SHERLOCK™

Instant FAME™ User's Guide

MIDI

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Sherlock® Software and Libraries

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Getting Started

Overview

The Sherlock® Microbial Identification System by MIDI, Inc. analyzes the fatty acid methyl ester (FAME) composition of bacteria using peak naming and pattern recognition algorithms to identify sample extracts processed by gas chromatography. The *Instant* FAME™ protocol includes a procedure that extracts the fatty acid methyl esters from the bacterial and yeast cells in less than three minutes compared to the previous standard extraction method that required *ca.* 90 minutes. 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.

Figure 1-1. Sherlock Microbial Identification System with Agilent 6850 Series gas chromatograph.

MIDI developed a new chemistry that achieves a 30-fold sample preparation speed and 10-fold sensitivity improvements. The combination of small reagent volumes and lower split ratio in the chromatography method increases the sensitivity. All unknown organisms must be processed using the same sample preparation procedure and chromatographic method that were used to construct the identification library (database).

Environmental aerobes to be extracted using the Instant FAME extraction procedure should be grown on Trypticase Soy Agar (TSA) at 30°C incubation temperature. For clinical aerobes, organisms should be grown on Blood Agar at the 35°C incubation temperature as in previous MIDI methods. Instant FAME also includes methods and libraries for water isolated or stressed organisms grown on R2A media at 30^{o}C, Mycobacteria grown on Middlebrook at 35^{o}C, and yeast grown on TSA at 30^{o}C.

The libraries were created with organisms most frequently seen in clinical and environmental laboratories. The libraries were developed with well-characterized strains, grown under specified conditions and harvested at a specific time. Instant FAME methods can only be used with Instant FAME libraries. Any other combination will create erroneous identifications. Data for samples originally analyzed with other methods cannot be reanalyzed using the Instant FAME methods (and vice versa).

The Microbial Identification System consists of a MIDI specified Agilent Technologies, Inc. gas chromatograph coupled to a computer system. The older model 5890 gas chromatograph is not capable of running the Instant FAME methods.

The fatty acid extracts of the microorganisms are loaded into the sample tray. The controller commands the injector to inject a 2µl aliquot into the gas chromatograph. A capillary column installed in the gas chromatograph oven separates the fatty acid methyl esters as the extract travels through the column to the detector. The flame ionization detector burns the carbon in each ester creating a signal or response that is plotted to create the chromatogram. The retention time, size, and shape of each peak is processed and stored by the Sherlock software.

Using an adaptive algorithm, the Sherlock software names each chromatographic peak based on retention time and calculates its percentage based on its area. Periodic calibrations during sample batches adjust for instrument retention time and response drift. The adaptive structure of the peak-naming algorithm allows it to adjust to normal instrumental drift between periodic recalibrations and calls for additional recalibrations if needed. The chromatographic quality of the calibration and sample run are checked against control parameters for each MIS method. Chromatographic problems (e.g. low area, column overload, poor peak shape) and contamination problems (e.g., low percentage of peak area named) cause warning messages such as “Question Analysis” to be printed. Severe problems prohibit a library search and require operator intervention.

Following peak naming and quality checks, the fatty acid profile of the sample is compared to the library profiles. Pattern recognition algorithms are used to identify the most likely matches and calculate similarity indices. Optional comparison charts provide a visual plot comparing the profile of the unknown to the profiles of the most likely matches.

Installation

This guide contains information necessary to successfully use the Instant FAME extraction procedure, methods, and libraries. The Instant FAME method and corresponding libraries are installed with the Sherlock software or with a library upgrade. The Instant FAME methods and libraries require additional licenses for operation. Instant FAME is only supported on Sherlock version 6.0 or higher.

The MIDI Inc. Instant FAME **Start Up Kit** is needed to complete the sample preparation. Kit consumables and equipment are listed in Appendix A. Each Start Up kit has enough consumables to run 192 samples.

The MIDI Inc. Instant FAME **Refill Kit** replacement consumables are also listed in Appendix A. Depending on the Refill Kit size you order, the kit will have enough material for *ca.* 400 samples or *ca*. 192 samples.

Usage Overview

The Instant FAME extraction greatly differs from the previous standard extraction. The procedure takes *ca.* three minutes and requires only 2.5 – 3.0 mg of cell mass due to smaller volumes of reagent and greater sensitivity of the GC method. The extraction is performed in a single 2ml GC vial. The procedure uses only three reagents, and no longer requires water baths or a tumbler.

- Organisms are grown on either 5% blood agar at 35°C, TSA at 30°C, R2A at 30°C, for 24 ± 2 hours, or Middlebrook at 35°C for 2-5 days. All cells harvested should be from pure culture and in log phase growth, which can be found in the 3rd quadrant. The Instant FAME procedure is *ca.* 10 times more sensitive than standard extraction procedure (using Rapid Methods); therefore approximately 2.5 – 3.0 mg of cells are needed for the extract.
- The cells should be smeared around the bottom quarter of the vial to assure contact with the reagents.
- 250µl of Instant FAME Reagent 1 is then added to the vial. The vial is capped and vortexed for 10 seconds.
- 250µl of Instant FAME Reagent 2 is then dispensed into the vial. The vial is capped and vortexed for three seconds.
- 250µl of Instant FAME Reagent 3 is then added, and a phase separation is observed. The top layer is clear and the bottom layer is red.
- Remove 70µl of the top layer into an insert. For assistance, the MIDI wand should be used by placing the end with the hole over the top of the vial opening and aspirating the top layer. The extract can then be analyzed on the gas chromatograph.
- Label the vials and place them on the GC for analysis. Fill in the Sample Processor table with the correct sample information. Place two fresh sample wash bottles on the GC. The wash bottles should be filled to the neck.

Table 1-1. Instant FAME libraries

All relevant information about library versions.

Name	Description	Growth Conditions
IBA1	Clinically relevant organisms	5% defibrinated sheep blood agar @ 35°C
IMYC1	Non-TB Mycobacteria	Middlebrook @ 35°C
IR2A1	Water/stressed organisms.	R2A @ 30°C
ITSA1	Environmental organisms	TSA @ 30°C
ITY1	Yeast	TSA @ 30°C

Comparison of Instant FAME Extraction Method to Standard Extraction Method

- The time to complete the Instant FAME extraction is *ca.* 3 minutes compared to *ca.* 90 minutes for the standard extraction procedure.
- Fewer cells are needed, only *ca.* 2.5 – 3.0 mg of wet cell weight (compared with 20 mg for the Rapid methods and 40-50 mg for the standard methods). Using an excessive cell mass can result in incorrect identifications or carry-over into the next sample, negatively impacting the next sample's identification as well.
- The Instant FAME Extraction procedure uses fewer reagents (3), a smaller volume of each reagent (250µl), and the reagents are more environmentally benign than in the standard extraction procedure.
- The extraction procedure no longer requires the use of water baths and a tumbler.
- The Instant FAME method requires the Rapid Method Calibration Standard (MIDI Part # 1300-AA), which is packed in distinctive amber glass ampoules. A box is included in the Instant FAME refill kit.
- Because fewer cells are used the injection port liner typically lasts for 200 analyses versus 100 for standard methods. Liner life is reduced when samples contain mycolic acids or if the standard methods are used for some samples.
- If a culture is slow growing and likely to not provide adequate area, 100µl or 125µl of reagent 2 can be used rather than the standard 250µl. Adjust the volume by turning the dispenser dial until it locks in the 100µl or 125µl setting, depending on the dispenser model.
- The taxonomy of the Instant FAME libraries have been updated to correspond to that provided by the IJSEM.

