrier gas pressure (H_2) is greater than 10 psi above the inlet pressure.
- Replace the injection port liner and septum.
- Replace the molecular sieve traps on the gas lines.
- Check the quality of the gases.
- Replace the carrier gas regulator if there is evidence that it leaks.

Problem: High bleed.

The column bleed is seen as a rise toward the end of the chromatographic analysis. It should not exceed 1 inch on the chromatographic printout.

Possible Solutions:

If the bleed is excessive, recondition the capillary column by heating the GC oven to 300°C for two hours. Do not turn off the hydrogen flow through the column.

- Replace the injection port liner, which may have trapped some very high boiling compounds that are slowly being baked out of the system.
- Replace the carrier gas cylinder if you have changed the cylinder recently and doubt its purity.
- It may be necessary to replace the Column. This is a last resort. Contact MIDI Technical Support to confirm.

Problem: Tailing peaks.

Chromatographic peaks should have sharp, symmetrical shapes. The Ar/ Ht ratio must be between 0.017 and 0.1 for Sensitive methods, between 0.017 and 0.080 for Standard methods, and between 0.005 and 0.024 for Rapid methods.

Possible Solutions:

If only the hydroxy acids have tailed peak shapes, do the following:

- Install a new injection port liner.
- If the problem persists it may be necessary to replace the capillary column. This is a last resort. Contact MIDI Technical Support to confirm.

If all peaks, including the solvent peak, have a tail remove the column and inspect the column ends with a magnifying glass. There should be clean, even ends, without jagged edges. If they are not,

- Score the end and break the column to produce a clean end.
- Reinstall the column following the instructions in this manual and reignite the detector.

Problem: Noise or spikes.

The chromatogram may contain many small peak-like spikes with the appearance of a “hairy” baseline and many unnamed peaks in the Sample or Calibration Reports. Unnamed peaks will reduce the % Named value below 85%, which will cause “an Error” to appear. The Calibration and reagent blank should not contain many unnamed peaks.

Possible Solutions:

- Clean the waste and Solvent A bottles in the injection turret. Remove the diffusion caps from the waste vials to reduce contamination. Use fresh Reagent 3 in the Solvent A vial for every batch. Discard, rinse and refill Solvent A.
- The syringe may be contaminated. Replace it with a new one.
- Using a large pipette bulb on a piece of clean copper tubing, blow clean air in and around the detector to remove any particles that may be creating peaks due to electronic noise. Peaks due to electronic noise have extremely small Ar/ Ht ratios.
- If the extra peaks are thought to be due to electronic noise, it may be necessary to remove the detector, and follow the recommended procedures in the GC manual to clean it. There may also be a problem with the FID board. Confirm this with MIDI Technical Support first.

Problem: All three 2-OH compounds are Q-Checking.

Poor 2- hydroxy recovery is often caused by incorrect column position at the detector end. This is most common after a new column is installed.

Possible Solution:

- Remove the column at the detector end, cleanly clip 2-3 inches and reinstall. Confirm that the column end has a good, square cut. Reinstall and verify correct positioning.

Problem: Poor 2-OH and 3-OH recovery, many Q-checks.

Poor hydroxy recovery is most often due to a bad injection port liner.

Possible Solutions:

- Change the injection port liner and septum.
- Be certain that your samples have been extracted “cleanly”. There should be no visible water (seen as droplets or condensation in the vials) in any samples.

- If this continues after you have replaced the liner and septa, then clean or replace the gold split seal in the injection port.
- If you have recently installed a new column or removed the column from the injector end, there may be broken graphite from the ferrule in the column. Clip 2-3 inches from the column at the injector end and reinstall. Exposed graphite will absorb all hydroxys.

Problem: Baseline signal much greater than the mid-teens.

Possible Solution:

- Verify N₂ flow rate to the FID; it should be 30 ml/min (Do not trust electronic readings from the GC display.) The H₂/N₂ flow rate ratio should be 1:1.

Problem: No peaks named and the solvent peak tailed and has a “flat top”.

This can occur when the injection port septum has a leak. Suspect this if you are near the limit of the liner or if greater than 50 samples have been injected. If this is common problem it may be necessary to purchase a Merlin Microseal (see *Appendix A*).

Possible Solution:

- Change the septum and liner.

Problem: No fatty acid peaks in chromatogram, only a small solvent peak.

Possible Solutions:

- Sample solvent evaporated. Remove cap, add approximately 0.5 ml of Reagent 3, re-queue and run again.
- The syringe may be plugged. Remove the syringe and check its function with Reagent 3. The plunger should move freely.
- Syringe plunger set screw could be loose. Raise and lower the plunger mechanism by hand. The setscrew should hold the plunger. If not, tighten it and watch the next injection.

Problem: The run has many small, extra peaks and baseline is not flat.

Possible Solutions:

- If the septum and liner have been changed recently, confirm that there was an adequate bake-off time and temperature. If not, bake the system at 290° C for at least 2 hours.

