

AccuPRO-ID[®] Comparative Performance Case Study for the Identification of Bacteria

Overview/Abstract:

Accurate classification of unknown bacterial isolates is an essential first step in understanding the impact organisms have on an environmental monitoring (EM) program. Currently available methods of identification (ID) range from genotypic to phenotypic, with 16S sequencing being the gold standard for bacterial ID. Charles River's AccuPRO-ID[®] provides reliable, reproducible, rapid and affordable bacterial identification for industries required to identify microorganisms on a routine basis. AccuPRO-ID[®] utilizes a polyphasic approach of matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS), which is supported by our genotypic 16S rDNA sequencing method, AccuGENX-ID[®] and our extensive, validated Accugenix[®] bacterial identification libraries. This polyphasic approach offers unparalleled accuracy and reportable identification rate compared to phenotypic systems such as VITEK[®] 2 Compact, API[®] strips, and Biolog GEN II/III or other commercial MALDI-TOF MS systems.

Situation/Challenge:

When identifications are based on phenotypic characteristics, such as with the VITEK 2 Compact, API strips or Biolog, the methods are highly error prone, variable and subjective. Phenotypic methods have been in use for decades, and most of the commercial identification systems are focused on clinical investigations, which is apparent in the limited database coverage. Both the impact

of the library and the inherent variability of biochemical and phenotypic systems significantly affect the accuracy of bacterial identification. It is critical that the identification libraries against which data are compared, whether generated by phenotypic, proteotypic or genotypic methods, contain species relevant to the production facility from which the organisms were isolated. If the library lacks depth and breadth of coverage, the interpretation of the data may not always be reliable or accurate.

Traditionally, routine IDs have been made with simple microbiological exam or utilizing manual or semi-automated phenotypic systems that differentiate between organisms based on the results of biochemical tests. There is very little complexity in the data from these phenotypic systems, which can lead to limited differentiation between microorganisms. Additionally, since microorganisms isolated from manufacturing environments will likely be physiologically stressed, they may not fully express their phenotypic or biochemical characteristics. This can lead to inconclusive test results and subjective interpretation, and thus a higher rate of erroneous identifications and higher costs.

We realized that development of the AccuPRO-ID[®] MALDI-TOF library presented an opportunity to provide an efficient, cost-effective method for obtaining accurate identifications of microorganisms isolated from production environments, as opposed to other ID platforms that focused mainly on isolates from a clinical setting.

Solution:

We combined our expertise in library development with a stable, robust methodology to develop a new approach to microbial identification that addressed and overcame the shortcomings present in the current routine ID methods: the AccuPRO-ID® solution. It utilizes a polyphasic approach to identifications, wherein proteotypic MALDI-TOF methods are supported by our AccuGENX-ID® 16S rDNA sequencing. Combined with our extensive and relevant sequencing and MALDI-TOF libraries, AccuPRO-ID® provides a highly accurate and economical option for routine monitoring programs.

Charles River continually strives to improve the protein-spectra database for AccuPRO-ID® to encompass organisms that are relevant to the industries we serve. Thus, we are constantly adding new organisms to the library to improve the frequency of identification. We have the expertise required to advance the MALDI-TOF technology and the library database to provide our customers with a rapid species-level identification.

AccuPRO-ID® is a proteotypic-based identification service that utilizes MALDI-TOF MS, a technology that has been shown in scientific literature to be very accurate and reproducible. Identifications by MALDI-TOF are based on unique protein spectra primarily consisting of ribosomal proteins and other proteins that are expressed at high levels. The spectra are compared to a validated database, analyzing peak intensity and position, to establish the ID. The result is not only produced rapidly, but is also highly reliable, since it is based on molecules and is not subject to the biochemical variability seen in phenotypic systems.

In order to demonstrate the superior performance, accuracy and repeatability of our AccuPRO-ID® solution, we completed numerous comparative blinded studies in collaboration with our clients. These studies have compared identifications generated by our clients with the most commonly used phenotypic systems to those generated with the AccuPRO-ID®. These data, presented below for the VITEK2 Compact, API strips, Biolog GEN II/III and the VITEK MS system, strongly support the assertion that the AccuPRO-ID® solution is significantly more accurate, leading to more confidence in the identification information and allowing for more effective tracking and trending in a production facility.

Results/Conclusion:

All samples were submitted by our customers and processed according to the AccuPRO-ID® service offering. The samples were first subjected to MALDI-TOF analysis, which yielded identification results for the majority of the samples. Under the standard AccuPRO-ID® offering, the samples that did not yield usable spectra or had no library match would have been automatically tested with our AccuGENX-ID® 16S rDNA sequencing. However, as part of these double-blind comparative studies, all samples were subjected to 16S rDNA sequencing, since it served as the reference method to verify the taxonomic identity of the organisms. The microbial identifications generated by the three phenotypic systems and the other commercial MS system were performed by our customers. After completion of testing, data were exchanged and both the customers' identifications and those generated by AccuPRO-ID® were compared to AccuGENX-ID® 16S rDNA sequencing results to determine the accuracy of the phenotypic and proteotypic methods.

