

The AccuPRO-ID™ Solution from Accugenix, Inc.

Accurate classification of unknown bacterial isolates is an essential first step in understanding the impact organisms have on an environmental monitoring program. Accugenix offers the AccuPRO-ID™ solution as an ideal technology for accurate, reproducible, fast and economical microbial identification for industries required to identify microorganisms on a routine basis.

AccuPRO-ID™ is a proteotypic based service that utilizes matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) spectrometry. The AccuPRO-ID™ MALDI-TOF technology is less dependent on growth conditions than phenotypic methods, because it primarily analyzes ribosomal proteins, which are constitutively expressed at very high levels. A small amount of sample results in a protein spectrum based on the ribosomal proteins which are then compared to a validated database for identification. The AccuPRO-ID™

identification service is a polyphasic approach backed up by our proprietary genotypic 16S rDNA sequencing method, AccuGENX-ID™, and our relevant, validated and up-to-date industry leading library. If we are unable to identify your organism by MALDI-TOF, we will sequence it at no additional cost to you. This enables you to achieve an unparalleled 98% accuracy and reportable identification rate.

The bacterial database at Accugenix is the most extensive of all the commercial libraries available. In fact, the libraries from all identification systems are a subset of the Accugenix reference library. It is critical that the identification library against which you compare your data, whether generated by phenotypic, proteotypic or genotypic methods, contain all species relevant to your environment. If the library is incomplete, the interpretation of your data is not reliable.

Keywords

Proteotypic, Genotypic, Phenotypic, bioMérieux, VITEK®2 Compact, AccuPRO-ID™, MALDI-TOF, 16S rDNA Sequencing

In combination with maintaining relevant libraries, having consistent qualified methods of analysis are essential when identifying organisms in the manufacturing environment. MALDI-TOF technology is highly accurate and has demonstrated increased accuracy and reproducibility over phenotypic systems.

Case Studies

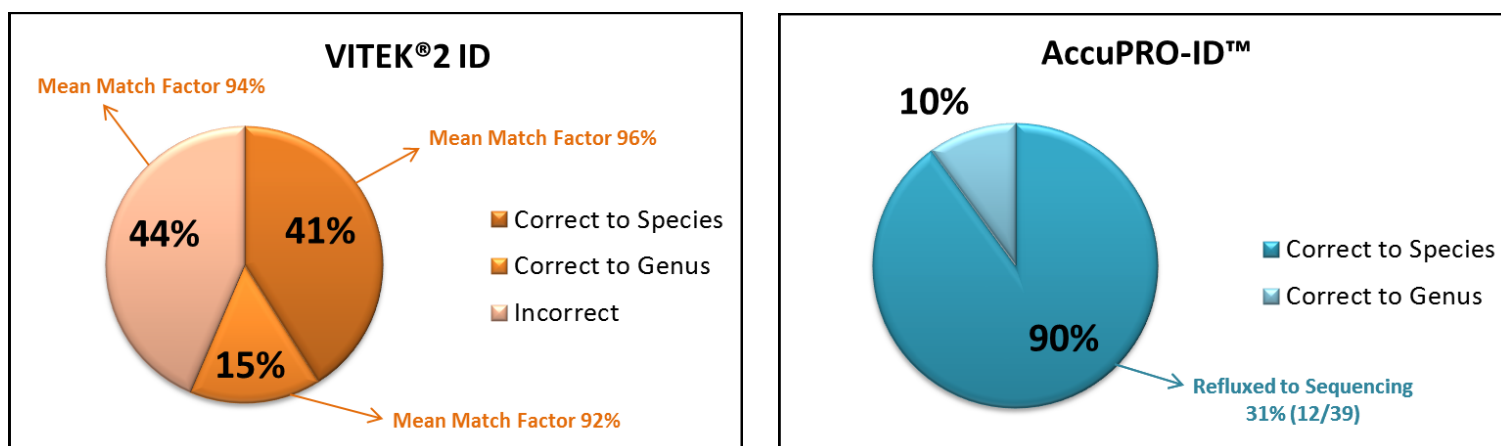
In the following case studies, Accugenix evaluated the accuracy of reported identifications from the AccuPRO-ID™ method to the results from the bioMérieux VITEK®2 Compact. The data show that the AccuPRO-ID™ service from Accugenix is significantly more accurate than the VITEK®2, leading to more confidence in the EM information and allowing for more effective trending and tracking in a production facility.