- Contaminated gases will generally show up immediately after changing a tank and will often show just a few random peaks or a baseline shift.

Problem: Poor naming of Organisms.

Possible Solutions:

- The Area count range should be between 100,000 to 300,000. Review streaking and harvesting procedures to confirm that adequate cell mass is being sampled. Verify that the calibration area count was within normal range to rule out an auto sampler error.
- If the chromatogram looks normal and the % Named is less than 90%, perhaps the oven calibration is off. Confirm that the ECL values of 10:0 2OH and 16:0 2OH values are within specifications.

Problem: Library search results in “NO MATCH”

This indicates the Sherlock parameters have determined that the sample is so different from all of the entries in that database that it will not calculate a Similarity Index. This may be a good answer in that it will tell you that you DO NOT have any of the organisms in that database.

By following the steps below, you can find the closest entry in the Library. This may help you choose which confirmation test to perform on the culture to make a proper ID of the organism in question.

To run a “NO MATCH” sample to find the closest match via Comparison Charts or to reclassify a previously run sample against an updated Library, follow the instructions in *Chapter 5 - Sherlock CommandCenter*. Further, the Sherlock *Tracker* add-on can be used to search for other samples that have been run which are similar to this sample.

Sample Preparation Errors

Low similarity indices or "NO MATCH" library results may be caused by mixed cultures or deviations in the sample preparation procedure. If low (less than 0.5) similarity index values are frequent,

- Verify that the media preparation and culture conditions are as defined in Chapter 2.
- Prepare and use fresh reagents.
- Monitor the temperature of methylation in the water bath so that it does not exceed $80^{\circ}\pm1^{\circ}\text{C}$.
- Harvest an adequate number of live cells, so small peaks are integrated and named by Sherlock.

Use of Positive and Negative Controls

The easiest way to catch problems with reagent or sample extract preparation is using a negative reagent control with each batch of samples and a positive control for each library used. A negative control or "reagent blank" is useful to monitor for reagent contamination since no peaks would be expected from such a sample. MIDI recommends *Stenotrophomonas maltophilia*, ATCC #13637 as a positive control for the TSBA6/RTSBA6 method and library and *Pseudomonas aeruginosa*, ATCC# 9027 or 27853 as positive control for the CLIN6/RCLIN6 method and library. For the VPI anaerobe broth method, use *Clostridium perfringens*, ATCC 13124 and/or *Bacteroides fragilis*, ATCC 25285.

Improper Preparation of Reagent 2

One of the most common problems encountered involves the improper preparation of Reagent 2, the 6 N HCl solution. The HCl concentration used in this reagent is specified in Chapter 2 as 6.00N. If "concentrated HCl" is purchased, it usually is listed as containing "approximately 35 to 38%" HCl; this percentage is often not accurate! If concentrated HCl is used, you must know the exact concentration before using it to make 6.00 N HCl. By assuming the concentrated HCl is 12 N and adding an equal volume of water, the resulting solution may be too weak or too strong. If the acid is too weak, the "soaps" formed in Step 1 of the extraction procedure will not be methylated and area counts on the printed report will be below acceptable levels. If the acid is too strong, the cyclopropane fatty acids will be degraded, and the resultant profiles will not match a Library entry. Cyclopropane fatty acids will also be degraded if the samples remain in the water bath longer than ten minutes or if the water bath is hotter than 80°C .

If you suspect that there is a problem with the HCl concentration of your Reagent 2, titrate it with a known base to determine the exact normality. For best results, purchase standardized 6.00 N HCl. See Appendix A for a suggested vendor.

The Extraction Procedure: Harvesting

The amount of cells in the extraction tube will affect the results. We recommend approximately 40-50 mg (wet weight) per extraction tube, 20-25 mg (wet weight) for the Rapid and Sensitive methods. Too few cells may not yield enough fatty acids for a reliable comparison to the database. In this case, a warning will be printed on the profile concerning the TOTAL RESPONSE.

To correct:

The sample may be concentrated and run again. You should be aware that contamination can become a serious problem when the solvents are evaporated.

The sample may be regrown (on two plates if necessary) and extracted a second time.

For most species, too many cells will result in excessively high area counts and/or column overload, with subsequent shifting of peak retention times. Such peaks may not be named properly, and comparisons to the database will be invalid. With Gram positive, spore-forming bacteria (i.e., *Clostridium* and *Bacillus*) an increase in cell mass harvested may not necessarily correlate to an increase in area counts. Over-harvesting causes the fatty acid ratios change and the area counts to decrease. This seems to be due to gel formation in the extraction Step 1, which results in differential extraction of some fatty acids.

Final Transfer of Extract to Sample Bottle

It is **VERY** important to transfer only 2/3 of the top (organic) layer to the GC autosampler vial. That volume of liquid is more than adequate for the auto sampler needle to submerge and withdraw 2 μ l of sample. Any aqueous phase that is present in the auto sampler vial will be injected onto the column.