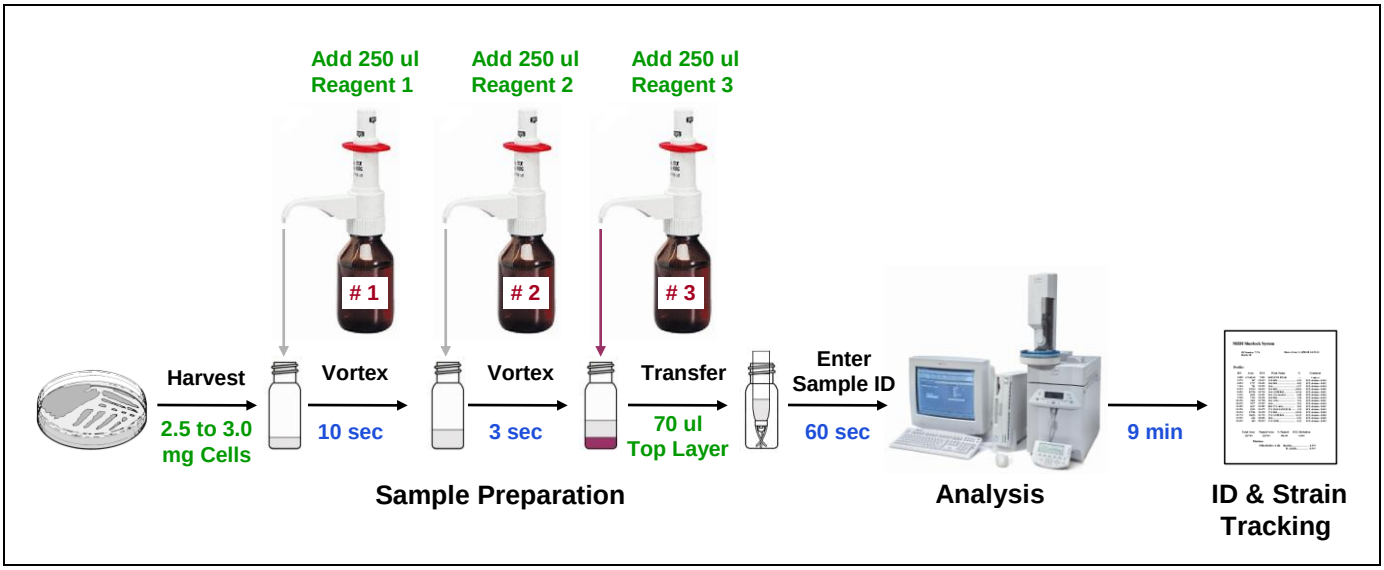
Section 2
Preparing Extracts

Instant FAME Extraction Overview

There are five basic steps in the preparation of GC ready extracts from cell cultures for Instant FAME fatty acid composition analysis (as shown in Figure 2-1):

- Harvesting cells from culture media
- Adding Instant FAME Reagent 1 extract the fatty acids from the cells
- Adding Instant FAME Reagent 2 to transfer the fatty acids from the aqueous phase to an organic phase
- Adding Instant FAME Reagent 3 to separate the phases
- Removal of the top layer for GC analysis

Figure 2-1. Instant FAME Identification Procedure.
Overview of 5 steps involved in preparing extracts for analysis by the Instant FAME extraction protocol.



NOTE: For a more detailed explanation of these steps, see "Instant FAME Extraction Detailed" section starting on page 17.

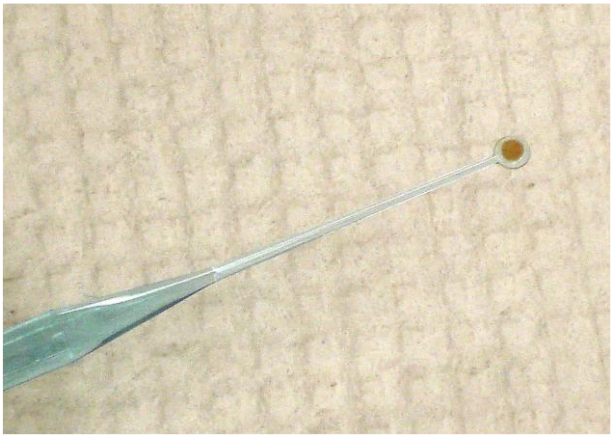
- **Media** – The following media should be used to grow organisms for the corresponding libraries (Media part numbers are listed in Appendix A)
  - Trypticase Soy Agar with 5% defibrinated Sheep Blood
  - Trypticase Soy Agar
  - R2A
  - Middlebrook and Cohn 7H10 Agar
  - Other media used are specified by the organism's entry description
- **Labeling Plates** – Plates should be labeled with as much pertinent information as possible.
  - Organism's source
  - Library accession number
  - Morphological information
  - Date of streak
  - Initials of technician who streaked the plate
- **Streaking Plates** – A quadrant streak is required to obtain pure colonies in log phase growth. For a picture of a quadrant streak, see Figure 2-12.
- **Quality Control Organisms** – Each of the following controls should be run per batch of samples:
  - **Blank Control** – The User should simulate the Instant FAME extraction by going through the entire protocol, but without the addition of bacterial cells.
  - **Procedure Control** – A known, well characterized strain with a valid library entry should be grown using specified media and growth conditions and processed using the Instant FAME extraction procedure. This will determine whether all growth conditions and extraction procedures were correct. Bacterial examples are:
    - *Bacillus subtilis* ATCC 6633 or *Stenotrophomonas maltophilia* ATCC 13637 are recommended to use for a procedure control for Environmental and Water Isolate methods and libraries.
    - *Pseudomonas aeruginosa* ATCC 27853 is recommended to use for a procedure control for the Blood method and library.
    - *Saccharomyces-cerevisiae* ATCC 18824 or *Candida albicans* ATCC 14053 are recommended to use for a procedure control for the Yeast method and library.
    - *Mycobacterium abscessus* ATCC 23006 is recommend to use for a procedure control for the Mycobacteria method and library

- **Incubation Parameters**
  - Clinical Organisms: 24 ± 2 hours for aerobes at 35°C ± 2°C
  - Mycobacteria: 2-3 days for most species, at 35°C ± 2°C
  - Environmental Organisms: 24 ± 2 hours for most aerobes at 30°C ± 2°C
  - Water Isolated Organisms: 24 ± 2 hours for most aerobes at 30°C ± 2°C
  - Yeast: 24 ± 2 hours for most yeast at 30°C ± 2°C
- **Extraction Log Sheet**
  - An extraction log sheet should be created to provide your laboratory with a record that matches the extraction numbers to autosampler tray bottle positions. Include the following details from the plate label:
    - The duration of incubation
    - The technician who will complete the extraction
    - Reagent lot numbers
    - Media and growth conditions
  - A sample Extraction Log Sheet is provided in Appendix B.
- **Label GC Vials** – Label GC vials according to the Extraction Log Sheet. You will be using two 2ml vials per extraction. The extraction is performed in the first vial and the second vial holds the insert containing the finished extract.

Figure 2-2. Instant FAME GC Vial Setup.
Two GC vials are used for each sample. One of the GC vials contains a glass spring foot insert.

