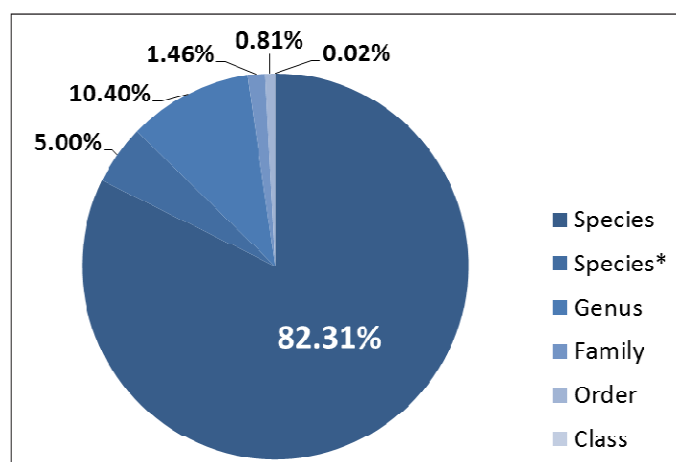


A Retrospective Case Study on the Operational Impact of the Accugenix[®] Library Development Process for MALDI-TOF on Performance

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 (those encountered in production) to result in an accurate species-level report? Because Charles River has purposely developed a strategy to add MALDI-TOF (matrix-assisted laser desorption/ionization-time-of-flight) entries based on the most frequently occurring samples that fail to yield species-level identifications, we continually evaluate the operational impact that our entries have for the QC microbiologist with respect to ID reports.

Background Information

We performed a retrospective study based on a large sample set from a single aseptic manufacturing site producing vaccines. The site had submitted 5,942 bacterial isolates over the last four years as unknowns which resulted in an identification. All samples were submitted for 16S rDNA sequencing analysis services, either through AccuGENX-ID[®] or AccuBLAST[®], both of which use our leading reference 16S library for bacterial isolates found in industrial settings. Unlike other ID platforms, our sequencing service yields identifications based on a taxonomic confidence level and not a percent probability. Of these 5,942 samples, 82.3% had a species-level call, 5% were species* (i.e., when there are two or more microorganisms that the 16S rDNA sequence cannot discriminate because they are phylogenetically too closely related), 10.4% were genus, 1.5% were identified to the family, 0.8% to the order and 0.02% representing a single sample to the class level.

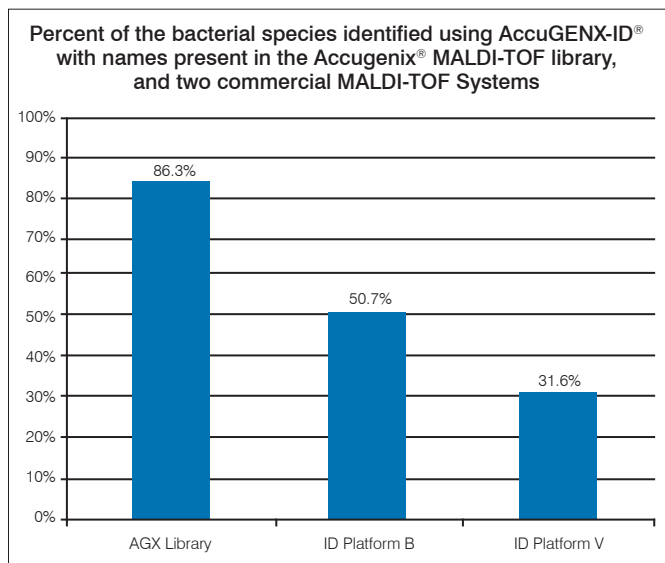


Method

The study analyzed the list of species-level identifications against all commercial MALDI-TOF identification systems as well as the Accugenix[®] proprietary library by simply examining each individual reference library for the presence or absence of these identified species names. It should be noted that the results are not truly indicative of a performance claim since we did not test these samples on each system. The study merely speaks to the potential capability of a system to identify an unknown bacterial sample based on library capacity and diversity. To reiterate the logic, we considered that if a species name was not present in the reference library, then the system could not possibly identify the unknown isolate. If the species name was in the library, we considered the system capable of identifying the unknown microorganism.