Over time, water that is inadvertently injected will damage the hydrophobic coating of the capillary column and cause significant peak tailing. It will be necessary to remove one loop of the column at the injector end, following the procedure outlined in *Chapter 6 - Routine Maintenance*. This can only be done a few times without significantly changing peak retention values. Failure to follow this guideline will greatly reduce the lifetime of the column.

Appendix A

Equipment and Consumables

Overview

Some additional equipment is needed for proper operation of the Sherlock Microbial Identification System. These items are listed on MIDI, Inc.’s website (www.midi-inc.com) to aid in setting up and operating the laboratory. Suggested vendors, where appropriate, are listed. **Please visit the website (www.midi-inc.com) for the most recent vendor and part number list.**

Several items, such as water baths, test tube racks and inoculating loops may already be in the laboratory. Other items are nonessential, but may be useful by increasing the simplicity and reproducibility of the procedure.

Note: Do not use heating blocks as a substitute for water baths. The heat transfer of water is required to maintain proper temperature control during the procedure.

Before ordering gases, see your local gas supply representative to confirm the CGA tank fitting numbers. Some gas suppliers furnish “instrument” or “chromatographic” purity grades (names vary with supplier) intended for chromatographic use. The purity should be specified when possible. For optimal chromatographic performance, follow the gas purity recommendation.

For consumable ordering information, see MIDI, Inc.’s website. **The media for aerobic bacteria and other items marked with an asterisk (*) have NO SUBSTITUTES.** See Appendix B for details of other media used for other libraries. Suggested vendors where appropriate are listed.

Appendix B

Other Culture Techniques

Overview

This chapter contains the Sample Preparation guidelines and lists of any additional equipment and consumables for the alternate libraries that MIDI has created. The lists are not meant to be exhaustive; they are for the users' convenience when setting up the system.

NOTE: For Instant FAME techniques please see the separate *Instant FAME™ User's Guide*.

These include the following:

- Mycobacteria
- Yeast
- Actinomycetes
- Fungi
- Plate-grown Anaerobes
- Broth-grown Anaerobes

Items marked with an asterisk (*) have NO SUBSTITUTES.

Mycobacteria Library Culture Technique (MI7H10 Library)

MI7H10 Media for Mycobacteria

Cultures are grown on Middlebrook 7H10 plus Middlebrook OADC Enrichment (see Table B-1), in 5-10% CO₂ at 35°C until growth is adequate. Grow *Mycobacterium marinum* at 30°C. Prepare the medium according to the directions on the label.

The extraction procedure for Mycobacteria has been modified to decrease the formation of cloudy extracts and so avoid having to change the injection port liner too frequently. To assure good extraction, add about 5 glass beads (3mm diameter) to the saponification step of the extraction procedure. This provides good dispersion of the cells during vortexing. After the base wash step, pipette the extract into a GC sample vial containing anhydrous sodium sulfate (no more than 1/3 filled). Make sure that none of the aqueous phase is transferred. Using a pipette, flush the extract through the drying agent three times or until no longer cloudy. Transfer the extract to a clean vial being certain not to carry over particles. Discard the vial containing sodium sulfate.

Note: It may be necessary to use beads for the reagent blank as well.

*Table B-1
Additional Consumables and Equipment or Mycobacteria*

Please visit www.midi-inc.com for most recent vendors and part numbers.

CONSUMABLE	SOURCE	PART NUMBER	AMOUNT
Mycobacteria Culture Technique			
Middlebrook 7H10 plus Middlebrook OADC Enrichment	BD	221174	Pkg. 20
3 mm Glass beads	Fisher	10-310-1	1 lb.
Anhydrous sodium sulfate (cert. ACS)	Fisher	S421-500	500 g

Yeast Library Culture Technique (YST28 and YSTCLN Libraries)

SAB Media for Yeasts

Yeast cultures are grown on Sabouraud Dextrose Agar (see Table B-2) at 28°C for 24 hours. The medium is prepared according to the label directions and aseptically dispensed in sterile 100x15 mm petri dishes.

These organisms have only a few fatty acids. Therefore, it is imperative that they are grown on the same media used for database building. Yeasts appear to work well if **they are given three subcultures after arriving at the bench. Lyophilized cultures may require four subcultures following reactivation.**

The optimal cell mass necessary for producing a good area has not been determined; it varies with species.

Scoop these cells with a loop as in bacterial harvesting, taking about twice the cell mass (80 mg). A large amount is needed since the fatty acid content is low. It should not overload the column. To be safe, start with a small amount and observe the area on your profile. One can adjust the harvesting accordingly. After the samples have been harvested and placed in tubes, add Reagent 1 and continue with the normal extraction procedure.

Culture Control

Each sample batch should also contain a known strain as a positive control. We recommend *Candida albicans* ATCC 14053 or *C. albicans* ATCC 10231.

*Table B-2
Additional Consumables and Equipment for Yeast*

Please visit www.midi-inc.com for most recent vendors and part numbers.

