The Sherlock® Mycobacteria Identification System

Mycolic acid analysis using High Performance Liquid Chromatography (HPLC) is being used by many laboratories for the diagnosis of tuberculosis and other mycobacterial infections.

In one simple step, Sherlock® analyzes mycolic acids in a bacterial sample, searches its library of reference mycobacteria using pattern recognition software, and prints a detailed sample report.

All this for under $5.00 per sample.

- **Automated analysis and identification of *M. tuberculosis***
- **Distinguishes *M. bovis* BCG from tuberculosis group**
- **No HPLC experience required**
- **Agilent 1100 HPLC-reliable, no sample carryover, better chromatography**
- **Automatic sample re-injection if the concentration is too high or too low**
- **Classic visual confirmation**

MIDI

Sherlock® Microbial Identification System

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Sherlock Mycobacteria Identification System

The Sherlock Mycobacteria Identification System (SMIS), developed and marketed by MIDI, Inc., Newark, DE, USA analyzes and identifies microorganisms isolated in pure culture on a solid growth medium. Sherlock uses a sample preparation procedure and high performance liquid chromatography (HPLC) to yield qualitatively and quantitatively reproducible mycolic acid composition profiles. This sophisticated chromatographic system has been developed to be used by microbiologists; thus, chromatographic experience is not essential for operation. The Sherlock software calibrates and monitors the system to ensure proper functioning.

Mycolic acids extracted from unknown mycobacteria are automatically quantified and identified by the Sherlock software to determine the mycolic acid composition. The mycolic acid profile is then compared to a "library" (of profiles of reference strains of mycobacteria) stored in the computer to determine the identity of the unknown. Unknown samples may be identified to the species or to the species-complex group. The species-complex will typically be organisms that have high DNA similarities and/or are similar in clinical significance. Examples are the *Mycobacterium avium/intracellulare* and *M. tuberculosis/bovis/africanum* complexes.

Agilent Technologies HPLC

The Sherlock Microbial Identification Software can be used only with the Agilent Technologies model 1100 HPLC. A computer with Agilent Technologies ChemStation software (Version A.06.03 or above) installed is also required.

Environmental Considerations

The chromatographic unit will operate within temperatures of 4-40ºC (39-104ºF) and < 95% relative humidity; however, an environment comfortable for human habitation (reasonably constant temperature and humidity conditions) is recommended for optimum performance and instrument lifetime.

Computer and Software

Sherlock runs on an IBM-compatible personal computer. The computer should be considered to be a dedicated instrument controller. MIDI only guarantees support of Sherlock software loaded on Hewlett-Packard or Dell computer equipment.

Minimum requirements:

- Hewlett-Packard or Dell 96 Mb RAM minimum, 8Gb hard drive, 300 MHz Pentium II
- Windows NT or Windows 2000
- Agilent Technologies A.06.x or higher ChemStation Software
- CD-ROM drive and 3.5" floppy drive
- 1024x768 VGA Color Display
- LAN or GPIB Interface Board
- Mouse
- Dedicated printer

Intended Use

The MIDI Sherlock® Mycobacteria Identification System is intended to aid in the identification of *M. tuberculosis* and differentiation from other mycobacteria species through the analysis of mycolic acids derived from cultured bacterial samples, using high performance liquid chromatography (HPLC) performed on the Agilent model 1100 HPLC, along with Sherlock® pattern recognition software.

The system is used, along with other identification methods, to identify mycobacteria that have been isolated from clinical specimens by traditional culturing techniques. Following observation of growth on the solid medium, identification of the mycobacterial isolate is done with the MIDI Sherlock® Mycobacteria Identification System device. Results should be interpreted in conjunction with other laboratory observations and procedures.

Limitations

Sherlock can identify only those microorganisms for which mycolic acid composition profiles of a representative number of correctly named reference strains have been determined and entered into the Mycobacteria Library. 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.

The Sherlock® Mycobacteria Identification System has been shown to reliably differentiate the *M. tuberculosis* complex from other mycobacteria. Definitive identification of Mycobacterium other than tuberculosis (MOTT) requires use of additional laboratory testing to identify clinically significant organisms. Sherlock may be useful in conjunction with probe hybridization, biochemical testing, and other laboratory observations. The ability of Sherlock® to correctly identify MOTT not listed in the database has not been evaluated.