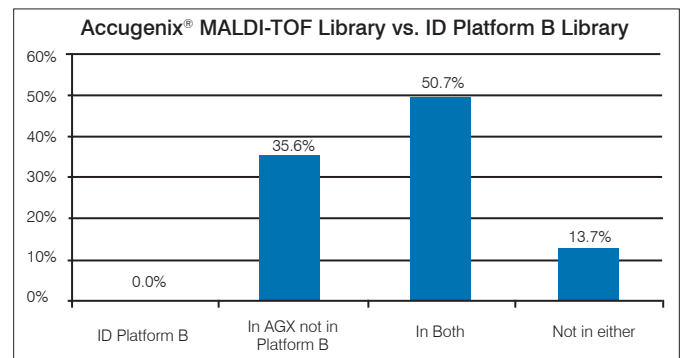
Results

Of the total species-level identifications generated by our sequencing services, 519 unique species were identified. We evaluated the percentage of bacterial names present in our AccuPRO-ID[®]/Access[™] MALDI-TOF libraries versus the two leading commercial MALDI-TOF reference libraries (based on the latest reference releases as of December 2014). The percentage of bacterial species identified by our AccuGENX-ID[®]/AccuBLAST[®] services with names present in the various MALDI-TOF libraries is graphically illustrated below. We have 86.3% coverage of these species in the Accugenix[®] MALDI-TOF library, where ID Platform B has only 50.7%. ID Platform V shows a significant gap in coverage at 31.6%.



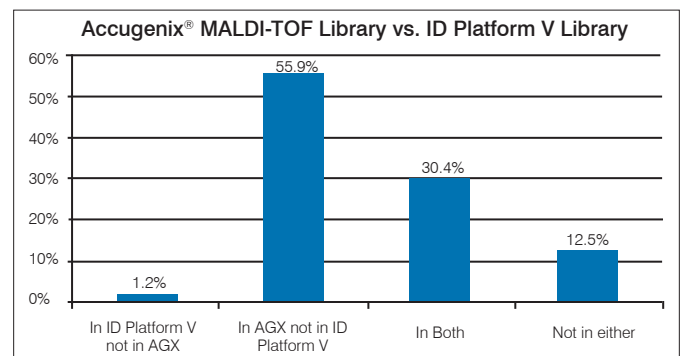
Accugenix[®] MALDI-TOF Library vs. ID Platform B Library

We also examined the percent of species names which are present in the Accugenix[®] MALDI-TOF library versus what is absent for each of the two commercial systems. Analyzing ID Platform B first, 35.6% of the species names are exclusive to the Accugenix[®] proprietary library and are a direct result of our development process, 50.7% of the species names are found in both libraries, and 13.7% of the species names are not in either library. These missing entries represent species names on our target list for continued library development.



Accugenix[®] MALDI-TOF Library vs. ID Platform V Library

In comparison, evaluating ID Platform V, 55.9% of the species names are exclusive to the Accugenix[®] proprietary library. Only 1.2% of the species names are exclusive to the ID Platform V, 30.4% were contained in both, and 12.5% were missing species names from both systems.



It becomes obvious that the species coverage with Accugenix[®] is the most extensive of the three MALDI-TOF reference libraries with respect to the diversity of bacterial organisms commonly found in production facilities. This coverage naturally improves our ability to identify unknown bacteria.

