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IDENTIFICATION OF MICROORGANISMS USING FATTY ACID METHYL ESTER (FAME) ANALYSIS AND THE MIDI SHERLOCK® MICROBIAL IDENTIFICATION SYSTEM

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INTRODUCTION

For more than 15 years, a substantial portion of the pharmaceutical industry has relied on the MIDI Sherlock® Microbial Identification System for identification in their microbiological testing laboratories. The Sherlock System identifies microorganisms based on gas chromatographic (GC) analysis of extracted microbial fatty acid methyl esters (FAMEs). Microbial fatty acid profiles are unique from one species to another, and this has allowed for the creation of very large microbial libraries. The current Sherlock System libraries have over 1,500 bacterial species, along with 200 species of yeast. A combination of features makes the system attractive for use in pharmaceutical quality

control (QC) environments. These features include, but are not limited to: accurate identifications, large environmental libraries, the ability to perform presumptive "strain tracking" (for finding the source of a contaminant), high throughput, and a low cost per sample for consumables.

BACKGROUND: DIFFERENT STRENGTHS IN DIFFERENT TECHNOLOGIES

The three major techniques for identification of pharmaceutical QC bacteria are biochemical tests, fatty acid profiling, and DNA sequencing. Each technique has its strong points and weaknesses. The following comments lay the basis for comparison of the fatty acid-based MIDI Sherlock Microbial Identification System.

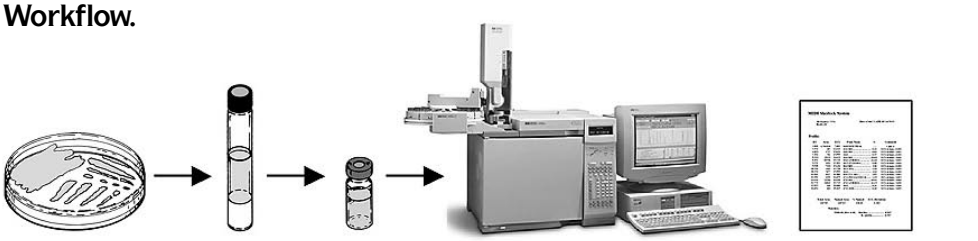
Biochemical test-based identification systems are familiar to most microbiologists and require little training to operate. Systems range from strip cards for specific groups of bacteria (e.g., for coryneforms, *Bacillus*, enterics, etc.) to large plate arrays that may be automatically scanned for changes due to pH shifts or redox reactions. The strength of identification in enterics is generally quite good and the ease of use and cost per sample for identification is considerably less than for DNA sequencing, but higher than for FAME analysis (Cook 2003; O'Hara 2005). The use of these systems depends on choice of the correct "card" or "strip" of wells of reagents. This is typically done using information such as that gained from the Gram stain (a prerequisite step not involved in the other two major technologies). One problem with most biochemical test systems, however, is that these systems are geared to the clinical market, and as a result, are limited in the number of environmental species they can identify.

DNA-based technology for the identification of bacteria typically uses only the 16S rRNA gene as the basis for identification. This technique has the advantage of being able to identify difficult-to-cultivate strains, and is growth and operator independent. As the 16S rRNA gene is highly conserved at the species level, speciation is commonly quite good, but as a result, subspecies and strain level differences are not shown. Some problems with the 16S rRNA technology are that it requires a high level of technical proficiency, and the costs per sample, as well as equipment costs are high. As a result, the technology is not well suited for routine microbial QC, but rather is best used for direct product failures (Sutton 2004). Technology that uses information from both the 16S rRNA and 23S rRNA genes is also used in pharmaceutical QC, but primarily to aid in strain tracking.

The MIDI Sherlock System identifies all of the aerobic bacteria in its library using a standard sample preparation technique (Figure 1), so there is no need for upfront biochemical tests or a Gram stain to help decide which card or test strip to use. Environmental bacteria are grown on commonly used medium at 28°C for 24 hours. Bacteria are harvested from a quadrant of the streak that will most closely approximate the log stage growth and provide adequate cells for analysis. Some of the species that are discriminated well using FAME analysis include those of *Bacillus*, *Pseudomonas*, Gram-positive cocci and rods (such as coryneforms), Gram-negative non-fermenters (such as *Acinetobacter*), and unusual environmental organisms found in pharmaceutical facilities. The Sherlock System has the unique ability to perform strain tracking with known or unknown isolates. Because of the low technical proficiency required to operate the system, consumable costs of less than $3.00 per sample, and throughput of 200 samples per day, the Sherlock System lends itself easily to routine microbial QC.