Overview of Sherlock Operation

The Sherlock system is completely controlled by the computer. After sample extracts have been prepared , 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 sequence can be started. A sequence is a run of one or more sample extracts on the HPLC. When a sequence is started from the keyboard, the following occurs:

- The sample's Method (instrument setpoints, calibration instructions, and sample tray location) is downloaded to a ChemStation file. ChemStation sets the HPLC parameters and controls the injection by the automatic liquid sampler.

- The automatic sampler selects the sample bottle for the calibration mix. The sampler controller causes the injection of a small portion of the extract into the solvent flow stream of the HPLC.

- The C18 (reverse phase) column installed in the HPLC column-compartment separates the mycolic acids present in the mix as they pass through the column to the detector. The mycolic acid sample preparation converts the mycolics into fluorescent derivatives. As the compounds pass through the fluorescence detector, the fluorescent tags are excited by 345-nm wavelength light and this causes emission of light at 425- nm that is quantitatively detected by a photo-multiplier tube in the detector. The amount of light emitted is related to the concentration of the tagged compound and the time of elution is related to the chromatographic properties (structure) of the compound. This signal is stored in the ChemStation data file. The plotted and integrated signals, called the chromatogram, are printed by ChemStation.

- When the run is complete, the retention time, peak width and response of each peak are transmitted from ChemStation to Sherlock for processing. Peaks in the chromatogram are identified by mycolic acid Equivalent Carbon Length (ECL) value (name).

- When peak naming is complete, Sherlock searches the library to identify the unknown organism.

The library search uses both the peak name and the peak amount to match with known profiles stored in the library. Following the library search, the computer prints the Composition Report, which includes the peak naming and library classification results. Each library entry is a computer-generated composite of the reference strains of each species or subspecies group of organisms, taking into consideration strain-to-strain and experimental variability. Reference strains were cultured and processed under carefully controlled conditions.

The computer system automatically sets the operating parameters of the chromatographic unit. There is no need to manually enter the HPLC parameters. The system will automatically recalibrate within a preset interval. You are permitted to enter sample information into the Sample Table while samples are being processed. This allows for continuous operation of your system.

Sherlock® Mycobacteria Identification System

Fact Sheet

Automated analysis and identification of *Mycobacterium tuberculosis*

Reliable Agilent 1100 HPLC

Cost effective analysis; under $5 per sample, environmentally friendly solvents

Intuitive and easy user operation

Fluorescence detector 1000x more sensitive than UV detector; minimal cell mass required for identification

MIDI's Sherlock® Mycobacteria Identification System has received FDA 510(k) clearance for sale in the United States.

Description

The Sherlock Mycobacteria Identification System analyzes and identifies *M. tuberculosis* isolated in pure culture on a solid growth medium. In addition, the system is used, along with other identification methods, to identify mycobacteria that have been isolated from clinical specimens by traditional culturing techniques. Sherlock uses a prepared sample and high performance liquid chromatography (HPLC) to yield qualitatively and quantitatively reproducible mycolic acid composition profiles. The mycolic acid profile is then compared to a "library" stored in the computer to determine the identity of the unknown.

Instrumentation

The Sherlock Mycobacteria Identification System is composed of a Windows® 2000-based computer loaded with the MIDI Sherlock software and an Agilent ChemStation. The computer is interfaced with an Agilent 1100 HPLC. The Agilent HPLC has several unique features that ensure high quality analysis. The Agilent needle and autosampler are in the flow path, resulting in no carryover and fewer misidentifications resulting from carryover.

Sherlock Operation

After harvesting the mycobacteria, the mycolic acids are saponified, extracted, derivatized and then loaded into the HPLC autosampler for analysis. Information about each sample is logged into the Sample Table and the sequence is run. The computer system automatically sets the operating parameters of the HPLC. The system automatically recalibrates within a preset interval and makes adjustments for variances in sample concentration.

When the run is complete, the retention time, peak width and percentages of each peak are transmitted from ChemStation to Sherlock for processing. When peak naming is complete, Sherlock searches the library to identify the unknown organisms. Each library entry is a computer-generated composite of reference strains for the species. Finally, the computer prints the Composition Report, which includes the peak naming and library classification results. A reference comparison chromatogram is also generated with each analysis for classic visual confirmation of results.

