Charles River Accugenix® MALDI-TOF Enhancements for Bacterial Identifications Enable Improved Root Cause Analysis

Diversity of Organism and Library Coverage

Library coverage is often a topic of discussion for microbiologists wanting to know what the impact or relevancy of the Charles River Accugenix® library databases is on the identification of environmental, EM or industrial microorganisms. As a contract laboratory service provider offering both sequencing (AccuGENX-ID®) and MALDI-TOF-based identifications (AccuPRO-ID® and the Axcess™ system), Charles River has the ability to compare reference libraries based on the microorganisms that we identify and analyze the reportable rates.

Figure 1 graphically represents the diversity of bacterial species we identify (the x-axis) and the frequency at which these species are reported (the y-axis) with our 16S rDNA sequencing service. In this analysis, 987 different species comprise the top 95% of 85,557 reports produced by our 16S rDNA sequencing service for bacteria. The pie chart on the far right of the figure shows the percentage of the most frequently reported species that are contained and not contained in the OEM library (Bruker Biotyper 4.0). These data indicate that 45% of the most frequently reported species are not contained in the OEM library, and that there is quite a bit of growth needed for the OEM MALDI library to meet the needs for species-level identifications for bacterial isolates found in production facilities.

Library Development

With the knowledge of these gaps in library coverage, we implemented a strategy for library development that would dramatically increase the reportable rate directly from our MALDI-TOF system. In Figure 2, the year is listed on the x-axis and the number of entries is listed on the y-axis. The line in blue represents the number of entries in the original equipment manufacturer’s library as well as the frequency of those updates. Currently, there are more than 5,600 entries in the manufacturer’s library and updates average once or twice a year. The line in green represents the number of entries created by Charles River’s Accugenix® group.
Charles River Accugenix® has created more than 1,600 entries, making the total number of entries in the combined libraries more than 7,200 – represented in the red line. This “total” library is the one utilized when samples are tested by our outsourcing MALDI-TOF solution, AccuPRO-ID®, or our insourcing Axcess™ system. The data points on the red and green lines, representing library releases, are much more frequent than those on the blue line, as we perform a maintenance update to our library every 2-4 weeks. This means that if an entry or species is absent, we have the ability to add missing entries much more quickly.

Marketing materials from competing MALDI-TOF systems often speak to the number of data entries and some speak to the number of spectra in the library. However, what really matters operationally to the industrial microbiologist is the ability to ascertain an identification that reflects the broad diversity of bacterial samples found in production facilities. Does the library of the system offer both broad coverage (number of species) and relevant species to result in an accurate species-level report? Figure 3 shows that the Charles River Accugenix® entries to the MALDI library only comprise 21.5% of the database, but that 21.5% comprises 35% of the unique species entries in the entire library. The combined libraries supporting the Charles River Accugenix® services totaled 3,088 species at the time this figure was created.

In support of our strategy to add entries based on the most frequently occurring samples that fail to yield species-level identifications, we continually evaluate the operational impact for the QC microbiologist with respect to ID reports. We compared more than 72,000 spectral files against the OEM reference library and our Charles River Accugenix® library. In Figure 4, using the manufacturer’s most recent library release (v.4.0), nearly 27% of samples did not generate a species-level identification report when data are evaluated based on an alternate match factor value cutoff, validated by Charles River, and using unknown samples processed by our laboratory. In contrast, only 15% of the spectra did not generate a species-level identification report from those same unknown samples when using the Charles River Accugenix® reference library. This indicates that the Charles River Accugenix® entries increase the operational performance of the system. The entries made by Charles River Accugenix® are providing more effective coverage for the organisms encountered. This makes quite a bit of sense because the manufacturer’s library is primarily targeted towards the clinical environment, not environmental samples.
Operational and Cost Savings Impact for Environmental Monitoring

Since library coverage heavily influences the ability to obtain an identification report, surveillance for monitoring the state of control can be impacted when secure species-level identifications cannot be made. In Figure 5, we have identified greater than 1,300 species from more than 61,000 samples on our MALDI-TOF platform. About 15% of the reports are only generated because of a Charles River Accugenix® library entry. Therefore, if we did not have these entries, we would not be able to do tracking and trending for 15% of the samples without processing them through a backup identification platform.

Putting this into real-world context, for these samples, the most frequently occurring organisms in the EM arena occur 15% of the time. If these frequently occurring organisms were not present in a library, ancillary testing would need to be used to accurately identify them, which would raise the cost of testing. Additionally, for those running a 24-hour manufacturing cycle with short product life cycles, this ancillary testing could potentially delay product release; with access to a comprehensive library like Charles River’s, these delays could be avoided and batches could be released sooner, which could equal a huge cost savings.

Aside from library coverage, our reporting of identifications for AccuPRO-ID® and Axcess™ is based on a validated match value cutoff that is supported by our 16S rDNA sequencing service. If a sample does not result in a species-level confidence, we will perform 16S rDNA sequencing using our AccuGENX-ID® service at no additional charge. This separates us from competing MALDI-TOF systems. While a cutoff value may not seem critical, it is important in terms of environmental monitoring. The graphs in Figure 6 are examples of data from one customer using Charles River’s trending and tracking tool. On the left, it can be seen that 74% of the customer’s samples fall into 10 genera and the remaining 26% fall into many different genera. While there is absolutely some good information provided, one is unfortunately not able to delineate the number of Staphylococcus samples that are Staphylococcus nepalensis vs. the number that are Staphylococcus aureus, which is frequently an objectionable organism for many topical non-sterile products. That is where our cutoff matters. We do not support genus-level cutoffs with our MALDI-TOF; only species-level. This means that if one clicks on the Staphylococcus slice in this genus-level graph in the Tracking and Trending feature in our web portal, (s)he can determine how many of those organisms are Staphylococcus aureus, and how many are other Staphylococcus species (see graph on the right-hand side). This level of detailed analysis simply is not provided by the manufacturer’s cutoffs.
Example: Typical Gram-Negative Isolates From Water Systems

Analysis of FDA product recall data for 134 non-sterile pharmaceutical products from 1998 to September 2006 demonstrated that 48% of recalls were due to contamination by either *Burkholderia cepacia* or *Pseudomonas* spp. (Jimenez L., 2007). As an example, in cosmetic products, *P. aeruginosa* was recovered from contaminated mascara material and was identified as the agent responsible for corneal ulcers in the 1970s (Ortho, 2009). *Pseudomonas* genera are Gram-negative, non-fermenting bacteria commonly linked to water and water purification problems in industry. They are considered opportunistic pathogens which disperse and adhere easily to surfaces, forming a biofilm which interferes with cleaning and disinfection procedures. Pseudomonads can survive and grow in deionized water. Contaminated deionized water can be a source of microbial contamination if it is used for the final rinse of equipment that has been cleaned and sanitized, and it may be the source of contamination for finished products in these industries.

Focusing on these problematic genera, the *Pseudomonas* species make up 4.6% of all our sequencing reports generated over the last year. Of the 120 species of *Pseudomonas* in our AccuGENX-ID® 16S rDNA library, the OEM MALDI-TOF library contains only 67% and the Charles River MALDI-TOF library contains 78%. Of the 100 *Pseudomonas* species we reported, the top 95% consisted of 39 species. Almost 85% of these organisms were in our MALDI-TOF library and 15% were exclusive to the Charles River entries. If only using the OEM database, the inability to obtain an identification for roughly 33% of the species within this frequently occurring genera would impact the tracking of potential objectionable organisms and risk assessment to product(s) and intended patient populations.

References