CONSUMABLE	SOURCE	PART NUMBER	AMOUNT
Yeast Culture Technique			
* BBL Sabouraud Dextrose Agar	Fisher	B11584	500 g

Actinomycete Library Culture Technique (ACTIN1 Library)

TSB media for Actinomycetes

Actinomycetes are grown in Trypticase Soy Broth on a shaker:

- Prepare media by dispensing 20 ml of Trypticase Soy Broth into 150 ml Erlenmeyer flasks. Use reusable foam stoppers or aluminum foil to cap. Sterilize flasks and autoclave for 30 minutes (121°C @ 15 psi).
- Inoculate the flask by stripping the agar containing a few colonies with a sterile loop from a plate or slant culture. Incubate flasks at 28°C on a platform shaker at 150 rpm. A shaker water bath can be substituted.
- Check flasks after 24 hours for contamination. Actinomycetes may require 48 hours or longer (some up to 3 weeks) of incubation to obtain adequate growth for extraction.
- Harvesting is by filtration. MIDI uses a glass microanalysis filter holder commonly used in wastewater analysis (see Table B-3 below). The 300 ml volume funnel uses 47 mm membranes and is ideal for this purpose. Using a loop, gently scrape and lift the cells from the membrane and place in an extraction tube. More than twice the cell mass of a normal analysis is needed for a good sample of actinomycetes.

After the actinos have been harvested and placed in tubes, add Reagent 1 and continue with the normal extraction procedure.

Culture Control

Each sample batch should also contain a known strain as a positive control. We recommend *Streptomyces albus* ATCC 3004.

Table B-3
Additional Consumables and Equipment for Actinomycetes
Please visit www.midi-inc.com for latest vendors and part numbers.

CONSUMABLE	SOURCE	PART NUMBER	AMOUNT
Actinomycete Culture Technique			
*BBL Granulated Agar	Fisher	B11849	500 g
*BBL Trypticase Soy Broth (TSB)	Fisher	B11768	500 g
Sample Preparation			
Glass filter holder (includes funnel clamp, support screen, gasket, base, and No. 8 stopper)	Fisher	09-753-1E	1
Nalgene hand operated vacuum pump	Fisher	01-070	1
Cellulose filters MSI cellulose filter membranes with a 0.45 µm pore size	Fisher	E04WP0-4700	100
1000 ml vacuum flask with side arm			1
150 ml Erlenmeyer flasks			6
Disposable foam stoppers			6
Platform shaker (150 rpm)			1

Fungi Library Culture Technique (FUNGI Library)

SAB Media for Fungi

When starting fungi on plates, use BBL Sabouraud Dextrose Agar. When preparing flasks for fungal growth, use Difco Sabouraud Dextrose Broth. Fungi are grown at 28°C on a shaker rotating at 150 rpm.

- Start lyophilized cultures in 1-ml sterile de-ionized water using standard ATCC procedures. streak onto Sabouraud Dextrose Agar (See Table B-4) plates. Start live fungal cultures by anchoring a small amount of organism in the center of SAB plate with a sterile needle. Incubate at 28°C until adequate growth for transfer is achieved (about two to five days).
- Prepare broth flasks by aseptically dispensing 40 ml of Sabouraud Dextrose Broth into 125-ml Erlenmeyer flasks. Plug with reusable foam stoppers or aluminum foil caps. Autoclave flasks for 30 minutes (121°C @ 15 psi) and let cool.
- Transfer the culture from plate to flask by cutting a small agar chunk from the edge of fungal growth with a sterile needle or hook. Incubate flasks at 28°C and shake at 150 rpm. A shaker-water bath can also be used.
- Check flasks after 24 hours. A few species, like *Fusarium* and *Rhizopus*, will be ready for harvest in one day. Most species require 2-3 days to achieve adequate growth for extraction. A few species, like *Trichophyton*, may need four or more days. Optimal growth for harvesting varies with species and has not been standardized. However, a large cell mass is required. When several mycelial balls are present or broth is cloudy, the culture is ready for extraction.
- Use a glass filtering system to harvest the fungal colonies (see Table B-4). Set up inside bio-safety hood. After apparatus is assembled, empty contents of flask into funnel and apply a slight vacuum. Fungal colonies will be collected on filter paper.
- Transferring the colonies can be difficult. Use a sterile loop, dissecting needle or spatula depending on colony type. Use whatever works to carry the colonies into the extraction tube, maintaining a sterile technique. The optimal cell mass necessary for producing a good area has not been determined; it varies with species. Several colonies from a good grower should be ideal. Often the entire amount is needed. Some filtration product will blanket the filter. Scoop these cells with a loop as in bacterial harvesting, taking about twice the cell mass (80 mg). A large amount is needed since the fatty acid content is low. It should not overload the column. To be safe, start with a small amount and observe the area on your profile. You can adjust your harvesting accordingly.
- Because fungal colonies do not dissolve during the extraction procedure, a different procedure is required following addition of Reagent 3. After tumbling, instead of removing the bottom phase, as in bacterial extraction, transfer the top phase into a clean set of tubes

and then add Reagent 4 to the top phase. Use a fresh pipette for each transfer. The bottom phase remaining in the original tubes may be discarded.