A key feature for evaluation of an ID system is its ability to provide a name for an unknown. However, it is critical to remember that a reportable ID does not necessarily equal an accurate ID. In the studies presented here, all systems, except the Biolog GEN II/III, resulted in a high frequency of identification ranging from 86% and 92% for the VITEK 2 and API strips, respectively, to 99% with AccuPRO-ID® (MALDI-TOF backed up by 16S rDNA sequencing). When evaluating the AccuPRO-ID® data, results from all the studies were combined. As stated previously, accuracy of the IDs made by the commercial systems was determined by comparison to the AccuGENX-ID®. For the AccuPRO-ID® service offering, the MALDI-TOF ID was compared to the 16S ID for accuracy. AccuPRO-ID® outperformed all of the commercial systems with an average rate of accuracy to the species level of 85% and an additional 13% correct to the genus level, while 1% was incorrect. The AccuPRO-ID® solution showed an approximately two-fold increase in species-level accuracy over VITEK 2, API strips, Biolog and VITEK MS systems.

Performance and Accuracy of Identification					
Reportable Rate	AccuPRO-ID®	VITEK® 2	API®	Biolog	VITEK® MS
Isolates with ID	99%	86%	92%	49%	89%
Accuracy of the ID	*				
Correct to Species	85%	43%	37%	38%	45%
Correct to Genus	13%	33%	30%	38%	42%
Correct to Family	1%	1%	3%	0%	2%
Wrong	1%	23%	30%	24%	11%

* For the AccuPRO-ID® service offering, the MALDI-TOF ID was compared to the Accugenix® 16S ID for accuracy.

Specifically, the VITEK 2 had a high percentage of reportable IDs at 86%; however, as compared to the AccuGENX-ID®, the VITEK 2 was only correct to the species level for 43% of the samples, correct to the genus level 33% of the time, and was incorrect on 23% of the identifications. Although the API strips had a high reported-out rate, the accuracy of the IDs was quite poor. API strips accurately identified just 37% of the organisms to the species level and an additional 30% to the genus level, and was incorrect for 30% of the isolates. Further, approximately one-quarter to one-third of the identifications obtained using the VITEK 2 or API strips were not accurate. Biolog had a very high percentage of no identification (51%). Of the isolates which had an ID (49%), Biolog was accurate to the species level for 38%, to the genus level for 38%, and incorrect for 24%. In many cases, there is less inherent risk for a test that results in no identification than one that results in a misidentification, as the latter can lead to false confidence in the quality of an ID and a false sense of control. However the low reportable rate can add significant costs to an EM program when achieving an identification is required for compliance and demonstrating a controlled state of the manufacturing environment. Finally, the VITEK MS accurately identified 45% of the organisms to the species level and an additional 42% to the genus level, and was incorrect for 11% of the isolates.

Overall, the performance, accuracy and reproducibility of the IDs to the species level for the phenotypic systems and the VITEK MS were poor. Much of the variation can be due to phenotypic profiles of the organisms isolated from the manufacturing environments. These organisms are stressed and may not fully express their biochemical profiles as compared to the reference profiles, leading to misidentification or no identification. Additionally, many of the errors could have been due to the usage of the wrong test system/card as a result of an incorrect Gram stain result. Correct choice of a biochemical-based identification system (e.g., GEN II, API, VITEK 2) requires and depends on defined upstream steps. Reduction and simplification of upstream steps, tests, and additional information can have a huge operational benefit in regards to time, labor and investment in reagents, as well as accuracy.

Operational considerations also include the complexity of sample preparation. Procedures that are easy, robust and rapid are preferred, especially if they also minimize the influence of growth conditions on the result of an identification system. Further, many of the errors with the phenotypic systems and the VITEK MS were due to gaps in the reference libraries. To improve performance, extensive and relevant data entries in the corresponding library would be required. These entries should reflect the microorganism spectrum found in the manufacturing settings. However, due to the limited test panel complexity of these phenotypic ID systems, expansion of the library database may still not result in increased differentiation and identification.

Finally, the frequency of incorrect or incomplete results (no ID) from the identification systems has to be reduced to ensure product safety and eliminate supply chain delays caused by inadequate results. When an identification system is independent of variable expression profiles or ancillary testing and is supported by an extensive and relevant reference library, such as with AccuPRO-ID®, performance can increase dramatically as demonstrated here.

Having identified more than a million samples over the past decade, our goal for our AccuPRO-ID® service was to develop and continue to optimize the MALDI-TOF platform to better accommodate identification of environmental isolates. This case study shows the suitability of our MALDI-TOF platform for identifying environmental isolates and demonstrates that our systematic approach to broadening the reference library improves both reportable rates and accuracy over conventional phenotypic systems.