The National Institute for Occupational Safety and Health (NIOSH) has validated the MIDI Sherlock System for the identification of aerobic bacteria (Pendergrass 1998). NIOSH is part of the Centers for Disease Control and Prevention and is the federal agency responsible for conducting research and making recommendations for the prevention of work-related illness. Another publication of general significance is "Identifying bacterial contaminants in a pharmaceutical manufacturing facility by gas chromatographic fatty acid analysis" (Olsen 1990). Additional detail on operation of the system can be found in MIDI Technical Note #101 (Sasser 2001).

Figure 1. MIDI's Fatty Acid-based Microbial Identification System Workflow.

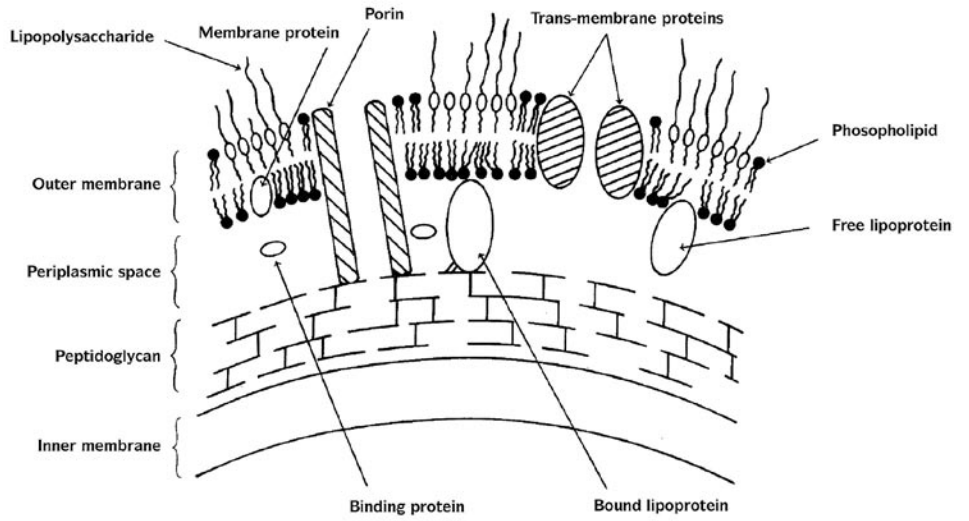


HOW FAME ANALYSIS WORKS FOR IDENTIFICATION OF BACTERIA

More than 300 fatty acids and related compounds are found in bacteria. The wealth of information contained in these compounds is both in the qualitative differences (usually at genus level) and quantitative differences (commonly at species level). As the biochemical pathways for creating fatty acids are known, various relationships can be established. Thus 16:0 → 16:1 through action of a desaturase enzyme and is a mole-for-mole conversion. Following this, as the bacterial cell becomes physiologically mature, the shift of 16:1 → 17:0 cyclopropane is again a mole-for-mole conversion. This information suggests that use of the cells in an actively growing stage minimizes the differences between cultures. Use of a 24 ± 2 hour culture and harvesting from a rapidly growing quadrant of a quadrant streak plate reduces the differences. Additionally, a covariance matrix is used in the Sherlock software to minimize the impact of these changes.

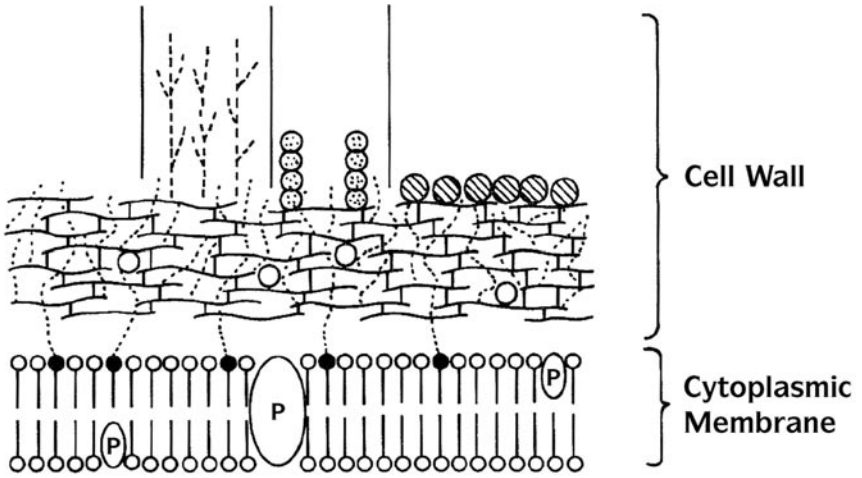
Controlled growth temperature and use of standardized commercially available media also contribute to the reproducibility of the fatty acid profile.