Operational Impact of Library Coverage Based on Frequency of Occurrence

We also considered the frequency with which a particular species was encountered. We did this by calculating the weighted average of the species names and how often that species name was observed in the facility where the samples were obtained. Matching up the presence or absence in the reference libraries, we get an idea of the number of times a missing ID will result due to library coverage by taking into consideration how often the individual species is recovered. For example, if an organism is not in the library but has been seen just once over 5 years, there is less of an impact than missing an organism the facility has observed numerous times. The table below is an example showing the presence or absence of the top three most frequently occurring bacterial organisms observed at this facility.

For confidentiality purposes, species names are labeled X, Y and Z. Species X, for example, is in all libraries and has been observed 362 times over the course of 4 years. Species Y is exclusive to the Accugenix MALDI-TOF library and was observed 174 times over the same period. It is the second most frequently occurring, and yet neither of the other MALDI-TOF platforms can identify this species.

By weighing the entire frequency of occurrence (FOO) list for all samples identified to the species-level against their presence/absence in the Accugenix® library, we can definitively state that 95% of these isolates could result in an ID since we have the most frequently occurring species in the database. In contrast, Platform V could identify less than half of these isolates. This is not a performance statement since this is an evaluation of the species name only and does not represent the library entry diversity needed to cover the intra-species spectra differences properly (which is reflected in our multiple library entries for each species).

Summary

In summary, frequent library updates to the Accugenix® MALDI-TOF reference database are prioritized by the frequency of occurrence of bacteria found in production facilities. This yields a much higher species-level accountability rate than competing MALDI-TOF systems, and has a positive impact on a facility's operations and reporting capabilities. In addition, both the Access™ system and AccuPRO-ID® service triage any unknown that yields no identification, at no additional cost to clients. Using 16S rDNA sequencing, AccuGENX-ID®, targets these isolates for the continual development of our MALDI-TOF reference library. As a frame of reference, Charles River has expanded ID Platform B's library database by more than 1,600 entries (v.14.11), for a combined total of about 7,200* entries. On average, we perform maintenance updates 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. Because we are responsible for this process, our clients save time and money by not having to purchase, upgrade and validate these updates. Most importantly, Charles River Accugenix® customers can be confident that their manufacturing environment is quality-controlled with data from the most comprehensive and accurate identification libraries in the industry.

* As of September 2014

| Bacterial Species Identification | Top Three Observed Organisms | In AGX MALDI-TOF | In Platform B | In Platform V | Weighted Averages of FOO Coverage | |
|----------------------------------|------------------------------|------------------|---------------|---------------|-----------------------------------|-----|
| | | | | | | |
| Species X | 362 | Yes | Yes | Yes | AGX | 95% |
| Species Y | 174 | Yes | No | No | Platform B | 68% |
| Species Z | 158 | Yes | No | Yes | Platform V | 45% |

Charles River Microbial Identification Products & Services

[AccuGENX-ID®](#) – Comparative DNA sequencing of the 16S rRNA gene in bacteria and the ITS2 rRNA region in fungi.

[AccuPRO-ID®](#) – A first-in-industry polyphasic approach to microbial identification utilizing proteotypic MALDI-TOF mass spectrometry technology supported by our AccuGENX-ID® 16S rDNA sequencing method (at no additional cost).

[Access™ System](#) – Features the precision of the Bruker MALDI Biotyper instrument with the industry-leading Accugenix® proprietary microbial libraries through a secure online network.

[AccuBLAST®](#) – Designed exclusively to address gaps in the MicroSeq® identification system, the AccuBLAST® service compares customers' MicroSeq® data files against our best-in-industry Accugenix® sequence libraries, increasing confidence in their microbial IDs.

Additional Resources

Technical sheet: [Charles River Accugenix® MALDI-TOF Enhancements for Bacterial Identifications Enable Improved Root Cause Analysis](#)

[Bacterial library comparisons](#)

PDA Micro 2014 Poster: [Effect of MALDI-TOF Library Development on the Efficiency of Operations and Tracking and Trending Capability](#)

For more information on the Charles River Microbial Identification Products & Services, please visit: www.criver.com/accugenix.