- **Harvesting**
  - Harvest 2.5 - 3.0 mg of cells using a sterile inoculation loop. This amount can be estimated by using the 1µl end of the loop. Fill the loop completely with growth so there is no empty space within the interior of the loop (Figure 2-3).

Figure 2-3. Instant FAME Harvest Amount.
A 1µl inoculating loop is used to harvest 2.5 to 3.0 mg of cells.



- The cells should be in log phase growth. This type of growth is typically located in the 3rd quadrant of the purity streak.
- Smear cells along the bottom quarter of the vial to ensure contact with the reagents. See Figure 2-4 and 2-5 for an example.

Figure 2-4. Placing cells in GC vial.
2.5 to 3.0 mg of cells are placed into the GC vial without the insert.

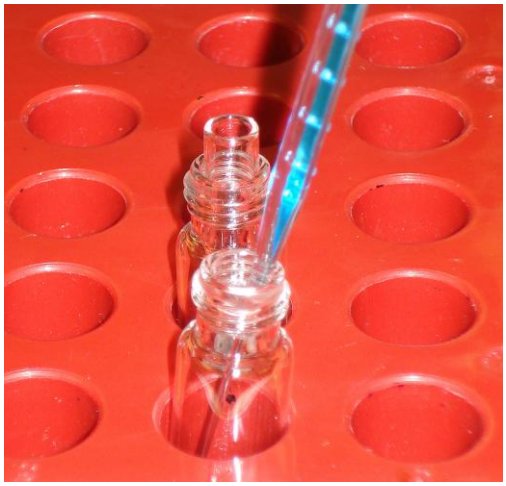


Figure 2-5. Bacterial cells in vial.
The cells are smeared onto the bottom quarter of the vial for better reagent contact.

- To concentrate a sample, use only 100µl or 125µl of Instant FAME Reagent 2. This can be done by turning the pump dial of Instant FAME Reagent 2 until the 100µl or 125µl (depending on model) setting is designated and then dispensing the reduced volume rather than the standard 250µl.
- Refer to the "Slow growing and fastidious organisms" section for more information on harvesting those organisms.

- **Extraction**
  - **Instant FAME Reagent 1**
    - Add 250µl of Reagent 1 to the vial (Figure 2-6).
    - Cap the vial and vortex for 10 seconds (Figure 2-7).
  - **Instant FAME Reagent 2**
    - Add 250µl of Reagent 2 to the vial (Figure 2-6).
    - Cap the vial and vortex for 3 seconds (Figure 2-7).
  - **Instant FAME Reagent 3**
    - Add 250µl of Reagent 3 (Figure 2-6).
    - Observe the phase separation, with the top layer being clear and the bottom layer red (Figure 2-8).

Figure 2-6. Addition of reagents.
250µl of each of reagent are used per sample.

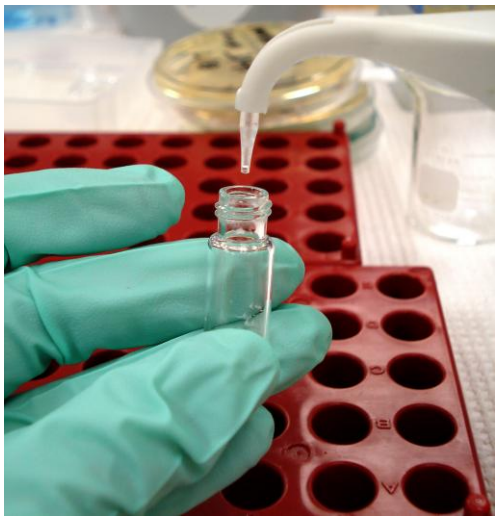


Figure 2-7. Vortexting steps.
The vortexer is used after the addition of Reagent 1 for 10 seconds and again after Reagent 2 for 3 seconds.

Figure 2-8. Phase separation.

After the addition of Reagent 3, there is a noticeable phase separation, with the organic fatty acid layer on top and the aqueous dye-colored layer on the bottom. The top layer is used for analysis.

- **Remove Top Layer** – Using the Eppendorf Pipette, remove 70µl of the top layer and place into an insert.
  - The MIDI Wand or pipette spacer can be used to assist with this step.
  - Place the end of the Wand with the hole over the opening of the extraction vial. Depress the pipette plunger button to the first step. Lower the tip through the Wand's hole and into the extraction vial. Pick up 70µl of the top layer.
  - Lift the Wand with the Pipette and tip in place. Transfer the 70µl to the insert.
  - The Wand prevents the pipette tip from accessing the bottom layer of the extract and positions the tip to pick up just the top layer. See Figure 2-10 for an example of the bottom aqueous layer being erroneously picked up with the top layer.

NOTE: Be extremely careful not to access the interface or the bottom layer. Do not jostle the vial. Be sure to depress the pipetter halfway (expunging the air) before placing the tip in the vial.

Figure 2-9. Using the MIDI wand.
70µl of the top organic layer is picked up using the MIDI wand, which prevents the pipette tip from entering the bottom aqueous layer.

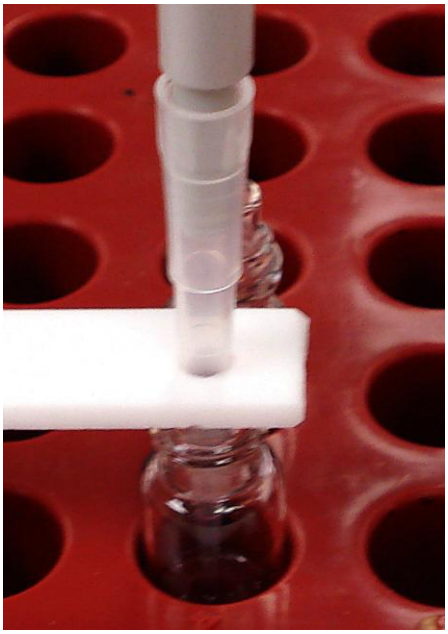


Figure 2-10. Erroneous Sample extract containing aqueous layer.
If the pipette contains red dye, it contains some of the aqueous layer. The last step should be redone. The aqueous layer will degrade the inlet and potentially damage the column.

- **Load the Automatic Sampler** – Load extracts in the GC autosampler along with the calibration standard and QC samples. Enter the samples into the Sample Processor table.
    - The "Sample ID" entry in the extraction log should match the plate label on which the culture was grown. The same text should be used in the computer's sample table. Enter all pertinent information within the naming field, up to 42 characters. For additional information, refer to the "Entering Data" section on page 24.
    - Empty the wash bottles and refill them to the neck with fresh hexane. It is **critical** for the wash bottles to be full to avoid carryover.
    - Empty waste bottles.
    - The Instant FAME Methods use Rapid Calibration Mix (Part #1300-AA, included in Instant FAME startup and refill kits).

Figure 2-11. Automatic liquid sampler.
Samples are placed in the automatic liquid sampler (ALS) and logged into the Sherlock Sample Processor table.

- **Start the Analysis Batch –** Click the Start Batch tool in the Sherlock Sample Processor to begin analyzing the samples.