Culture Control

Each sample batch should also contain a known strain as a positive control. We recommend *Aspergillus niger* ATCC 10535.

Table B-4
Additional Consumables and Equipment for Fungi
Please visit www.midi-inc.com for latest vendors and part numbers.

CONSUMABLE	SOURCE	PART NUMBER	AMOUNT
Fungi Culture Technique			
*BBL Sabouraud Dextrose Agar	Fisher	B11584	500 g
*DIFCO Sabouraud Dextrose Broth	Fisher	DF 0382-17-9	500 g
Sample Preparation			
Glass filter holder (includes funnel clamp, support screen, gasket, base, and No. 8 stopper)	Fisher	09-753-1E	1
Nalgene hand operated vacuum pump	Fisher	01-070	1
Cellulose filters	Fisher	E04WP0-4700	100
1000 ml vacuum flask with side arm			1
125 ml Erlenmeyer flasks			6
Reusable foam stoppers or aluminum foil			6
Platform shaker-incubator or shaker-water bath			1

Anaerobe Plate-Grown Library Culture Technique (BHIBLA Library)

- All plate-grown anaerobes including lactobacilli are grown on supplemented brain heart infusion agar with blood (BHIBLA) plates or Brucella blood agar plates. Pre-poured plates are available (see Table B-5) and should be used for the best results.
- Use prerduced agar plates in a gas generating system. Choose one system from Table B-5 or an equivalent system from another manufacturer.

The standard incubation conditions for plate-grown anaerobes are:

- $35 \pm 1^\circ\text{C}$ temperature
- 48 ± 2 hours time

After the plate-grown anaerobes have been harvested and placed in tubes, add Reagent 1 and continue with the normal extraction procedure.

Culture Control

Each sample batch should also contain a known strain as a positive control. We recommend *Propionibacterium acnes* ATCC 11827.

Table B-5
Additional Consumables and Equipment for BHIBLA Library
Please visit www.midi-inc.com for latest vendors and part numbers.

Consumable	Source	Part Number	Amount
Brucella Blood Agar w/ 5% Sheep Blood	Remel	01252	10/pk
Brucella Blood Agar	Anaerobe Systems	AS-111	1
BBL GasPak 100	Fisher	11-814-22	1
BBL GasPak 150	Fisher	11-816-2	1
BBL Anaerobic Systems Disposable H ₂ & CO ₂ Generator Envelope	Fisher	11-814-32	10/pk

VPI Anaerobe Broth Library Culture Technique (MOORE5 Library)

Introduction

The MIDI Sherlock System differentiates among species of bacteria by comparing the composition of cellular fatty acids. It uses an identification library developed for cells harvested from surface growth on agar plates.

Many species of anaerobic bacteria do not give luxuriant growth on the surface of plates, so we have developed an identification library based on cells harvested from growth in broth. The Moore library was developed over a 4-year period. The library entries are based on 20 or more analyses of 1-100 characterized (often confirmed by DNA homology) strains.

Performance

The precision of identification varies from species to species. For example, correct identification of *Fusobacterium nucleatum* is close to 99%, whereas the identification of serotypes of species of *Actinomyces* currently is only 75 to 85% correct. Species, including undescribed species that are not in the library will identify erroneously, usually at low similarity indices. There are several undescribed species in the database; they are denoted by letters and numbers.

Directions for use of the broth-grown anaerobe library are given in the remainder of this appendix. Please follow these directions carefully since they are different in many ways from the standard directions presented in *Chapter 2 – Preparing Extracts*.

Preparing Extracts - Overview

Cells alter the fatty acid composition of their lipids to maintain membrane fluidity with varying environmental conditions. It is essential to control the time and temperature of incubation and the selection of a culture medium before comparing fatty acid compositions. The sample procedure is summarized in Table B-6.

*Table B-6
Sample Processing*

Harvesting	Grow cultures in appropriate medium (see below), centrifuge, and retain cell pellet.
Saponification	Lyse the cells to liberate the fatty acids from the cellular lipids. A strong methanolic base combined with heat and pressure kills and lyses the cells. Fatty acids are cleaved from the cell lipids and are converted to their sodium salts.
Methylation	Methylation converts the fatty acids (as sodium salts) to fatty acid methyl esters which increases the volatility of the fatty acids for the GC analysis.
Extraction	Fatty acid methyl esters are removed from the acidic aqueous phase and transferred to an organic phase with a liquid-liquid extraction procedure.
Base Wash	A weak basic solution saturated with NaCl is added to the sample preparation tubes to break emulsions and to remove free fatty acids and residual reagents from the organic extract. Residual reagents will damage the chromatographic system, resulting in tailing and loss of the sulphydryl fatty acid methyl esters.