Figure 2. Gram-negative Bacterial Membrane (Ratledge 1988).



Branched chain fatty acids (iso and anteiso acids) are common in many Gram-positive bacteria, while Gram-negative bacteria are composed of predominately straight chain fatty acids. The presence of lipopolysaccharide (LPS) in Gram-negative bacteria gives rise to the presence of hydroxy fatty acids in those genera (Figure 2). Thus, the presence of 10:0 3OH, 12:0 3OH, and/or 14:0 3OH fatty acids indicates that the organism is Gram-negative and conversely, the absence of the LPS and hydroxy fatty acids indicates that the organism is Gram-positive (Figure 3). As a result, it is not necessary to perform the traditional Gram stain prior to FAME analysis.

Figure 3. Gram-positive Bacterial Membrane (Ratledge 1988).



How do plasmid gain or loss and mutations affect fatty acid profiles? In an unpublished work, an author of this chapter (Sasser) found that removal of plasmids from several pseudomonads (by treatment with ethidium bromide) did not change the fatty acid profile. Similarly, insertion of plasmids from other species of pseudomonads did not significantly change the profiles. As bacteria frequently exchange plasmids, the system would not work well if such changes did cause alterations in the fatty acid composition. Similarly, treatment with ultraviolet light (a frame-shift mutagen) or point-mutagens such as nitrosoguanidine and ethyl methanesulfonate at levels that kill 99.999% of the cells and create large numbers of auxotrophic and/or motility mutants did not affect the fatty acid profile, as long as the growth rate was relatively normal. This suggests that the fatty acid composition is highly conserved genetically and that significant changes take place only over considerable periods of time. As a result, the same genus and species of bacteria from

anywhere in the world will have highly similar fatty acid profiles as long as the ecological niche is similar. The adaptation to different ecological niches over long periods of time provides information vital to strain tracking by fatty acid profiling. To allow for this variance, the Sherlock libraries are composed of data from tens of thousands of strains obtained from widely varying sources and ecological niches.

The largest genus entry in the Sherlock libraries belongs to the genus *Bacillus* (42 unique species). Fatty acid profiles are quite unique for *B. anthracis*, compared with other *Bacillus* species (Kim 2003; Song 2000). This fact enabled the Sherlock System to pass a November 2004 nationwide study, designed to test the ability of the system to correctly identify *B. anthracis* and distinguish it from other members of the genus. As a result of the study, the MIDI Sherlock System was the only method to be granted AOAC® *Official Methods*℠ status for the confirmation of *B. anthracis* (AOAC Method #2004.11). The study was conducted by AOAC INTERNATIONAL and was sponsored by the Department of Homeland Security. Most recently in January 2006, the MIDI Sherlock System received FDA 510(k) clearance for confirmation of *B. anthracis.*

FAME Analysis Procedure

The Sherlock System requires that bacteria be grown in culture. The fatty acids are extracted by a procedure which consists of saponification in dilute sodium hydroxide/methanol solution followed by derivatization with dilute hydrochloric acid/methanol solution to give the respective methyl esters (FAMEs). The FAMEs are then extracted from the aqueous phase by the use of an organic solvent and the resulting extract is analyzed by GC (Table 1).

Table 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

As the bacteria are killed in the saponification step of the extraction, there is little infectivity concern with handling of the sample once this step is concluded. FAMEs are more volatile than their respective fatty acids and therefore more suitable to GC analysis. The Sherlock software automates all analytical operations and uses a sophisticated pattern recognition algorithm to match the unknown FAME profile to the stored library entries for identification.