Instant FAME Extraction Detailed

Growth Media Selection

The following media were used for the corresponding methods and libraries:

- Clinical Method (IBA1):
    - BBL Brand Trypticase Soy Agar with 5% defibrinated Sheep Blood (BD Part#: 221261, prepared plates) – primary media used to build the library
    - BD BBL BCYE Agar (BD Part#: 221808, prepared plates)
    - BD BBL Mueller Hinton Chocolate Agar (BD Part#: 221860, prepared plates)
    - BD Difco Lactobacilli MRS Broth (BD Part#: 288130, dehydrated media)

- Examples of organisms typically grown using other media or conditions:
  - Afipia-broomeae (BCYE at 30C)
  - Amycolatopsis-orientalis (chocolate)
  - Avibacterium-avium (chocolate)
  - Campylobacter-coli-GC subgroup B (chocolate)
  - Campylobacter-fetus-fetus (chocolate, blood, CampyPak)
  - Campylobacter-fetus-venerealis (chocolate, blood, CampyPak)
  - Campylobacter-hyointestinalis(chocolate,blood,CampyPak)
  - Campylobacter-lari (chocolate, blood, CampyPak)
  - Campylobacter-sputorum-bubulus (CampyPak, chocolate, 48-72h)
  - Campylobacter-sputorum-sputorum (CampyPak, chocolate, 72h)
  - Fluoribacter-bozemanae (BCYE)
  - Fluoribacter-dumoffii (BCYE)
  - Fluoribacter-gormanii (BCYE)
  - Gardnerella-vaginalis-GC subgroup A (48h, chocolate)
  - Helicobacter-cinaedi (blood, chocolate CO2)
  - Lactobacillus-jensenii (48h on MRSA)
  - Lactobacillus-reuteri (48h on MRSA)
  - Leuconostoc-mesenteroides (MRSA)

- Environmental Library (ITSA1):
  - BBL Brand Trypticase Soy Agar (BD Part#: 221283, prepared plates)
- Water Isolate Library (IR2A1):
  - Difco Brand R2A Agar (BD Part#: 218263, dehydrated media)
- Yeast Library (ITSA1):
  - BBL Brand Trypticase Soy Agar (BD Part#: 221283, prepared plates)
- Mycobacteria Library (IMYC1):
  - BD BBL Middlebrook and Cohn 7H10 Agar (BD Part#: 221174, prepared plates)

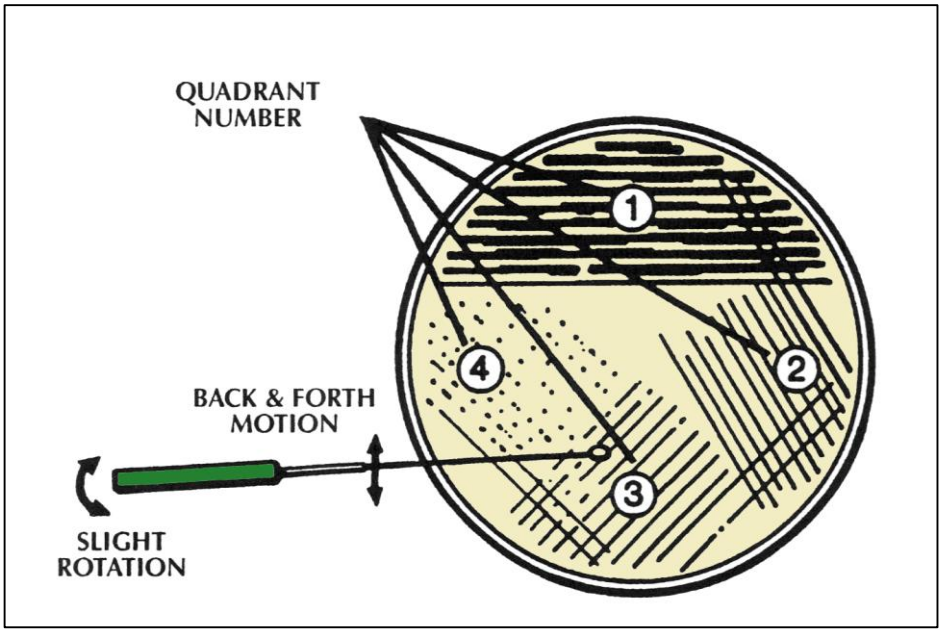
IMPORTANT: If Powdered Media is acquired from another vendor, it must be an exact match to the prepared plates specified above. Differences can produce unexpected results.

Streaking Plates

NOTE: This section is from the ***Operating Manual***. It is repeated here for convenience.

The quadrant streak pattern is necessary for culturing cells on plates for identification by the MIS. The streaking pattern results in ample material for analysis while confirming the presence of a single colony type or pure culture.

Figure 2-12. Quadrant streak.
The quadrant streak method is used to grow and harvest the cells needed for analysis.



1. Sterilize and cool the inoculation loop. The loop can be cooled by plunging it into the agar away from any cell colonies.
2. Take a cross section of cells from the primary isolation plate after confirming its purity.
3. With the 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.
4. Inoculate quadrant 2 by rotating the loop 90° and passing the loop edge through the corner of the quadrant 1 twice. Then streak the rest of the quadrant 2 with parallel lines without reentering quadrant 1.
5. 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 the quadrant 3 with parallel lines without reentering quadrant 2.
6. Sterilize and cool the inoculation loop. The loop can be cooled by plunging it into the agar away from cell colonies.
7. 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.

Incubation

The standard incubation conditions for aerobes are as follows:

- Clinical organisms: 24 ± 2 hours on blood agar at 35°C
- Mycobacteria: 2-5 days on Middlebrook at 35°C
- Environmental organisms: 24 ± 2 hours on TSA at 30°C
- Stressed organisms: 24 ± 2 hours on R2A at 30°C
- Yeast: 24 ± 2 hours on TSA at 30°C

Use a high quality incubator in which growth conditions can be controlled. The temperature within the incubator should only fluctuate ± 2°C. 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.

Harvesting

The effect of age on the organism's fatty acid profile is minimized by frequently checking the plate for fresh growth. A plate should be harvested 24 ± 2hours after being streaked. Standardization of physiological age of culture is obtained by choosing the correct sector from the quadrant streak. Each quadrant in the streak dilutes the inoculum so that quadrant 4 should contain well isolated colonies to serve as a check of purity. Colonies should be harvested from the most dilute quadrant exhibiting confluent growth (log phase) along the streaking axis. This area of harvesting typically yields the most stable fatty acid composition since the inoculum has been diluted enough to result in abundant growth of colonies without a limiting nutrient supply. **The optimum area for harvesting is log phase growth which usually occurs in the third quadrant.**

Fewer cells are needed to avoid overloading the more sensitive analysis. Use only about 2.5 – 3.0 mg wet weight of cells (compared to 20 mg for the Rapid methods, 40-50 mg for the standard methods.) Using an excessive cell mass can result in incorrect identification and possible carryover into the next sample, impacting its identification.

Caution – Failure to use cells from the correct quadrant or using too many cells can result in poor library matches. However, it may be necessary to use cells from quadrant 2 with slower growing organisms.

- Remove the cultured cells from the plate by gently scraping the surface of the culture medium with a sterile inoculating loop.
- Approximately 2.5 – 3.0 mg of live wet cells is the correct amount of material to use for processing.
- Insert the loop with the cells into a clean 2ml screw cap vial. Wipe the cells off the loop and onto the lower inner surface of the culture vial (within the bottom portion of the vial). Remove the sterile loop. The intent is to have the cells come into contact with all three reagents in the vial.