Medium Selection

For each library a standard medium has been selected for several reasons:

- It will support growth for most of the organisms in the library.
- It does not contain a significant amount of fatty acids that, if extracted out of the medium, would interfere with the analysis.
- It is readily available commercially.

Each sample is processed in a single test tube and up to 40 samples can be conveniently prepared in batches.

Delay During Sample Preparation

Ideally, sample processing should continue uninterrupted once cells have been harvested. If it is necessary to delay the sample preparation procedure, the samples can be held with little effect on the fatty acid analysis at several steps.

- Cell pellets can be held frozen for up to one month after harvesting and before the addition of Reagent 1.
- Following methylation, the sample preparation should not be delayed.
- After extraction and removal of the aqueous phase, the organic phase can be held, refrigerated, overnight.
- The completed extract, in the crimped-top GC bottle, can be stored at or below 0°C for several days before GC analysis.
- Following GC analysis, the extracts can be stored refrigerated for several weeks if the pierced septum is removed no later than 2 hours after puncture. Replace the pierced septum with a new one to prevent possible contamination from septa bleed or loss of solvent through evaporation.

Do not hold samples:

- After saponification before extraction.
- Over the base wash.

Media

Tubed media for this analysis are available commercially. See Table B-7 for ordering information.

Reagent Preparation

Four reagents are required to saponify the cells and to esterify, extract, and wash the fatty acid extract. Prepare the reagents in clean, brown, labeled, one-liter bottles. Place a Teflon-coated stir bar in each bottle to aid in mixing. Only Teflon and glass should come in contact with the reagents. Bottles can be reused without rinsing.

Caution: Reagents 1 and 4 are caustic and Reagent 2 is acidic; wear safety glasses and gloves at all times. Methyl-tert-butyl ether (MTBE) and hexane (in Reagent 3) are flammable. Extinguish all flames and heat sources before use. Handle in a chemical fume hood.

Reagent 1- Saponification Reagent

- Add NaOH pellets to water and methanol while stirring. Stir until pellets are dissolved.

Sodium hydroxide (certified ACS)	45 grams
Methanol (HPLC grade)	150 ml
Deionized distilled water	150 ml

Caution: NaOH is caustic. Wear safety glasses and gloves.

Reagent 2- Methylation Reagent

We have found that we get better methylation by using hydrochloric acid in methanol (2a) and sulfuric acid in methanol (2b) and by adding these separately rather than by using only HCl in methanol, as in the standard extract preparation.

Reagent 2a

- Slowly add acid to methanol while stirring.

6.00N Hydrochloric Acid	325 ml
Methanol (HPLC grade)	275 ml

Note: The hydrochloric acid must have a certified concentration. Only HCl shipped in glass bottles is accepted. Consult your vendor for packaging information. Please refer to the supplier recommendation in Appendix A. If 6.00N HCl is not available, consult your manufacturer's label for the concentration of that lot. Confirm that the final solution is 6.00N by titrating against a standard base solution.

Reagent 2b

- Slowly add acid to methanol while stirring.

Caution: The bottle will get very hot while preparing this reagent.

50% Sulfuric Acid (aqueous)	325 ml
Methanol (HPLC grade)	275 ml

300 ml each of Reagents 2a and 2b should be sufficient to run 36-40 samples per day for 5 days.

Reagent 3- Extraction Solvent

- Add MTBE to hexane and stir.

***Caution:** Methyl-tert-butyl ether (MTBE) and hexane are flammable. Extinguish all flames and heat sources before use. Handle in a chemical fume hood.*

Hexane (HPLC Grade)	200 ml
Methyl-tert-butyl ether (HPLC Grade)	200 ml

Reagent 4- Base Wash

- Add pellets to water while stirring. Stir until pellets are dissolved.

***Caution:** Sodium hydroxide (NaOH) is caustic. Wear safety glasses and gloves.*

Sodium hydroxide (certified ACS)	5.4 g
Deionized distilled water	450 ml
Sodium Chloride (Cert. ACS)	120 g

Reagent Storage and Shelf Life

It is recommended that reagents be prepared fresh each month. They can be stored at room temperature, in bottles supplied with Teflon-lined caps. Reagent 3, the extraction solvent, is flammable and should be properly stored in a chemical fume hood or vented solvent cabinet. All reagents should be properly labeled including the date of preparation and an expiration date of one month.

Purity Checks

The purity of reagents should be checked periodically by preparing a reagent control blank (procedure without cells) with every batch of samples.

The acids and the NaOH (Reagents 1 and 4) etch the Teflon of the plunger in the dispensers. Replacement dispensers are expensive. At the end of each day, remove the dispensers from the Reagent 1 and Reagent 4 bottles and cap the bottles with clean Teflon-lined screw caps. Rinse the dispensers well by pumping clean, distilled water through them. Leave the dispensers filled with water and store submerged in water. Storage in water preserves the life of the dispensers.

Preparing Broth-Grown Extracts

Growth of Cultures and Harvesting

Medium: These PRAS tubes are shipped in 16x100 mm tubes and contain 10-ml aliquots of broth with Hungate caps for storage and incubation. Teflon-lined caps are included for the extraction procedure.

