Sherlock™ Microbial Identification System with *E-FAME Methods*

General Description

The Sherlock Microbial Identification System identifies bacteria and yeast by gas chromatographic (GC) analysis of fatty acid methyl esters (FAMEs). The Sherlock software, methods and libraries are combined with an Agilent® Technologies 6850 or 7890 GC and Agilent ChemStation software for a complete, automated microbial identification solution.

Sherlock's pattern recognition algorithms, combined with its calibration mixture, standardize each instrument. This virtually eliminates the manual calibration adjustments associated with a GC. No chromatography knowledge or experience is required.

Sherlock E-FAME Libraries

The optional E-FAME sample preparation method enables a user to identify a microbe from pure culture in less than 20 minutes. The following libraries are available and include USP <61> and USP <62> strains:

Environmental Aerobes (ETSA)
240 species-entries. TSA agar at 30ºC ± 2ºC for 24 ± 2 hours.

Clinical Aerobes (EBA)
267 species-entries. TSA with 5% defibrinated sheep blood agar at 35ºC ± 2ºC for 24 ± 2 hours.

Other Species – these entries are included in the libraries above and have specific growth conditions:
- *Aspergillus niger*
- *Candida albicans*
- *Clostridium sporogenes*

Low Cost Per Sample

All reagents and consumables used to prepare a sample (except culture media) are included in the E-FAME Startup and Refill kits. The cost per sample with the E-FAME kits is around $6.00 USD (not including culture media). This includes shipping costs.

Instrument Throughput

Following the E-FAME sample preparation (typically done in batches), the GC vials are loaded into the instrument's autosampler. The automated system takes over and quickly analyzes each sample. No additional incubation is needed at this point.

E-FAME methods process 6 samples per hour on a 6850 or single channel 7890 Series GC.

Culturing

Like all widely used confirmatory techniques, Sherlock requires pure microbial cultures. Using standard laboratory techniques, a single subculture from the primary isolation plate, incubated for 24 hours, is typically sufficient for performing the analysis. Slow growing organisms typically require 48-hour incubation times.

Sample Preparation

The E-FAME kits contain all the consumables and reagents needed to process samples (except culture media). A technician averages less than 3 minutes per sample to prepare a batch of 10 samples for analysis from pure culture. The sample preparation involves:

- Harvest a small quantity of cells (overflowing loopful, ~ 5 mg) from the pure culture.
- The 3-step liquid-liquid extraction process and heat block step require less than 3 minutes to complete per sample.
- It is not necessary to do a Gram stain or other offline tests since all aerobic bacteria are prepared the same way.
- The amount of reagents used per sample is less than 1 ml.

Hardware

A Sherlock system is composed of a Windows® 7 Professional or Windows® XP Professional-based computer loaded with the MIDI Sherlock and Agilent ChemStation software. The computer is interfaced to one of the following Agilent GCs, configured to MIDI's specifications:

Agilent 6850 Series GC
- 57cm x 28cm x 49cm (L x W x H)
- Weight: 29kg
- Operating temp: 15ºC to 35ºC
- Operating humidity: 5% to 95%

Agilent 7890 Series GC – Single
- 51cm x 58cm x 49cm (L x W x H)
- Weight: 49kg
- Operating temp: 15ºC to 35ºC
- Operating humidity: 5% to 95%

Agilent 6850 Series II GC with 27 Vial Tray

MIDI, Inc.
Newark, Delaware

Analysis Software

Analysis software enables a user to explore relationships between sample data using:

- Dendrogram plots
- Neighbor-joining trees
- Principal component analysis (PCA) with 2-D plots and histograms

The graphics can be exported to Microsoft Office® and other packages for further analysis and for research publications.

Data Export Software

Data Export software enables a user to export sample data, fatty acid profiles, library match results and other information to Excel® spreadsheets and Access® databases. There are many applications for custom reports and calculations available using Excel, Access, and other data analysis tools:

- Trend analysis
- Custom reports
- Summary reports for sample sets
- Microbe population studies
- Research and publications
- Data mining

Library Generation Software

Optional Library Generation software enables a user to create custom libraries from any sample data. Uses for *Library Generation* include:

- Quality control of proprietary strains used in production processes
- Quickly recognize contaminants that reoccur in a facility or process
- Assign an identity to organisms that do not have a published taxonomy
- Catalog culture collections
- Alternative growth conditions
- Research

Tracker/Cluster Software

Optional Tracker/Cluster software enables a user to locate the source of a contamination. *Tracker* and *Cluster* operate independently of sample identification, allowing unknown samples to be compared.

Tracker locates other samples that are likely to be the same strain as a sample of interest. *Tracker* searches for matches between the current sample and all previous samples.

Cluster automatically finds groups (clusters) of highly related samples. Relationships between clusters and samples can be explored using:

- Dendrogram plots
- 2-D Color-coded PCA plots

Uses for *Tracker/Cluster* include:

- Trend analysis
- Summary reports for sample sets
- Microbe population studies
- Research and publications
- Data mining

Electronic Records and Signatures Software

Optional Electronic Records and Signatures software enables a user to comply with U.S. FDA regulation 21CFR Part 11.

- Provides access control based on Windows user passwords and group settings to authenticate users and determine their privileges.
- When configured with a Windows domain, users can be authenticated using their domain credentials.
- Sherlock requires the user to logon before granting access to controls and data.
- Packages all associated data, audit trails, logs and results in a secure electronic vault for storage on the local disk or on a remote file server.
- Supports two levels of electronic signature authority with notes added by the signer.
- Automatic inactivity logoffs.

- Supports security policies defined for the Windows system or domain, including lockout of accounts after a predetermined number of login failures.

Sherlock DNA Software

This optional software enables users to identify and analyze microbial DNA sequence data from over 2,500 species of bacteria, yeast and fungi.

Sherlock DNA is able to import DNA sequence data from any DNA sequencer manufacturer and comes with 16S rRNA gene sequence libraries for bacterial identification and 28S rRNA libraries for fungi/yeast identification.

Polyphasic ID
The Sherlock software has the capability of reporting an identification based on both DNA and FAME analyses, yielding a polyphasic report. Because the Sherlock DNA and FAME libraries have been taxonomically harmonized, results are very easy to understand. Further, Sherlock will store the DNA sequence directly with the associated FAME sample.

Markets Using Sherlock

- Animal Science
- Biodefense / Public Health
- Bioremediation
- Clinical Microbiology
- Edible Oil Analysis
- Marine Science
- Pharmaceutical QC
- Plant Pathology / Protection
- Renewable Energy
- Soil Science / PLFA
- Water Testing
- Taxonomy Studies