Slow Growing and Fastidious Organisms

Not all microorganisms can be cultured with the standardized Instant FAME extraction conditions. The objective is to harvest the organism in log-phase growth. Harvest from the most sparse growth area that easily allows harvesting of 2.5 – 3.0 mg of cells. Slow growing bacteria may require 48 – 72 hours to reach log phase growth.

A fastidious organism is one that prefers a medium other than the routinely used one. Some organisms may require enriched media or specific atmospheric conditions. For example, *Lactobacillus* grows better on MRS media than on blood agar at 35ºC, and some strains of *Streptococcus* grow better with 5-10% CO2. The appropriate culture conditions for these organisms are listed next to the entries in the Instant FAME library listings. Your MIS can only identify these organisms if you use the indicated culture conditions. The MIS growth conditions attempt to duplicate the most commonly used media and conditions for these organisms.

A light grower is an organism that does not thickly coat the plate, or its colonies are observed to be faintly present on the media. For example, some strains of *Streptococcus* grow lightly upon blood media. This type of organism, if grown past the 24 hour incubation mark, may have little additional growth on the media's surface. With the Instant FAME extraction procedure, such organisms can be harvested from the quadrant having sufficient growth, even if it is the third or second quadrant. The extract is then made more concentrated by only using 100µl of Instant FAME Reagent 2 instead of the full 250µl.

Should the unknown organism have an inadequate amount of growth in the third quadrant the following options are available:

- **Concentrate the growth** – Take as much fresh growth from the 3rd quadrant as possible. During the extraction procedure use 100µl (or 125µl, depending on dispenser model) of Instant FAME Reagent 2 rather than the full 250µl. Only 70µl of the extract (top layer) should be removed to run on the GC.
- **Streak two plates** – Streak two plates of the organism on the same media previously used and combine the harvested growth from the 2 plates in the extraction procedure. You would use the full 250µl of the Instant FAME Reagent 2 in this scenario.
- **Increase Incubation Time** – Allow the organism to grow an additional 24-48 hours.

Quality Controls

To ensure that culturing, sample preparation, and the instrument all function within normal parameters, you should include two quality control samples with each batch of samples.

- **Blank Control** – Simulate the Instant FAME extraction by going through the entire protocol, without the addition of any bacterial cells. This will determine if there are any problems with the reagents, consumables, or technician technique.
- **Procedure Control** – A well-characterized strain with a valid library entry should be grown using specified media and growth conditions and processed using the Instant FAME extraction procedure. This will determine potential problems with technique, incubation temperatures, media, etc.
    - *Bacillus subtilis* ATCC 6633 or *Stenotrophomonas maltophilia* ATCC 13637 are recommended for Environmental/Water methods and libraries.
    - *Pseudomonas aeruginosa* ATCC 27853 is recommended to use for a procedure control for the Blood method and library.
    - *Saccharomyces-cerevisiae* ATCC 18824 or *Candida albicans* ATCC 14053 are recommended for the Yeast method and library.
    - *Mycobacterium abscessus* ATCC 23006 is recommend to use for a procedure control for the Mycobacteria method and library

Reagent and Dispenser Information

The three reagents used for the Instant FAME extraction procedure have a one (1) year expiration date if the shipment bottle is unopened. Upon opening the bottle, each reagent has a shelf life of six (6) months.

To prevent evaporation of the reagents when the dispensers are not in use for two days or longer, the dispensers should be removed. All excess reagents remaining in each dispenser should be flushed back into the dispenser's bottle, and the bottle capped.

The Instant FAME Reagent 1 dispenser should be cleaned by first flushing de-ionized water through the dispenser, followed by methanol until any resistance is gone. The dispenser should be completely cleared of all liquid and placed to the side. The Instant FAME Reagent 2 and 3 dispensers should be cleared by pumping excess reagent back into the bottles, and the bottles capped. These dispensers do not have to be cleaned.

CAUTION: Follow your lab's standard safety precautions, including appropriate Personal Protection Equipment for handling of caustic and flammable chemicals. Wear safety goggles and gloves while performing the extraction procedure. Do not work near open flame. Reagents 1 and 3 are caustic and Reagents 1 and 2 are flammable.

NOTE: Be careful not to dispense too rapidly. Reagents will splash out of the vial, and quality of the extraction will be compromised.

- **Instant FAME Reagent 1**
    - Prime each of your reagent bottles prior to use. Once the dispenser is primed, it will accurately dispense 250µl. After the cells are added to the vial, add 250µl of Reagent 1.

- Cap the vial and vortex for 10 (full) seconds. When vortexing, be sure to properly grip the vial. Avoid holding the cap, which can twist off while vortexing.

- **Instant FAME Reagent 2**
  - Add 250µl of reagent 2.
  - Cap and vortex the vial for 3 seconds. When vortexing, be sure to properly grip the vial. Avoid holding the cap, which can twist off while vortexing.
  - To concentrate the sample, the setting of the reagent 2 dispenser can be changed from 250µl to 100µl (or 125µl, depending on model). This can be done by turning and locking the dispenser into the lower setting. Using a lesser amount of reagent will concentrate the extract for the GC run.

- **Instant FAME Reagent 3**
  - Add 250µl of reagent 3.
  - Observe the phase separation. Two layers should be present within the extraction. The top layer is clear and bottom layer is red.
  - Remove 70µl from the top layer of the vial and place into an insert. Avoid picking up any of the bottom layer or the interface. If this happens, the red color of the bottom layer will serve as an indicator. The MIDI wand should be used by placing the end with the hole over the top of the vial opening. Place the pipette tip within the hole and carefully remove 70µl of the top layer.
  - Ensure that your cap is tightly sealed to avoid evaporation.
  - Place the sample vial in a rack. When all the sample extracts have been transferred to their sample vials, you are ready to load the autosampler and start running samples.

WARNINGS

- **Biohazard** – The Instant FAME extraction may not kill all of the cells. Extraction waste should be treated as biohazardous and disposed of according to State and Federal protocol.

- **Evaporation** – Should the extraction samples be left out in the lab over night, check the volume level of the sample within the insert prior to analysis. Evaporation will not damage but will concentrate your sample. Evaporation can be prevented by tightly fastening the caps to the vials. Should evaporation occur, place a few drops of Instant FAME reagent 2 into the insert to raise the level to its original height.

- **Storing Extraction Vials** – If an extract cannot be analyzed as soon as it is made, it can be stored at room temperature for a short (24 - 36 hours) period of time or within a refrigerator for longer periods of time (up to 72 hours). Be aware of possible solvent evaporation.

Loading the automatic sampler

- Before samples can be analyzed, the sample tray and injector turret must be loaded and samples properly identified in the sample table.
- The Instant FAME method uses the rapid calibration standard. The Rapid Method Calibration Standard (MIDI Part No. 1300-AA) is packaged in distinctive amber glass ampoules.
- Two wash bottles must be used for this extraction procedure. Empty the wash bottles and refill them to the neck with fresh hexane. It is critical for the wash bottles to be full to avoid carryover.
- Load the wash bottles into their proper positions
    - 6850 gas chromatograph – Wash in Solvent A and B positions, waste bottles in the Waste A and C positions
    - 6890 gas chromatograph – Single and Dual Tower Systems – Wash in Solvent A and B positions, waste bottles in the Waste A and B positions
    - 7890 gas chromatograph – Single Tower Systems – Wash in Solvent A and B positions, waste bottles in the Waste A and B positions
- The turrets can be moved by hand to gain access to the bottles.
- The calibration standard must be placed in the tray positions specified by the sample table.
- Each sample must have a name entered into the sample table to identify the sample with a given bottle position in the sample tray. Verify that all bottles are in the correct position.
- Empty the waste vials in the GC turret before starting a sequence. If the waste bottles become filled, the needle will contact the waste and become contaminated.
- Because a smaller cell mass is used, the injection port liner will typically last for 200 analyses versus 100 for the standard methods. Liner life is reduced when samples contain mycolic acids or if the rapid or standard methods are used for some samples.