Similarity Index

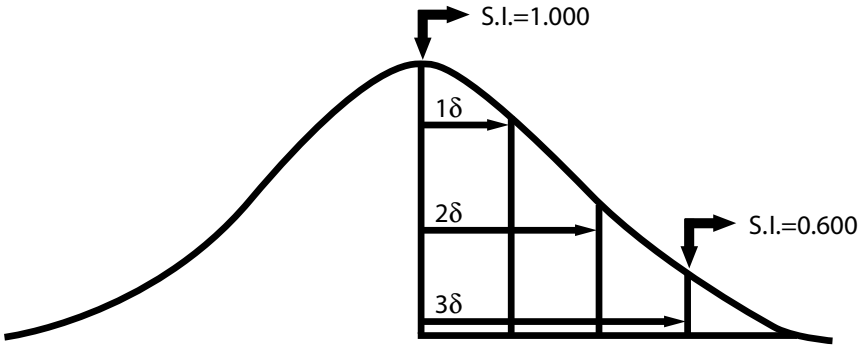
Many microbiology identification systems present results as a "probability" percentage. Thus, the system may report a 98% probability for the identification of an isolate. The basic assumption behind these "probabilities" is that species are well-defined groups of organisms with little variation in how 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.

The technique used by the Sherlock System to present results is based on a Similarity Index (SI). The SI 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 SI is not a "probability" or percentage, but an expression of the relative distance of the unknown sample from the population mean. An exact match between the fatty acid profile of the unknown and the mean of the library entry would result in an SI of 1.000. As each fatty acid varies from the mean percentage, the SI will decrease in proportion to the cumulative variance between the composition of the unknown and the library entry.

The SI assumes that fatty acid distributions for 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 SI is by looking at the Gaussian distribution of the population (in this case, the fatty acid composition). In Figure 4, 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 SI 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 SI drops.

Samples with an SI of 0.500 or higher and with a separation of 0.100 between the first and second choice are considered good library comparisons. If the SI 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). Values lower than 0.300 suggest that the organism is not a species in the library, but the software will still indicate the most closely related species, which can be useful when a new species is encountered.

Figure 4. Similarity Index as a Function of Population Distribution.

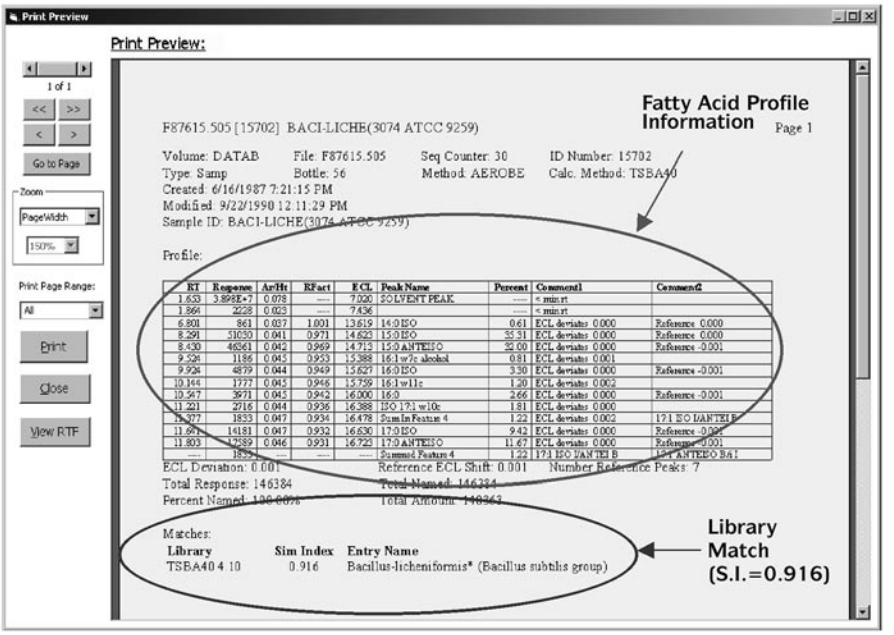


An example Sherlock sample printout shows a *B. licheniformis* library match with an SI of 0.916 (Figure 5). This SI value represents an excellent match to this library entry.

Analysis of the bacterial fatty acids is accomplished using an Agilent Technologies® gas chromatograph (GC). The highly reliable hardware provides analysis in less than nine minutes per sample and requires minimal maintenance, typically consisting of replacing the injection port liner after each two hundred analyses.

The Sherlock software Sample Processor table is used to enter information about the sample that includes all data useful for strain trending, such as facility, room, source (water, raw material, etc.), operator, product type, and so forth. This information can subsequently be queried in a spreadsheet or database to help with trend analysis or source tracking.