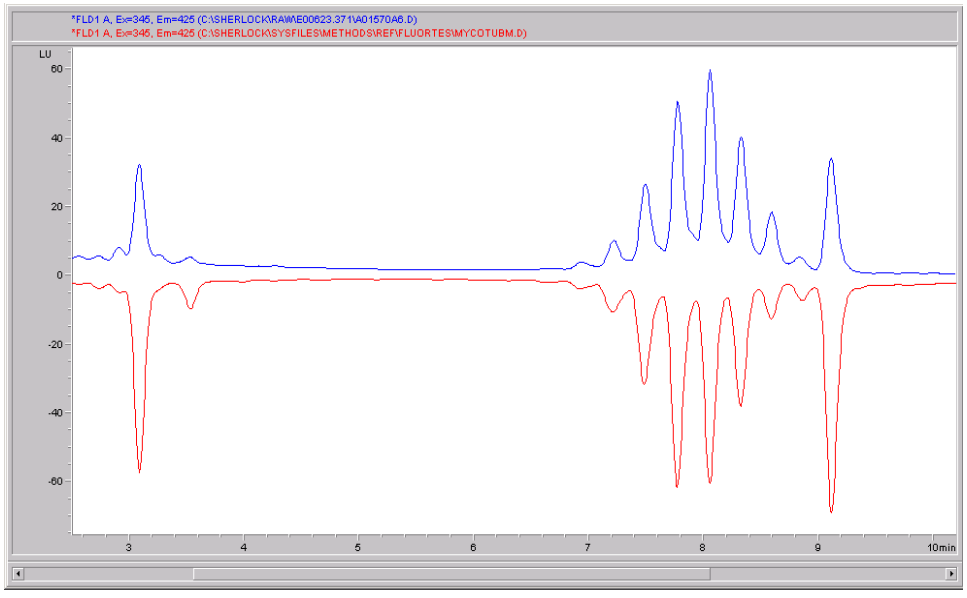

Figure 1- Visual Confirmation- the user can perform a visual comparison of the sample HPLC chromatogram to a known "reference" chromatogram. The reference chromatogram (red) is printed as the mirror image below the sample chromatogram (blue).

Mycobacterium-abscessus/chelonae
Mycobacterium-asiaticum
Mycobacterium-aurum/vaccae
Mycobacterium-bovis BCG (not 35737)
Mycobacterium-celatum
Mycobacterium-chelonae/abscessus
Mycobacterium-flavescens I
Mycobacterium-flavescens II
Mycobacterium-fortuitum/peregrinum
Mycobacterium-gordonae I
Mycobacterium-gordonae II
Mycobacterium-haemophilum I(30C, chocolate)
Mycobacterium-haemophilum II(30C, chocolate)
Mycobacterium-haemophilum III (30C, chocolate)
Mycobacterium-interjectum
Mycobacterium-intermedium
Mycobacterium-kansasii
Mycobacterium-lentiflavum/triplex
Mycobacterium-MAC A (avium/intracellulare)
Mycobacterium-MAC B(avium/intracellulare)
Mycobacterium-MAC C (intracellulare/avium)
Mycobacterium-MAIS complex(scrofulaceum/avium/intracellulare)
Mycobacterium-malmoense
Mycobacterium-marinum(30C)
Mycobacterium-mucogenicum I
Mycobacterium-mucogenicum II
Mycobacterium-neoaurum
Mycobacterium-nonchromogenicum/terrae
Mycobacterium-peregrinum I/fortuitum
Mycobacterium-peregrinum II/fortuitum
Mycobacterium-simiae
Mycobacterium-szulgai I
Mycobacterium-szulgai II
Mycobacterium-terrae/nonchromogenicum I
Mycobacterium-terrae/nonchromogenicum II
Mycobacterium-thermoresistible
Mycobacterium-triviale
Mycobacterium-tuberculosis complex(TB,bovis,africanum,microti)
Mycobacterium-xenopi
Mycobacterium-xenopi II

Sherlock® Mycobacteria Identification System

Specification Sheet

General Description

The Sherlock® Mycobacteria Identification System (SMIS) was developed and is marketed by MIDI, Inc., Newark, DE, USA. The system is FDA 510(k) cleared and is intended to aid in the identification of *M. tuberculosis* and differentiation from other mycobacteria species through the analysis of mycolic acids derived from cultured bacterial samples.