Entering Data

Enter all pertinent information pertaining to the sample into the “Name” field. The more information added, the easier it is to answer future inquiries about the particular sample. 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 naming is extremely important for record keeping as well as cataloging samples and using the features of the library generation software. It is advantageous to organize the name field 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. Only

information before the open parenthesis sign ‘ (‘ is used for grouping. A total of 42 characters may be entered.

We suggest using “UNK” in front of unknown samples. Fields after "UNK" are separated by dashes such as "UNK-PROJ1-AREAQ-24". The section beginning with an open parenthesis sign ‘(‘allows the user to enter discrete information about a sample, such as the patient identification number. It is helpful to use the initials of the operator who is logging in the sample into the computer. That person is verifying that the samples are placed in the correct position on the auto sampler tray. It is not necessary to close the parenthesis. The software stops cataloging functions at the left parenthesis.

Note: Do not use single or double quotation marks (‘ or “) in the Name or Sample ID.

Interpreting MIS Results

Refer to the **Sherlock Operating Manual** – Chapter 4, for information on how to interpret Sherlock reports. Matches to entries marked as “confirm with other tests” should be confirmed using appropriate techniques.

Section 3
Troubleshooting

This section covers problems specific to the Instant FAME methods. Problems with the Sherlock system are covered in the Sherlock MIS Operating Manual.

NOTE: Only use the Instant FAME equipment and consumables supplied by MIDI, Inc. in the Startup Kit and the Refill Kit. Substitutions or modifications to the MIDI supplied consumables may result in any and all of the problems listed below.

Sample Related Problems

Match not found or low similarity index for first choice.

- Cells harvested for extraction were not in log phase growth. Cells should be harvested from the most dilute quadrant that exhibits log phase growth after 24 ± 2 hours incubation. Typically the cells should be harvested from the third quadrant. Longer incubation times may be specified for slow growing organisms. For most organisms cells from quadrant three are preferred. For slower growing organisms, it may be necessary to harvest cells from quadrant two. Avoid using cells from quadrant one.
- Cells should be harvested from plates and extracted within ~30 minutes of removal from the incubator. Work in small batches of 6 to 7 plates that can be harvested and extracted in ~30 minutes.
- Cells must be grown on the specified media and at the specified temperature. Confirm that the correct media for the method and organism was used as specified in section 2 of this manual and that the incubator is set to the correct temperature for the method. The incubator set points must be:
  - Clinical bacteria on BA at 35°C
  - Environmental bacteria on TSA at 30°C
  - Water/Stressed bacteria on R2A at 30°C
  - Yeast on TSA at 30°C
  - Mycobacteria on Middlebrook at 35°C
- The incubator should be capable of controlling the temperature to ±2°C of the set point.
- The media should be fresh and within its expiration date. If the media has dried, new media should be obtained.

- Under-harvesting or over-harvesting cells can result in distorted FAME profiles and lead to poor identifications. Under-harvesting will result in low total responses and small but significant peaks may be missed.
- Over-harvesting can cause high total responses, resulting in some small, unexpected peaks appearing. Extreme over harvesting will result in GC column overload and possible failure to recognize key fatty acid peaks. See the troubleshooting sections for low total response and high total response.
- Stressed organisms such as those exposed to antimicrobial agents, excessive heat or cold, or low nutrient conditions may exhibit poor identifications unless they are subcultured an extra time for the IBA1, ITSA1, ITY1 and IMYC1 methods. The IR2A1 method is designed for low nutrient organisms typically found in water.
- The species may not be in the Instant FAME library.

Low total response reported for samples.

- Harvesting too few cells may not yield enough fatty acids for a reliable comparison to the library. If the Total Response is less than 50,000 Sherlock reports an error. For most organisms, the Total Response should be between 100,000 and 500,000. The proper cell mass to harvest is 2.5 mg to 3.0 mg.
- Check that the GC vial/insert contained sufficient sample for the syringe to aspirate the correct volume for injecting into the gas chromatograph. There should be a minimum of 70µl in the insert.
- Failures in the extraction process caused by dispensing incorrect reagent quantities or adding reagents in the wrong order can result in poor extractions. See dispenser related problems below.
- Vortexing for less than the times specified in Section 2 will result in lower total responses. Note that vortexing for longer times does not change the total response or fatty acid profile. The vortexing times are designed to always produce a complete reaction while minimizing the sample preparation time.
- The harvested cells must be placed at the bottom of the extraction vial in order for the reagents to come in full contact with them and completely extract the fatty acids.
- A clogged or partially obstructed syringe can result in too little sample being introduced into the gas chromatograph. See the gas chromatography troubleshooting section for a clogged syringe.

High total response reported for samples.

- Harvesting too many cells can result in a Total Response that is too high. This results in small percentage peaks appearing, which may cause lower similarity score. A Total Response in the range of 100,000 to 500,000 produces the best results. If a single peak exceeds 500,000 Sherlock reports an overload error.

- If the GC vial is not tightly capped, the sample can evaporate, causing it to become more concentrated. Reagent 2 can be added to dilute the sample.

Extra (contamination) peaks seen in samples.

- Be sure that both solvent wash bottles in the GC's turret are filled. If the level drops below the minimum indicated on the vial (about half way down), the syringe will not be completely rinsed after each sample. This may result in carry over to the next sample.
- A blank control should be prepared with each batch to check for reagent and dispenser contamination problems. See the troubleshooting section for peaks reported in a blank control.
- Care must be taken to avoid harvesting media along with the organism. Compounds from the agar will appear as extra peaks in the chromatogram and can confuse the identification process.
- Contamination peaks are found in the interface between the two layers. Pipetting from the interface between the top layer and bottom layer will introduce contamination peaks. The appearance of the 18:3w6, which only occurs in the interface, is an indication. The interface also contains 16:0, 18:0 and 18:1w9 that can impact the identification. Use the Wand and observe that no red dye is picked up when pipetting the top layer.

Peaks reported in blank control.

- The total response should be <2250 and is considered normal.
- A small total named response <750 for a blank is considered normal.
- Be sure that both solvent wash bottles in the GC's turret are filled. If the level drops below the minimum indicated on the vial (about half way down), the syringe will not be completely rinsed after each sample. This may result in carry over to the next sample.
- Run Reagent 2 as a sample to test it for contamination. If Reagent 2 is free of contamination, Reagent 1 or Reagent 3 may be contaminated. Test a new blank control prepared with fresh reagents.
- Pipette tips other than those supplied by MIDI may contain contaminants. The Instant FAME methods are designed to account for the compounds found in the MIDI supplied pipette tips.
- The reagent dispensers are designed to prevent contamination of the reagents as long as they are maintained in an upright position. Laying a dispenser on its side or inverting it can contaminate the reagent.

Calibration Related Problems

The Instant FAME methods use the same calibration standard (1300-AA) as the Rapid methods. The calibration troubleshooting techniques found in the MIS Operating Manual apply.

