Technical Note 105
Interpreting Sherlock Mycobacteria Identification System Reports

The Sherlock Mycobacteria Identification System reports analysis results in two forms:

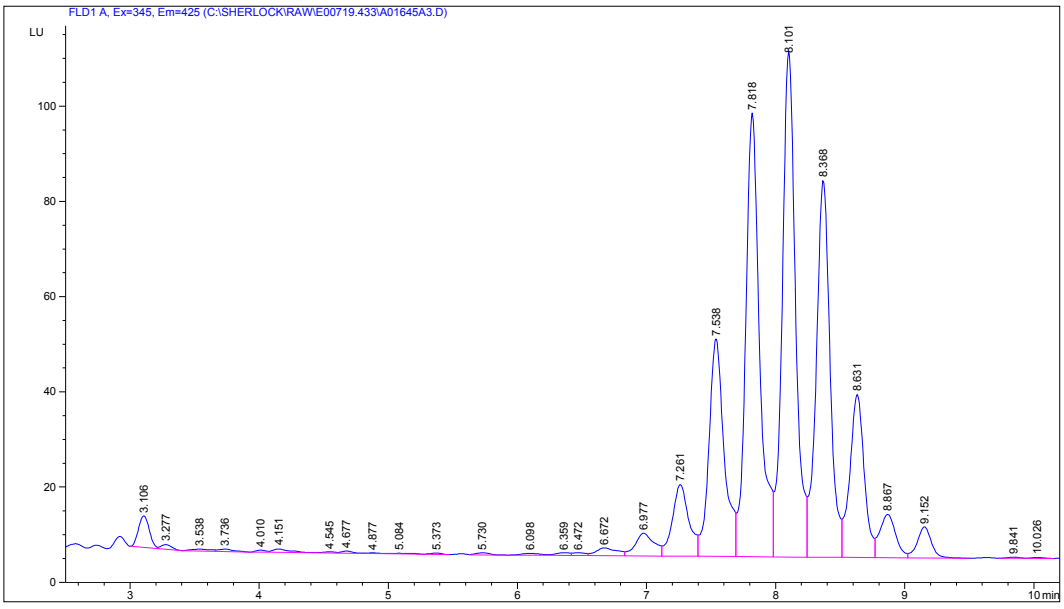
- A Chromatographic Report produced by ChemStation
- A Sherlock Composition Report, including a library search.

The primary component of the **Chromatographic Report** is the chromatogram, a visual plot or trace of the electronic signal generated by the fluorescence detector as mycolic acids of the sample elute from the column. The raw data of the chromatogram is stored in a ChemStation file and can be reintegrated on-screen and reprinted if desired. Sherlock stores a file containing all peak retention times, heights, and peak widths.

The Sherlock **Composition Report** comprises a Mycolic Acid Composition Report, a Library Search Report, and (optional) Comparison Charts. The Composition Report contains the mycolic acid composition of the organism. The Library Search Report lists the results of comparing the mycolic acid composition to the Sherlock Library.

Chromatographic Report

The ChemStation is used to accumulate raw data and to develop the chromatogram. Using the Sample Table and Calibration Sequence Table, Sherlock software identifies the sample scheduled for injection. The chromatogram heading lists the injection time and date, vial number and the sample name as it was entered in the Sample Table name field. The sample ID number appears in the Sample Name fields and within the data file name.



HPLC Chromatographic Report

Once the sample is injected, the ChemStation plots the signal from the fluorescence detector of the HPLC, creating the chromatogram. Mycolic acids in the sample are separated by the column and identified by the retention time of each peak.

Sherlock Composition Report

The peak retention time, width and height data from the ChemStation are transmitted to Sherlock data files at the end of each run.
The data are processed, peaks are assigned names (based on Equivalent Chain Length, "ECL," values, not structural names), the unknown is compared to the library and a report is printed.