Figure 5. Sherlock Fatty Acid Profile Printout with Associated Library Match.



MICROBIAL LIBRARIES

The Sherlock System is capable of identifying a wide range of microorganisms (Table 2). Screening isolates for physiological or biochemical grouping before analysis is not required. Each library was carefully developed by collecting well-characterized strains of reference cultures from microbiologists specializing in many areas, including: clinical, environmental, industrial, drinking/waste water, and food. To provide for normal species variability, Sherlock library entries include many different strains of each organism. As an example, the library entry for *B. anthracis* has over 70 strains for this entry alone.

In the combined Sherlock libraries, there are nearly 2,000 microbial species, including 700 environmental aerobic species, 620 anaerobic species, and 200 species of yeasts.

Because FAME analysis is based on a near-universal characteristic of all bacteria possessing a unique complement of cellular fatty acids, ongoing expansion of the Sherlock bacterial fatty acid reference libraries continues to enhance the system's utility. For this purpose, MIDI maintains

extensive collaborative relationships with a network of researchers around the world who forward analyses of newly encountered organisms. This data is then used to strengthen existing library entries or to add completely new ones when MIDI does not have an entry for that species.

Table 2. Sherlock Microbial Libraries.

Package	Library	Description
AEROBE	TSBA6	Aerobes, 28°C, 24hr, on Trypticase Soy Broth Agar
	RTSBA6	
	CLIN6	Clinical Aerobes, 35°C, 24hr, on Blood Agar, Chocolate, etc.
	RCLIN6	
	BTR3	Bioterrorism Clinical Aerobes, 35°C, 24hr, on Blood Agar, Chocolate, etc.
	RBTR3	
	M17H10	Mycobacteria, 35°C, 5-10% CO_2, on Middlebrook 7H10 Agar with OADC enrichment
ANAEROBE	BHIBLA	Anaerobes, 35°C, 48hr, on BHIBLA plates in Gas Packs
	MOORE6	VPI Broth-grown Anaerobe Library, 35°C, in PYG Broth
YEAST	YST28	Yeasts, 28°C, 24hr, SAB Dextrose Agar
	YSTCLN	Yeasts, 28°C, 24hr, SAB Dextrose Agar
	FUNGI	Fungi, 28°C, 2-5 days, in SAB Dextrose Broth, 150 RPM shake culture
	ACTIN1	Actinomycetes, 28°C, 3-10 days, in Trypticase Soy Broth, 150 RPM shake culture

MIDI CALIBRATION STANDARD

The Sherlock System requires an external calibration standard, a mixture of the straight chain saturated fatty acids from 9 – 20 carbons in length and five hydroxy acids. The hydroxy compounds are especially sensitive to

changes in pressure/temperature relationships and to contamination of the injection port liner. As a result, these compounds function as real-time quality control checks for the system. Retention time data obtained by injecting the calibration standard is converted to Equivalent Chain Length (ECL) data for bacterial fatty acid naming. The ECL value for each fatty acid can be derived as a function of its elution time in relation to the elution time of the known series of straight chain fatty acids. The GC and column allow windows to be set at 0.010 ECL units, giving great precision in resolution of fatty acid isomers. The software compares the ECL values for the most stable series (e.g., saturated straight chain or branched chain acids) to the Sherlock peak naming table's theoretically perfect values and may recalibrate internally if sufficient differences are detected.

