Sherlock® Microbial Identification System with *Traditional Methods & PLFA*

Specification Sheet

General Description

The Sherlock® Microbial Identification System (MIS) identifies fatty acid methyl esters (FAMEs) by gas chromatography. The resulting FAME profile can be used to identify the fatty acids in the sample (e.g. PLFA), or the resulting profile can be compared to one of Sherlock's microbial libraries (e.g. environmental bacteria).

For a complete automated microbial identification and fatty acid analysis solution, the Sherlock software, methods and libraries are combined with an Agilent 6850 or 7890 GC and Agilent ChemStation software.

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.

Libraries & Methods

The major Sherlock methods and libraries include:

Phospholipid Fatty Acids (PLFAD1)
165 fatty acids and related compounds. Can be used with any extraction method. Includes PLFA Tools package for automated calculation of user-defined factors.

Environmental Aerobes (TSBA)
686 species. Culturing media is TSBA and incubation conditions are 28°C ± 2°C for 24 ± 2 hours.

Clinical Aerobes (CLIN)
436 species. Culturing media is TSA with 5% defibrinated sheep blood agar and incubation conditions are 35°C ± 2°C for 24 ± 2 hours.

Anaerobes (MOORE)
Two anaerobe libraries are available. One is for BHIBLA plate-grown anaerobes (135 species). The other is for PYG broth-grown anaerobes (590 species).

Yeast (YEAST)
190 species. Culturing media is SDA and incubation conditions are 28°C ± 2°C for 24 ± 2 hours.

Low Costs Per Sample

It costs under $3.00 USD per sample for all consumables. This includes reagents, gases, calibration standards, glassware, and culture media.

Instrument Throughput

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

- **Standard** methods process 2 samples per hour on a 6850 or single channel 7890 GC.
- **Rapid** methods (Aerobes only) process 6 samples per hour on a 6850 or single channel 7890 GC. *Rapid* methods have 2 times the detection sensitivity of the *Standard* methods
- **Sensitive** methods for Anaerobes and Yeast process 2 samples per hour on a 6850 or single channel 7890 GC. *Sensitive* methods have 2 times the detection sensitivity of the *Standard* methods, and use the same calibration standard as *Rapid* methods.

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 and anaerobes typically require 48 hour incubation times.

Sample Preparation

Using inexpensive reagents, available from almost any chemical supply house, a technician averages only 5 minutes per sample to prepare a batch of 30 samples. Each sample is prepared for analysis using a liquid-liquid extraction in a single test tube.

- Harvesting a small quantity of cells from the culture plate is the most labor-intensive step. It will typically take 1 hour or less to harvest cells from 30 plates into 30 test tubes.
- The four-step liquid-liquid extraction process requires about 1½ hours or less for a batch of 30 samples. During the extraction process, approximately 35 minutes of "wait time" are available for the technician to do paper work and other tasks.
- The same sample preparation is used on all samples. It is not necessary to do a Gram stain or other offline tests before preparing and analyzing a sample.

Bio-Safety

Bio-Safety is enhanced because live organisms are not introduced into the instrument. The first step of the extraction procedure treats the cells with a sodium hydroxide solution for 30 minutes in a 100°C water bath. After the first step, the technician is no longer working with live organisms.

Laboratories that handle dangerous pathogens will typically perform the sample extraction in a BSL-3 lab and transfer decontaminated extracts to a non BSL-3 lab for instrument analysis. This allows the instrument to be maintained and serviced by technicians outside the BSL-3 lab.

Hardware

A Sherlock system is composed of a Windows® based computer loaded with the MIDI Sherlock and Agilent ChemStation software. The computer is interfaced to one of the following Agilent GCs:

Agilent 6850 Series II 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%

Analysis Software

This 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

This 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 created 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

This optional 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

This optional 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.

Uses for *Tracker/Cluster* include:

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

PLFA Analysis Tools

This software enables a user to automatically perform complex calculations, including the following:

- Adjusting for the molarity of different fatty acid compounds
- Scaling by a known amount of internal standard
- Categorizing results based on Fatty Acid types (e. g. iso/anteiso, omega)
- Categorizing results based on microbial types (e. g. gram positive bacteria, fungi)
- Calculate the Iodine Value for a sample

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.

Markets Using Sherlock

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

The information in this publication is subject to change without notice.

Date: 01 October 2014

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