Microbial identification at Accugenix, Inc. The samples were processed according to the AccuPRO-ID™ solution and were subjected to MALDI-TOF analysis (n=39 in both studies). MALDI-TOF yielded results for the majority of the samples. Under the standard AccuPRO-ID™ offering, the samples that did not yield significant spectra or no library match would have been automatically tested with 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 VITEK®2 (performed by our clients), and by Accugenix's MALDI-TOF were compared to Accugenix's 16S rDNA sequencing results to determine the accuracy of the phenotypic and proteotypic methods, respectively.

Results for Client A

As compared to the AccuGENX-ID™ sequence-based identification, the VITEK®2 was correct to the species level for 41% of the samples, correct to the genus level 15% of the time and was incorrect on 44% of the identifications (Figure 1). Interestingly, the average Match Factor in each of these groups was very similar. Of the 44% that were completely misidentified, the mean confidence level was 94%, thus showing that the confidence level percentages for the VITEK®2 have no real value. In contrast to the performance of VITEK®2, the AccuPRO-ID™ method described here was correct to the species level on 90% of the samples and correct to the genus level with the remaining 10% of the samples (Figure 1). As we typically see with the MALDI-TOF BioTyper, approximately 31% of the samples are sent to sequencing. Overall, these results suggest that the AccuPRO-ID™ method is more accurate than the VITEK®2.

Figure 1.



For Case Study I, further analysis of the 17 samples incorrectly identified by VITEK®2, showed that only three of the correct entries are present in the VITEK®2 database (*C. tuberculostearicum*, *C. aurimucosum*, *C. mucifaciens*, blue dots, Table 1). Additionally, 3 samples were all identified as *C. tuberculostearicum* by AccuPRO-ID™ (Table 1). However, the VITEK®2 generated three different IDs for the same organism (*Kocuria rosea*, *Kytococcus sedentarius* and *Micrococcus luteus*, yellow dots). Having three different identifications for the same organism makes accurate trending and tracking nearly impossible to accomplish with the VITEK®2 considering the identification of the same organism can be so highly variable.

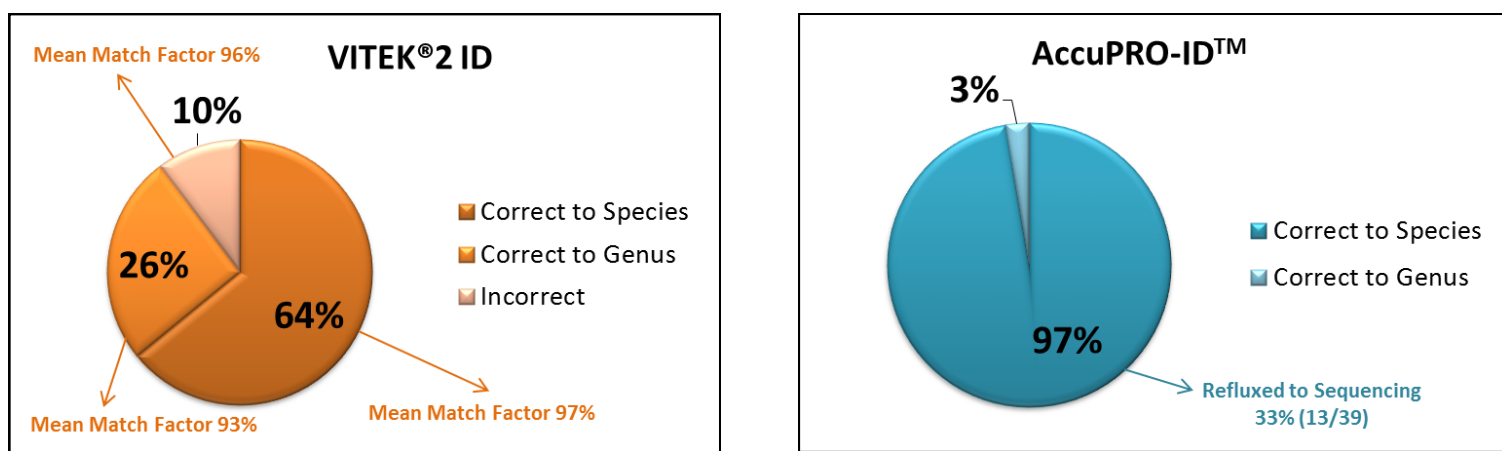
Table 1.