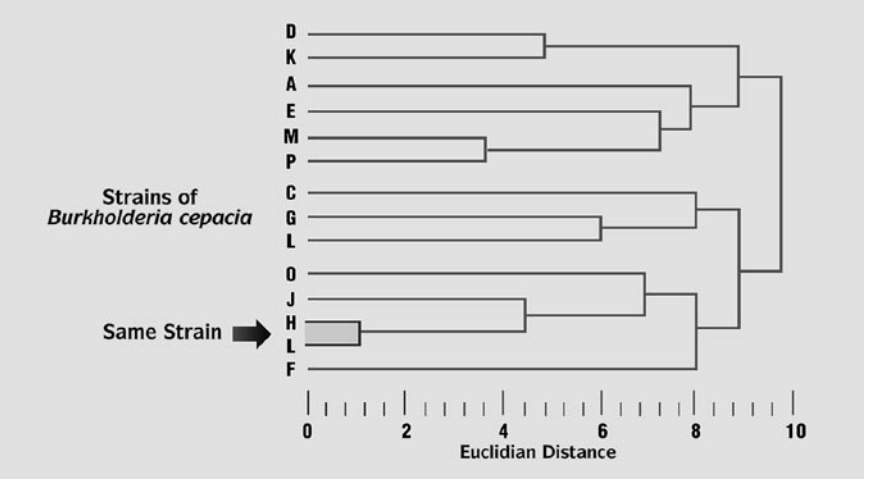
SYSTEM SUITABILITY MONITORING

A Performance Qualification (PQ) table is filled out with each batch of sample runs. The PQ Table is a record of optimal operating parameters for the MIDI method. Each sample batch contains two calibration runs, a positive control (the one used depends on growth conditions of the unknowns), and a reagent blank (containing no bacteria) as a negative control. These two Quality Control (QC) samples are analyzed after the calibrations and before any other samples in the batch. The positive control data is diagnostic for problems with the sample preparation procedure and an SI of at least 0.600, which demonstrates a high quality match against this well-characterized strain, ensures proper laboratory technique. The negative control data are diagnostic for reagent contaminants. The second calibration analysis data are diagnostic for problems with the GC.

"STRAIN TRACKING" IN TRACING SOURCE OF CONTAMINATION

The Sherlock System contains several tools to help with tracking the source of a microbial contaminant. The Sherlock dendrogram cluster analysis technique produces unweighted pair matchings based on fatty acid compositions and results are displayed graphically to depict the relatedness between organisms (Figure 6). The Sherlock "Tracker" program automatically compares the fatty acid profile of each sample to every other profile in the stored data files of the computer. The relation of the profile to that of the nearest matching profiles can be provided automatically at the finish of each analysis. The Tracker output can thus provide presumptive strain level tracking (or typing) without operator intervention and at no added cost per sample.

Figure 6. Tracking *B. cepacia* Isolated from a Pharmaceutical Product and from the Manufacturing Environment Using the MIDI Dendrogram Software. Strain H was Found in the Product and Strain L was Isolated from the Deionized Water Supply. The Other Strains were Isolated from Other Raw Materials. The Linkage of Strains H and L at Less than Two Indicates that these Two Strains are Probably Identical and Strain L is the Likely Source of Contamination.



Examples of how the MIDI system has been used for presumptive strain typing include the favorable comparison of Sherlock to pulsed-field gel electrophoresis in methicillin-resistant *Staphylococcus aureus* (Leonard 1995); "Efficacy of Microbial Identification System for epidemiologic typing of coagulase-negative staphylococci" (Birnbaum 1994); for use by a lab in Finland for comparison of *Bacillus cereus* by phage typing to typing by FAME analysis (Vaisanen 1991); for use in a Canadian lab for "Identification of sources of *Bacillus cereus* in pasteurized milk" (Lin 1998); and in a U.S. lab for "Subgrouping of *Pseudomonas cepacia* by cellular fatty acid analysis" (Mukwaya 1989). There are other similar papers (Hsueh 1997), but the ability to perform typing in the genera *Bacillus, Staphylococcus,* and *Burkholderia* (current taxonomy) suggests that the system is effective at presumptive strain typing in diverse genera of considerable importance in pharmaceutical QC.

"TRENDING" OF BACTERIAL SPECIES OVER TIME

Sherlock Data Export is an optional extension to MIDI Inc.'s Sherlock® Microbial Identification System (MIS). Data Export can be used to export Sherlock information to a database or a spreadsheet. Once the information is exported to these industry-standard files, an array of analysis, reporting, and charting tools may be used. Data Export creates Microsoft Access® databases and Excel® spreadsheets. Microsoft Office® is not required to use Data Export, (many packages accept databases and spreadsheets in these formats); however, to get the full value from Data Export, Microsoft Office is suggested.