Volume: DATA File: E026196.94A Seq Counter: 12 ID Number: 2246
Type: Samp Bottle: 10 Method: MYCOLC1
Created: 6/19/2002 7:32:29 PM
Sample ID: UN-MYCO (1004 4WK GO

Profile:

RT	Response	Ar/Ht	ECL	Peak Name	Percent	Comment1	Comment2
2.665	3406	0.088	36.413		---	< min rt	
2.838	2799	0.091	38.175		---	< min rt	
3.017	8922	0.097	40.000	ECL 40.000	---	ECL deviates 0.000	Reference 0.015
3.184	1215	0.093	41.699	Sum In Feature 1	0.11	ECL deviates 0.099	ECL 41.600
5.571	660	0.131	64.427	ECL 64.625	0.06	ECL deviates -0.198	
5.930	742	0.190	67.881	Sum In Feature 9	0.07	ECL deviates 0.168	ECL 67.713
6.298	1537	0.177	71.426	Sum In Feature 10	0.14	ECL deviates 0.039	ECL 71.387
6.523	3104	0.110	73.591	Sum In Feature 11	0.27	ECL deviates 0.162	ECL 73.429 index
6.592	3113	0.096	74.252	ECL 74.350	0.27	ECL deviates -0.098	
6.816	10131	0.157	76.385	Sum In Feature 12	0.89	ECL deviates 0.107	ECL 76.278 index
7.096	35798	0.130	79.056	Sum In Feature 13	3.16	ECL deviates 0.046	ECL 79.010 index
7.371	115990	0.123	81.688	Sum In Feature 14	10.22	ECL deviates 0.074	ECL 81.614 index
7.648	228230	0.117	84.312	Sum In Feature 15	20.12	ECL deviates -0.028	ECL 84.340 index
7.929	307522	0.112	86.977	Sum In Feature 16	27.10	ECL deviates -0.088	ECL 87.065 index
8.194	264531	0.119	89.430	Sum In Feature 17	23.32	ECL deviates -0.160	ECL 89.610
8.462	129343	0.121	91.951	Sum In Feature 18	11.40	ECL deviates -0.306	ECL 92.257 index
8.697	32646	0.140	94.214	Sum In Feature 19	2.88	ECL deviates -0.109	ECL 94.323
8.988	9733	0.151	97.000	ECL 97.000	---	ECL deviates 0.000	Reference -0.046
9.463	535	0.163	101.535		---	> max rt	
9.681	700	0.138	103.617		---	> max rt	
9.859	575	0.202	105.324		---	> max rt	
---	1215	---	---	Summed Feature 1	0.11		
---	742	---	---	Summed Feature 9	0.07		
---	1537	---	---	Summed Feature 10	0.14		
---	3104	---	---	Summed Feature 11	0.27		
---	10131	---	---	Summed Feature 12	0.89		
---	35798	---	---	Summed Feature 13	3.16		
---	115990	---	---	Summed Feature 14	10.22		
---	228230	---	---	Summed Feature 15	20.12		
---	307522	---	---	Summed Feature 16	27.10		
---	264531	---	---	Summed Feature 17	23.32		
---	129343	---	---	Summed Feature 18	11.40		
---	32646	---	---	Summed Feature 19	2.88		

ECL Deviation: 0.131 Reference ECL Shift: 0.034 Number Reference Peaks: 2
Total Response: 1134562 Total Named: 1134562
Percent Named: 100.00% Total Amount: 1153217

Matches:

Library	Sim Index	Entry Name
MYCAG1 1.02	0.906	Mycobacterium-tuberculosis complex (TB,bovis,africanum,microti)

Sherlock Library Search

Once a microorganism has been cultured, processed, and properly analyzed by Sherlock, its mycolic acid composition is matched with those of known organisms that are stored in the Mycobacteria Library. The Library profiles have been carefully developed to take into account strain-to-strain and experimental variation.

The library search is rapid. The naming of the unknown is available within minutes of the completion of the HPLC chromatographic analysis. The Sherlock Library Search Report lists the most likely matches to the unknown composition, and provides a similarity index for each match.

Interpreting the Library Search

If the search results in more than one possible match, the suggested identities are listed in order of descending similarity index.