Most calibration problems can be prevented if the PQ Table for Instant FAME Methods (Figure 3-1) is maintained and the routine maintenance procedures found in Chapter 6 of the MIS Operating Manual are followed. The following are some items that may be encountered with the Instant FAME methods:

Low total response reported for calibration.

- The expected Total Response for the calibration standard with Instant FAME is between 1.8 X 10^6 and 3.2 X 10^6. Values significantly below 1.8 X 10^6 may indicate a problem. Make sure that the calibration vial contains sufficient fresh calibration standard (MIDI Part # 1300-AA) for the syringe to aspirate the correct volume. Check for a clogged or partially obstructed syringe. See the gas chromatography related problems section to troubleshoot a clogged syringe.

High total response reported for calibration.

- Make sure fresh aliquots of the 1300-AA Rapid calibration standard are used. Store aliquots in tightly sealed vials. Evaporation will concentrate the standard. This problem can usually be avoided by routine maintenance to keep the PQ Table values in range.

Calibration fails.

- If a system has been working and the PQ Table (Figure 3-1) is maintained, calibration failures can usually be prevented. If the calibration fails, compare the report to Figure 3-2A and the chromatogram to Figure 3-2B. You should be able to detect the pattern of peaks shown in Figure 3-2B. Adjust the pressure as needed to bring the retention times of the solvent peak, the 9:0 peak, and the 20:0 peak into the ranges given in Figure 3-1 and Table 3-1. If a retention time is higher than it should be, increase the pressure. If it is lower than it should be, decrease the pressure.
- If the pressure is too low, the 20:0 peak may not be recognized by the system. When the retention time of the 20:0 peak exceeds 4.300 minutes, it will not be recognized. When this happens its retention time is not printed on the chromatogram. Increase the pressure until it is recognized.
- Refer to the Sherlock MIS Operating Manual for detailed instructions.

Gas Chromatography Related Problems

No peaks in chromatogram.

- Make sure the vial contains sufficient sample and that the syringe is not clogged.

Clogged syringe.

- A clogged or partially obstructed syringe can result in too little sample being introduced into the gas chromatograph. In this case, all samples and calibrations will exhibit less than expected total responses. See plunger error below for cleaning instructions.

Plunger error reported by software.

- The auto sampler motors are unable to move the plunger.
- To clear the syringe, remove it from the GC and flush it several times with deionized water until the plunger moves smoothly and easily. Flush the syringe several times with methanol or isopropanol.

Dispenser Related Problems

Dispenser difficult to push.

- Clean the Reagent 1 dispenser by first flushing with de-ionized water. Then flush with methanol until any resistance is gone.
- The Reagent 2 and Reagent 3 dispensers should not need to be cleaned. If they become difficult to push, they may need to be replaced. Contact MIDI Technical Support for additional help.

The pipette tip does not access the top layer or too little reagent is dispensed.

- Make sure the dispensers contain sufficient reagent and are primed.
- Test each dispenser and confirm it can be easily pushed down to dispense the correct volume of reagent.
- The wand pipette guide must be used with the MIDI supplied pipette tips and sample prep vials.

Temperature / Pressure Adjustment and PQ Tables

First try to correct using only the pressure adjustment. Typically temperature does not require adjustments.

Table 3-1 provides the column pressure and oven temperature adjustments needed to bring the retention times of the 9:0 and 20:0 peaks into their target time windows.

Table 3-2 is a PQ table that should be maintained with the instrument's daily log. When parameters fall out of their optimal ranges, corrective action should be taken.

Table 3-1. Temperature / Pressure Adjustment Table for Instant FAME 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.

Figure 3-1: PQ Table for Instant FAME Methods

Date	Gases	Inj. Port	Calibration Standard									Blank	QC Sample	
	Sig. 1 Baseline	Seq. #	Solvent RT	Total Response	RMS	SI	RT		OH %			Total Named	Total Named	SI
							9:0	20:0	10:0 2OH	14:0 3OH	16:0 2OH			
‡ Required Ranges →	NA	NA	NA	0.8-5.0 $x10^6$	<0.0028	>0.950	0.950-1.320	3.850-4.270	> 1.9	> 0.9	> 1.9	< 750	100-900 $x10^3$ **	>= 0.500
‡ Desired Ranges →	< 20	<200*	0.740 ±0.040	1.8-3.2 $x10^6$	<0.0020	>0.990	1.007-1.127	3.953-4.173	> 2.0	> 1.0	> 2.0	< 500	200-500 $x10^3$ **	>= 0.600

‡ Required Ranges indicate ranges beyond which the system will fail to calibrate or the results are not reliable. Desired Ranges indicate preferred running conditions for the system. If the system is outside the Desired Range but within the Required Range, the system will still operate correctly but there is a risk that an intermediate calibration could fail. Significant changes in system behavior should be investigated.

* If using a combination of Standard and Instant 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.