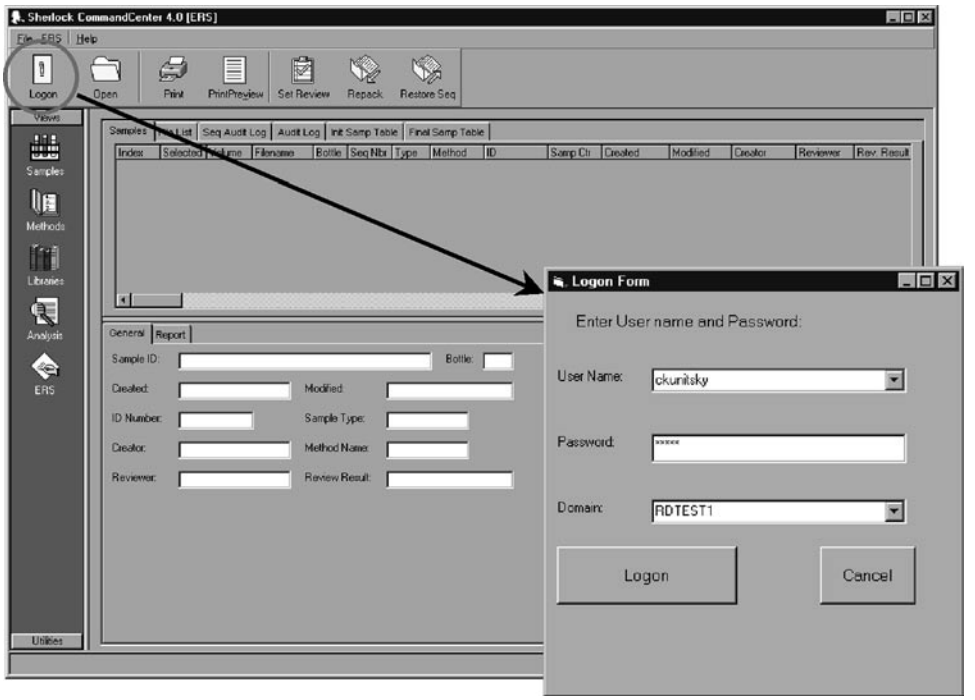
The "Data Export" tool in Sherlock allows the fatty acid profiles of all isolates to be exported to spreadsheets and/or databases for analysis of trends of occurrence in bacterial species over time. Thus, it is possible to query the identification data to see if there is a correlation of manufacturing environment bacteria with the introduction of new batches of raw materials or with dry weather bringing increasing amounts of dust into the environment. Similarly, data from multiple facilities may be combined to ascertain whether patterns of occurrences are related to specific types of equipment, to water supplies, or to new personnel. Trend analysis over years allows for the anticipation of some types of problems and the use of mitigation efforts to limit the potential microbial contaminant problems.

ELECTRONIC RECORDS AND SIGNATURES

Sherlock Electronic Records and Signatures (ERS) is an optional extension to MIDI Inc.'s Sherlock® Microbial Identification System. Sherlock ERS permits controlled data access to authorized personnel. A core component to 21 CFR Part 11 compliance is the ability to link all related transactions to an electronic signature and to track any associated changes made to a secure file. Sherlock ERS preserves the data, methods, libraries, and audit trail from a Sherlock sequence in a single, secure file. The Sequence Audit Log details all of the actions taken while the sequence was run, showing the date, the user name, and the activity. Changes after the data have been collected, such as approval or rejection of a sample, are detailed in the Audit Log, which also displays the date, name, and associated action. The Audit Log shows the old and new values for each change.

Figure 7. Sherlock Electronic Records and Signatures Logon Screen.

In order to comply with the FDA's requirement that an electronic signature must employ at least two distinct identification components such as a username and password, Sherlock ERS requires this information at every key operational point in the system. Sherlock ERS is integrated with the Windows® security models. Authorized ERS users and passwords are determined by the local or network system administrator, depending on the environment in which the Sherlock system is employed in (Figure 7).



USER CREATION OF A CUSTOM "LIBRARY"

A set of profiles to which an unknown is compared is called a "library" to distinguish it from commercial "databases" such as Access® (Microsoft Inc., Redmond, WA, USA). An example of a custom library might be one in which the user wishes to be able to test for a particular strain of an organism that creates a compound of interest for manufacture of a pharmaceutical product. The desired strain can be cultured 10-12 times, harvested, derivatized, and

extracted, followed by analysis. A new library can be created very simply and given the name desired by the operator. The data from the analyses can be combined to create an "entry" in the library that can be automatically searched as part of a standard analysis.

It is possible to create library entries for organisms grown under different fermentation conditions and/or for complex mixtures of organisms. It is also feasible to use the MIDI Sherlock System to analyze fatty acid-containing materials other than bacteria. Thus, plant and animal materials may be analyzed for fatty acid content and library entries made and searched automatically upon analysis.