Sherlock uses a sample preparation procedure and high performance liquid chromatography (HPLC) to yield qualitatively and quantitatively reproducible mycolic acid composition profiles. Automated pattern recognition software matches the sample's composition with stored patterns of mycobacteria species and groups. The Sherlock software also calibrates and monitors the system to ensure proper functioning.

The Sherlock Mycobacteria Identification System is designed for ease of use. As a result, no HPLC experience is required for operation.

Technology

Mycolic acid analysis by HPLC is being used by laboratories for diagnosis of tuberculosis and other mycobacterial infections. The technique was developed by the U.S. Centers for Disease Control, working with the "HPLC Users Group."

The CDC technique was used as the starting point for the Sherlock Mycobacteria Identification System. The extraction protocol was adapted from the methodology established at the Texas Department of Health, Bureau of Laboratories, Mycobacteriology/ Mycology Branch.

Mycobacteria Library

The Sherlock Mycobacteria Library contains 40 entries, created from well-characterized mycobacterial strains.

The Sherlock Mycobacteria Identification System has been shown to reliably differentiate the *M. tuberculosis* complex from other mycobacteria. Definitive identification of Mycobacterium Other Than Tuberculosis (MOTT) requires use of additional laboratory testing to identify clinically significant organisms. The Sherlock Mycobacteria Identification System may be useful in conjunction with probe hybridization, biochemical testing, or other laboratory observations.

Low Per Sample Costs

It costs under $5.00 per sample for all consumables. This includes reagents, internal standards, calibration standards, glassware and culture media.

Instrument Throughput

Following a short preparation procedure (typically done in batches), the samples are loaded into the instrument's autosampler. The automated system takes over and analyzes each sample.

- The Sherlock system can analyze up to 4 samples per hour.

Sample Preparation

Each sample is prepared for analysis using a liquid-liquid extraction.

- Harvesting a small quantity of cells from the culture plate is the most labor-intensive step. It will typically take less than 30 minutes to harvest cells from 30 plates into 30 test tubes.

- The four-step sample preparation process requires about 1.5 to 2.0 hours for a batch of 30 samples. During the extraction process, approximately 45 minutes hour of "wait time" are available for the technician to do paper work and other tasks.

Culturing

Sherlock requires pure cultures. The mycobacteria cultures typically are grown on solid medium such as Middlebrook 7H10 (or 7H11) at 35-37C, until visible growth is noted. Sensitive fluorescent detection often allows for analysis from a single colony. Some species of mycobacteria may normally be grown under different conditions and the database has been constructed to reflect this. Examples would be the culturing of *M. marinum* and *M. haemophilum* at 30C, with the latter organism being grown on chocolate agar rather than on Middlebrook.

Bio-Safety

Warning: Observe Biosafety Level III practices while working with viable cultures. An approved biological safety cabinet must be used!

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

With the MIDI System, Biosafety is enhanced because live organisms are not introduced into the instrument. The first steps of the sample procedure kill the cells (addition of potassium hydroxide solution followed by autoclaving for 30 minutes). After this, the technician is no longer working with live organisms.

The MIDI protocol uses a benign mixture of methanol and ispopropanol as its solvent system. This eliminates potential deleterious health effects or environmental contamination that might result from exposure to or disposal of toxic solvents (such as methylene chloride), which are often used in comparable HPLC separations.

Instrumentation

The Sherlock Mycobacteria Identification System is composed of a Windows® 2000-based computer loaded with the MIDI Sherlock software and an Agilent ChemStation (Version A.06.03 or above). The computer is interfaced with an Agilent 1100 HPLC.

Many of the hardware components of the MIDI System are designed to meet specifications required for robust and reproducible performance. The quaternary pump employs low pressure mixing to maintain a constant rate of solvent delivery. The autosampler, with flow-through design, eliminates carry over contamination and the Peltier temperature control is capable of maintaining column temperatures to within 0.1C. A long-life chromatographic column is able to provide consistent performance over thousands of runs.

In addition, the MIDI system employs fluorescence detection, which enhances sensitivity ca. 1000-fold compared with UV detection.

Markets Using Sherlock

- Diagnostic Laboratories
- Major Hospitals
- Public Health Departments

Sherlock Mycobacteria Identification System

www.midi-inc.com
The information in this publication is subject to change without notice.

MIDI & MIDI Authorized distributors are the sole source for the Sherlock Mycobacteria Identification System.

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