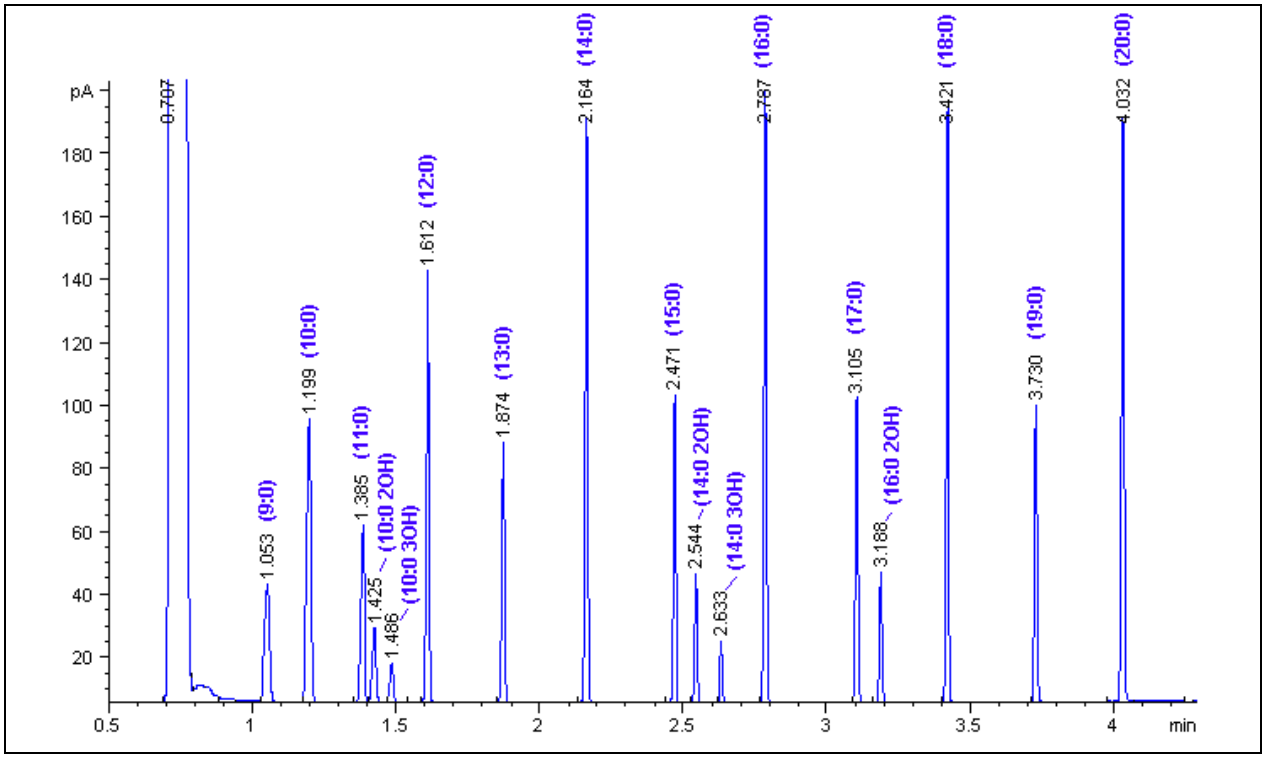
Figure 3-2A.
Calibration Report Example

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
0.7073	2.107E+9	0.017	----	6.6224	SOLVENT PEAK	----	< min rt	
1.0531	87758	0.019	1.229	9.0000	9:0	5.36		
1.1986	185257	0.015	1.160	10.0000	10:0	10.68	Peak match -0.0005	
1.3847	99109	0.013	1.102	11.0000	11:0	5.43	Peak match 0.0016	
1.4252	40928	0.013	1.093	11.1786	10:0 2OH	2.22	Peak match -0.0036	
1.4861	20581	0.013	1.080	11.4471	10:0 3OH	1.10	Peak match 0.0021	
1.6117	203616	0.011	1.054	12.0000	12:0	10.67	Peak match -0.0006	
1.8738	106284	0.010	1.015	13.0000	13:0	5.36	Peak match 0.0001	
2.1638	218654	0.009	0.983	14.0000	14:0	10.69	Peak match -0.0002	
2.4705	112477	0.009	0.959	15.0000	15:0	----	Peak match -0.0007	
2.5440	47385	0.009	0.954	15.2318	14:0 2OH	2.25	Peak match 0.0016	
2.6334	22600	0.009	0.949	15.5139	Sum In Feature 2	1.07	Peak match 0.0006	14:0 3OH/16:1 iso I
2.7873	229295	0.009	0.941	16.0000	16:0	10.72	Peak match -0.0006	
3.1047	116806	0.009	0.928	17.0000	17:0	5.39	Peak match -0.0004	
3.1883	49176	0.009	0.926	17.2640	16:0 2OH	2.26	Peak match 0.0015	
3.4213	234195	0.009	0.920	18.0000	18:0	10.71	Peak match -0.0014	
3.7298	117825	0.010	0.916	19.0000	19:0	5.36	Peak match 0.0011	
4.0323	235753	0.010	0.915	20.0000	20:0	10.72		
----	22600	---	----	----	Summed Feature 2	1.07	16:1 iso I/14:0 3OH	14:0 3OH/16:1 iso I

Total Response: 2015221 Total Named: 2015221
Percent Named: 100.00% Total Amount: 2119994
Profile Comment: Good peak matching. Peak position matching error (RMS) is 0.0014.
Matches:

Library	Sim Index	Entry Name
IBA1 1.30	0.999	MIDI-Calibration Mix 1

Figure 3-2B.
Calibration Chromatogram Example



Instant FAME Start Up Kit

- Aerobic Environmental Library (ITSA1)
- Aerobic Clinical Library (IBA1)
- Aerobic Water Isolate Library (IR2A1)
- Mycobacteria Library (IMYC1)
- Yeast Library (ITY1)
- 3 Liquid Dispensers (manufacturer and model varies)
- Vortex mixer (one of the following depending kit version and voltage):
  - Fisher MINIROTO, S56, 115V
  - Fisher MINIROTO, S56, 230V
- 2 vial rack trays
- Eppendorf Reference Pipette
- 4 MIDI Wand pipette aids
- 1-192 sample Instant FAME Refill Kit

Instant FAME Refill Kit (400 samples)

- Shipped in Refill Kit Box 1
  - 400 Conical inserts
  - 800 Clear 2ml GC Vials
  - 800 Restek PTFE/Silicon/PTFE Caps
  - 420 1µl Sterile Innoculating Loops
  - 480 Eppendorf Reference Pipette tips
  - 2 Injection Port Liners
- Shipped in Refill Kit Box 2
  - 1 Box of Rapid Calibration Mix Part #1300-AA
  - 3 Instant FAME Extraction Reagents
  - 2 Cleaning/Wash reagents (Methanol/Hexane)

Instant FAME Refill Kit (192 samples)

- Shipped in Refill Kit Box 1
  - 200 Conical Inserts
  - 400 Clear 2ml GC Vials
  - 400 Restek PTFE/Silicon/PTFE Caps
  - 200 1µl Sterile Innoculating Loops
  - 192 Eppendorf Reference Pipette tips
  - 1 Injection Port Liner
- Shipped in Refill Kit Box 2
  - 1 Box of Rapid Calibration Mix Part #1300-AA
  - 3 Instant FAME Extraction Reagents
  - 2 Cleaning/Wash reagents (Methanol/Hexane)

Customer Required Items

Visit www.midi-inc.com for latest vendors and part numbers.

- Sherlock® Microbial Identification System version 6.0 or higher
- Agilent 6850 GC, 6890 single/dual GC or 7890 single GC
- 30°C ± 2°C Incubator for Environmental Bacteria, Water Bacteria, and Yeast libraries
- 35°C ± 2°C Incubator for Clinical Bacteria and Mycobacteria library
- **Media for Clinical Bacteria Library (IBA1)**
  - BBL Brand Trypticase Soy Agar with 5% Defibrinated sheep blood – prepared plates (BD Part #: 221261)
  - BBL BCYE Agar – prepared plates (BD Part # 221808)
  - BBL Chocolate Agar – prepared plates (BD Part # 221860)
  - BBL Campylobacter CampyPak Plus (BD Part # 260685)
  - Lactobacilli MRS broth (BD Part # 288130)
- **Media for Environmental Bacteria Library (ITSA1)**
  - BBL Brand Trypticase Soy Agar – prepared plates (BD Part #: 221283)
- **Media for Water Bacteria Library (IR2A1)**
  - Difco Brand R2A Agar - dehydrated media (BD Part # 218263)
- **Media for Yeast Library (ITY1)**
  - BBL Brand Trypticase Soy Agar – prepared plates (BD Part #: 221283)
- **Media for Mycobacteria Library (IMYC1)**

    - BD BBL Middlebrook and Cohn 7H10 Agar (BD Part#: 221174, prepared plates)
- Petri Dishes 100mmX15mm (for R2A plates)
- Pasteur Pipettes, 5 inch, disposable
- Chemical storage cabinets for corrosives and flammables
- Hazardous chemical waste disposal
- Hazardous medical waste disposal

IMPORTANT: If Powdered Media is acquired from another vendor to make plates, it must be an exact match to the prepared plates specified above. Differences can produce unexpected results.

Appendix B – Extraction Log Sheet

Extracted By: **Data Entered By:**

Name: _____ Name: _____

Date / Time: _____ Date / Time: _____

Tube #	Sample ID	Pos #	Method	GC #	Comments/ Non-Standard Conditions
1	QC-BLANK				
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					

Lot # Exp. Date	Comments
Reagent 1: _____ _____	
Reagent 2: _____ _____	
Reagent 3: _____ _____	
Initial/Date: _____	

Verification

Plate Order Review: _____ ___/___/___	Data Entry Review: _____ ___/___/___
Vial Position Check: _____ ___/___/___	Method / Cal Mix Check: _____ ___/___/___