The Library Search Result

Matches:

Library	Sim Index	Entry Name
MYCAG1 1.02	0.668	Mycobacterium-nonchromogenicum/terrae
	0.443	Mycobacterium-terrae/nonchromogenicum I

Similarity Index

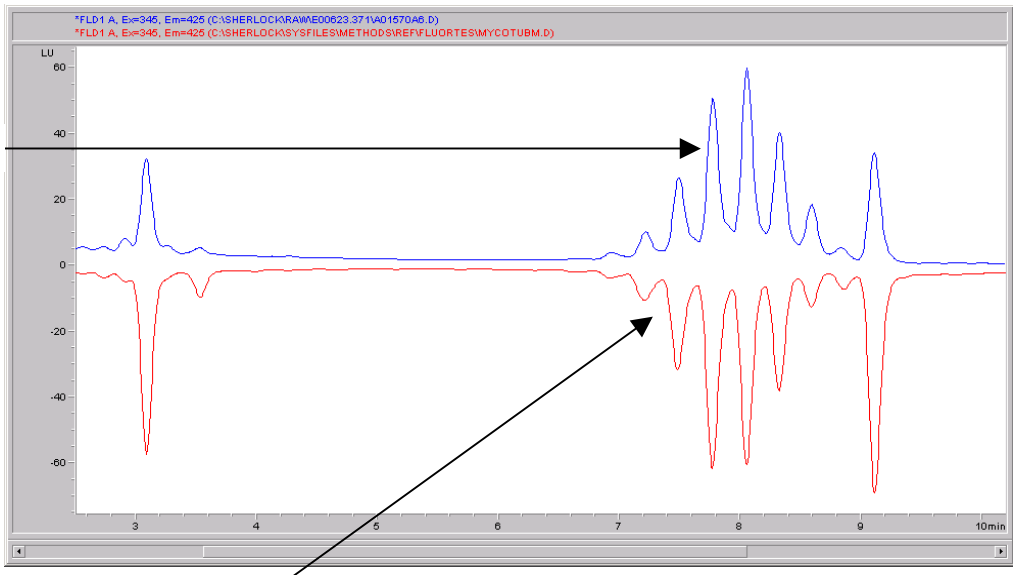
Many microbiology identification systems present results as a "probability" percentage. The system may report a 98% probability for the identification of an isolate. The basic assumption behind a "probability" assignment is that species are well-defined groups of organisms with little variation in how they perform certain biochemical tests. Since comparisons have traditionally been made between two or more biochemical test systems, the comparisons are simply how well the systems perform similar enzyme assays. Even when the naming is incorrect, the "probability" of the identification may be quite high and may be "confirmed" using a similar enzyme assay system.

The technique used by the Sherlock system is based on a "Similarity Index". The Similarity Index is a numerical value that expresses how closely the mycolic acid composition of an unknown compares with the mean mycolic acid composition of the strains used to create the library entry listed as its match. The database search presents the best matches and associated similarity indices. This value is a software-generated calculation of the distance, in multi-dimensional space, between the profile of the unknown and the mean profile of the closest library entry. Thus, it is not a "probability" or percentage, but an expression of the relative distance from the population mean. An exact match of the mycolic acid makeup of the unknown and the mean of the library entry would result in a Similarity Index of 1.000. As each mycolic acid varies from the mean percentage, the Similarity Index will decrease in proportion to the cumulative variance between the composition of the unknown and the library entry.

Visual Confirmation

An additional feature of the Sherlock Software allows the user to perform a visual comparison of the HPLC pattern to a known "reference" chromatogram. The "Visual Confirmation" software will select a chromatogram from a file of reference chromatograms. The selection will be the same species and will be approximately the same area. The chromatogram will be aligned using the internal standard peak times and the reference chromatogram will be scaled to the same area as the current analysis. The reference chromatogram is printed as a mirror image immediately below the current analysis for easy visual comparison.

Unknown



Reference

Visual Confirmation

www.midi-inc.com

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MIDI, Inc.
125 Sandy Drive
Newark, Delaware 19713
Phone: 302-737-4297
Fax: 302-737-7781
Email: midi@midi-inc.com