Use the following:

* Cultures containing Tween must be washed in 0.7% (w/v) aqueous MgSO₄ before saponification.

Note: Serum-containing media cannot be used because the fatty acids bind to the cells. They cannot be removed by washing, and interfere with the analysis.

PRAS PYG	Gram-negative organisms
PRAS PYG-Tween-80*	Gram-positive organisms
PRAS PYG-Tween*-arginine or PRAS PY-formate-fumarate	Some non-fermentative strains of <i>Wolinella</i> , <i>B. gracilis</i> , <i>B. ureolyticus</i> , <i>Campylobacter concisus</i>
PRAS PY-lactate	Veillonellae

Culture for Analysis

Growth from an active primary isolation plate can be inoculated directly into the proper broth. However, if starting from stored cultures, direct specimens, etc., the following protocol should be followed:

1. Inoculate Chopped Meat (CM) medium with culture to be analyzed.
2. Incubate at 35 -37°C until growth occurs (usually overnight).
3. Inoculate a Brucella blood agar plate with the actively growing CM culture.
4. Incubate at 35-37°C until sufficient growth is present and purity confirmed.
5. Using several well-isolated colonies, inoculate a tube(s) of the appropriate medium.
6. Incubate at 35-37°C until maximum growth occurs (usually overnight).

Only one 10-ml culture is required for strains that give 3+ to 4+ growth. With strains that yield scant to 1+ turbidity (e.g., some non-fermenters), 2 or 3 tubes may have to be inoculated to obtain sufficient cells for a satisfactory analysis.

Remove stopper with disinfectant-soaked tissues to control aerosol. Discard tissues into a container for autoclaving. Centrifuge culture in screw-capped tube to form a sediment (pellet) of cells. Remove supernatant and retain pellet. If multiple tubes of the same culture have been inoculated, resuspend pellet from each tube in 1.5 ml of 0.7% (w/v) aqueous MgSO₄, combine into one tube, and centrifuge again.

If culture contained Tween, resuspend pellet in 3.0 ml 0.7% (w/v) aqueous MgSO₄ and centrifuge. Remove supernatant and retain pellet. Cap with the Teflon-lined screw cap after the final centrifugation.

Pellets may be processed immediately or frozen for processing another day.

Culture Control

Each sample batch should also contain a known strain as a positive control.

We recommend *Bacteroides fragilis* ATCC 25285 and *Clostridium perfringens* ATCC 13124.

Adjustment of Reagents or Cells to Give Satisfactory Results

For best results, the “Total Response” of cellular fatty acids, as reported on the Composition Report, should be between 45,000 and 500,000. If the extract contains a large concentration of fatty acids (i.e., gives more than 500,000 Total Response), the larger amounts may appear as ghost peaks on the next analysis and thus give inaccurate results. If the “Total Response” is greater than 1,000,000, the printed instructions on the chromatogram will state that the sample should be diluted and rerun.

With too small an amount of fatty acids in the extract, the printed instructions on the chromatogram will state that the sample should be concentrated and rerun. This works if the concentration is done within a few hours. Otherwise, the pierced septum contaminates the sample and erroneous results are obtained.

With experience, the microbiologist can adjust cell pellet size or amount of reagents added to give the appropriate concentration of fatty acids. The desirable size of cell pellet is the size of two NaOH pellets.

In our experience, an overnight culture of strains of *Actinomyces naeslundii* (ATCC 12104) in PYG-Tween gives a pellet that can be run with the standard amount of reagent. An overnight culture of strains of *Bacteroides fragilis* (ATCC 23745) grown in PYG gives too large a pellet. With such strains, remove the supernatant and adjust the pellet to the size of two NaOH pellets.

A more common problem is having too small a cell pellet. In these cases, only one-half the volume of reagent should be used and/or the extract can be concentrated by evaporation under a stream of pre-purified nitrogen. If there is only a light film of cells on the wall or bottom of the culture tube after centrifugation, use half-volume of reagent and concentrate the extract to about one-half the original volume. Because there will be a small volume of the concentrated extract, it must be placed in special vials (with V-shaped insert) or low volume vial inserts for the automatic sampler. If the pellet is about the size of one NaOH pellet or a moderate film, extraction with one-half reagent volume without concentration will give a satisfactory concentration of fatty acids in the extract.

Saponification

- Thaw cell pellet at room temperature for about 10 minutes if frozen.
- Add 1.0 ml Reagent 1 (NaOH) to cell pellet.
- Tightly seal each tube with a clean Teflon-lined screw cap.

- Vortex 5 to 10 sec.
- Heat in boiling water bath for 5 minutes. Check the tubes for leakage, evidenced by the presence of bubbles rising in the tube. Re-tighten the caps of leaking tubes. If bubbling continues, the sample must be transferred to a new test tube.
- Vortex 5 to 10 sec.
- Heat in boiling water bath for 25 min.
- Cool in water at room temperature.