SUMMARY AND FUTURE DEVELOPMENTS

The MIDI Sherlock Microbial Identification System is a fully automated gas chromatographic analytical system that identifies over 2,000 microbial species based on their unique cellular fatty acid profiles. The Sherlock System has many features that are important for pharmaceutical industry microbiology QC. The combination of accuracy of identification, high throughput, low cost per sample, extensive environmental libraries, compliance with 21 CFR Part 11, Data Export for trend analysis, reliable Agilent hardware, and fully automated strain tracking ability is unique. Because no subjective tests are required, the naming is highly objective and reproducible. The newest version of Sherlock will have the ability to incorporate DNA sequence data, allowing for combined "polyphasic" genotypic (DNA sequence)-phenotypic (fatty acid) reports.

REFERENCES

Birnbaum, D., L. Herwaldt, D.E. Lowe, M. Noble, M. Pfaller, R. Sheretz and A.W. Chow. 1994. Efficacy of Microbial Identification System for epidemiologic typing of coagulase-negative staphylococci. *J. Clin. Microbiol.* 32: 2113-2119.

Cook, V.J., C.Y. Turenne, J. Wolfe, R. Pauls and A. Kabani. 2003. Conventional methods versus 16S Ribosomal DNA sequencing for identification of nontuberculous mycobacteria: cost analysis. *J. Clin. Microbiol.* 41(3): 1010-1015.

Hsueh, P.R., L.J. Teng, P.I. Lee, P.C. Huang, S.C. Chang, C.Y. Lee and K.T. Luh. 1997. Outbreak of scarlet fever at a hospital day care centre: analysis of strain relatedness with phenotypic and genotypic characteristics. *J. Hosp. Infect.* 36:191-200.

Kim, W.Y., T.W. Song, J. Y. Park, C.M. Kim, S.I. Chung and C.S. Choi. 2000. Analysis of cellular fatty acid esters (FAMEs) for the identification of *Bacillus anthracis*. *J. Kor. Soc. Microbiol.* 35(1):31-40.

Leonard, R.B., J. Mayer, M. Sasser, M. Woods, B.R. Mooney, B.G. Brinton, P.L. Newcomb-Gayman and K.C. Carroll. 1995. Comparison of MIDI Sherlock system and pulsed-field gel electrophoresis in characterizing strains of methicillin-resistant *Staphylococcus aureus* from a recent hospital outbreak. *J. Clin Microbiol.* 33:2723-2727.

Lin, S., H. Schraft, J.A. Odumeru and M.W. Griffiths. 1998. Identification of contaminated sources of *Bacillus cereus* in pasteurized milk. *Int. J. Food Microbiol.* 43:159-171.

Mukwaya, G.M. and D.F. Welch. 1989. Subgrouping of *Pseudomonas cepacia* by cellular fatty acid comparison. *J. Clin. Microbiol.* 27:2640-2646.

O'Hara, C.M. 2005. Manual and automated instrumentation for identification of *Enterobacteriaceae* and other aerobic Gram-negative bacilli. *J. Clin. Microbiol.* 18(1):147-162.

Olsen, W.P., M.J. Groves and M.E. Klegerman. 1990. Identifying bacterial contaminants in a pharmaceutical manufacturing facility by gas chromatographic fatty acid analysis. *Pharm. Technol.* 14: 32-36.

Pendergrass, S.M. 1998. Aerobic bacteria by GC-FAME. Method 0801. NIOSH Manual of Analytical Methods, Fourth Edition.

Ratledge, C. and S.G. Wilkinson. 1988. An overview of microbial lipids. pp.12-13 in Microbial Lipids Volume 1, 6th Edition, Ratledge, C and S.G. Wilkinson (eds.). Academic Press. San Diego, CA.

Sasser, M. 2001. Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note 101.

Song, Y., R. Yang, Z. Guo, M. Zhang, X. Wang and F. Zhou. (2000). Distinctness of spore and vegetative cellular fatty acid profiles of some aerobic endospore-forming bacteria. *J. Microbiol. Meth*. 39, 225-241.

Sutton, S.V.W. and A.M. Cundell. 2004. Microbial identification in the pharmaceutical industry. *Pharmacopeial Forum.* 30(5):1884-1894.

Vaisanen, O.M., N.J. Mwaisumo and M.S. Salkinoja-Salonen. 1991. Differentiation of dairy strains of the *Bacillus cereus* group by phage typing, minimum growth temperature and fatty acid analysis. *J. Appl. Bacteriol.* 70:315-324.

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