Note: Use a boiling or circulating water bath. Heated blocks or other heating means do not have adequate heat transfer and temperature control.

Caution: Heating of sealed tubes builds pressure. Cracked or scratched glassware could burst. It is recommended that heating be done in a chemical safety hood or behind a plastic shield. Wear safety glasses.

Methylation

- Reagent 2a (HCl-MeOH) 1.0 ml.
- Reagent 2b (H₂SO₄-MeOH) 1.0 ml.

Because of an excess of reagents, a granular precipitate (salt) may form. It is not a problem at this step.

- Tighten the cap.
- Vortex 5 to 10 sec. The precipitate will disappear.
- Heat in 80°C ($\pm 1^\circ\text{C}$) water bath for 10 min.
- Cool rapidly in water at room temperature.

Note: Excess time or temperature can degrade results. Cyclopropane compounds may be degraded, altering fatty acid profiles.

Extraction

- Add 1.25 ml Reagent 3 (hexane/MTBE).
- Tighten cap. Invert tube to check for leaks. If a leak is detected, transfer to a new tube.

- Mix end-over-end for 10 minutes.
- Using a Pasteur pipette, remove and discard the bottom (aqueous) phase. Use a new pipette for each tube.
- Save the top phase.

Base Wash and Transfer to the GC Vial

- Add 3.0 ml Reagent 4 (NaOH-NaCl).
- Cap tightly.
- Mix end-over-end for 5 minutes.
- Using a clean Pasteur pipette for each sample, transfer two-thirds of the top phase into the GC vial for the automatic sample changer.

The interface between the two layers sometimes is difficult to see and care must be taken not to transfer any of the aqueous (lower) phase into the auto sampler bottle. The interface is easier to see if the aqueous phase has been frozen (place tube at about a 45° angle in the freezer).

- Cap the vial, and label.

Note: Ensure that the cap is sealed tightly by trying to rotate the cap while holding the bottle. If the cap slips, recrimp or adjust the capping tool to provide a tighter seal.

Summary of Procedure

1. Inoculate the Chopped Meat (CM) medium with the culture to be analyzed. (Use this step if the culture is coming from storage or going to storage.)

Note: Contact MIDI Technical Support for alternative purity check/ activation step.

2. Inoculate one (or more) tubes of appropriate medium with actively growing CM culture.
3. Incubate until maximum turbidity is reached. (Usually overnight.)
4. Centrifuge, save pellet, discard supernatant.

Note: If using PYG-T-80, wash cells with 3 ml 0.7% MgSO₄.

5. Add 1.0 ml of Reagent 1. Vortex 5 to 10 seconds. Heat in boiling water bath for 5 minutes. Vortex 5 to 10 seconds. Heat in boiling water bath for 25 minutes. Cool in water at room temperature.

6. Add 1.0 ml Reagent 2a. Add 1.0 ml Reagent 2b. Vortex 5 to 10 seconds. Heat in 80°C water bath for 10 minutes. Cool in water at room temperature.
7. Add 1.25 ml Reagent 3. Mix end-over-end for 10 minutes. Discard aqueous (bottom) phase.
8. Add 3.0 ml Reagent 4 to the top phase. Mix end-over-end for 5 minutes.
9. Place extract (top layer) in GC vial.
10. Load onto the automatic sampler.

Equipment/Supplies

Table B-7 lists some additional consumables and equipment for Broth-Grown Anaerobes. The following equipment and supplies are needed in addition to those used for standard Sherlock extractions. Media marked with an asterisk are highly recommended.

*Table B-7
Additional Consumables and Equipment for Broth-Grown Anaerobes
Please visit www.midi-inc.com for latest vendors and part numbers.*

Consumable	Source	Part Number	Amount
Broth-grown Anaerobe Culture Technique			
*PRAS Media for Broth-Grown Anaerobes	Anaerobe Systems	AS-822	10 tubes
CM culture	Anaerobe Systems	AS-811	10 tubes
*PRAS PYG	Anaerobe Systems	AS-875	10 tubes
*PRAS PYG-Tween-80	Anaerobe Systems	AS-825	10 tubes
*PRAS PYG-Tween-arginine	Anaerobe Systems	AS-835	10 tubes
*PRAS PY-formate-fumarate	Anaerobe Systems	AS-858	10 tubes
*PRAS PY-lactate	Anaerobe Systems	AS-846	10 tubes
Brucella Blood Agar	Anaerobe Systems	AS-111	1
Sample Preparation			
NaOH (cert. ACS)	Fisher	S318-500	500 g
Methanol (cert. ACS)	Fisher	A452-1	1L
50% Sulfuric acid (aq.)	Fisher	LC25640-2	1L
NaCl (cert. ACS)	Fisher	S271-1	1kg
MgSO ₄	Fisher	SM109-1	1L
Hexane (HPLC grade)	Fisher	H302-4	4L
Methyl tert-butyl ether (HPLC grade)	Aldrich	29321-0	1L