Sample Name	VITEK®2 ID	Match Factor	Match to Sequence Reference?	AccuPRO-ID™	Confidence	Match to Sequence Reference?
890	<i>Brevibacillus choshinensis</i>	90%	No	<i>Bacillus beijingsensis</i>	Species	Species
865	<i>Brevibacillus choshinensis</i>	93%	No	<i>Bacillus beijingsensis</i>	Species	Species
790	<i>Geobacillus thermoglucosidasius / thermodenitrificans</i>	92%	No	<i>Bacillus muralis</i>	Species	Genus
429	<i>Alcaligenes faecalis ss faecalis</i>	97%	No	<i>Brevundimonas aurantiaca</i>	Species	Species
782	<i>Kocuria kristinae</i>	98%	No	<i>Corynebacterium aurimucosum nigricans</i> ●	Species	Species
902	<i>Kocuria varians</i>	98%	No	<i>Corynebacterium mucifaciens</i> ●	Species	Genus
885	<i>Dermacoccus nishinomiyaensis</i>	92%	No	<i>Corynebacterium singulare</i>	Species	Species
723	<i>Kocuria rosea</i> ●	99%	No	<i>Corynebacterium tuberculostearicum</i> ●●	Species	Species
805	<i>Kytococcus sedentarius</i> ●	96%	No	<i>Corynebacterium tuberculostearicum</i> ●●	Species	Species
461	<i>Micrococcus luteus</i> ●	94%	No	<i>Corynebacterium tuberculostearicum</i> ●●	Species	Species
430	<i>Sphingomonas paucimobilis</i>	89%	No	<i>Cupriavidus gilardii</i>	Species	Species
534	<i>Brevundimonas diminuta/vesicularis</i>	97%	No	<i>Massilia timonae</i>	Species	Species
878	Actinomycetes Species - ID by gram stain	NA	No	<i>Nocardiopsis dassonvillei</i>	Species	Species
789	<i>Bacillus smithii</i>	94%	No	<i>Oceanobacillus caeni</i>	Species	Species
889	<i>Bacillus lentus</i>	95%	No	<i>Paenibacillus campinasensis</i>	Species	Species
783	<i>Bacillus circulans</i>	86%	No	<i>Paenibacillus rhizosphaerae</i>	Species	Genus
703	Actinomycetes Species - ID by gram stain	NA	No	<i>Streptomyces carpinensis</i>	Species	Species

Results for Client B

The results of the second comparison study show that the VITEK®2 identifications reported by this study were slightly more accurate with 64% correct to the species level (Figure 2). However, there were still 36% of the identifications that were either correct only to the genus level or completely incorrect and again, the mean match factor or confidence level did not correlate with the accuracy of the identification. As noted by Zbinden *et al.* "even excellent identification by the VITEK®2 colorimetric card assay allows no prediction of the correctness of the results". Similar to the first study, the AccuPRO-ID™ method described here was correct to the species level on 97% of the samples and correct to the genus level with the remaining 3% of the samples (Figure 2). Again, as is typical with the MALDI-TOF BioTyper, 33% of the samples were sent to sequencing. Overall, these results, like the comparison shown above, suggest that the AccuPRO-ID™ method is more accurate than the VITEK®2.

Figure 2.



Upon examining the organisms that were misidentified, it is clear that missing library entries in the VITEK®2 database lead to incorrect identifications. As shown in Table 2 (blue dots), the correct organisms are not in the VITEK®2 database. Three of the correct organisms are in the VITEK®2 database, but they were still misidentified (yellow dots). One organism was misidentified due to the use of the wrong Gram stain card (red dot), and 5 of the *Corynebacterium spp.* were incorrectly identified (green dots). The organism marked with the arrow* was identified correctly to the genus level by AccuPRO-ID™, but it was incorrectly identified by VITEK®2. The *Candida* yeast** species was included as an internal control for the method and was not identified by AccuPRO-ID™ which is currently exclusively used for bacterial analysis.

Table 2.

Sample Number	VITEK®2 ID	Match Factor	Match to Sequence Reference?	AccuPRO-ID™	Confidence
1	<i>Candida intermedia</i>	95%	No	-	-
2	<i>Brevundimonas diminuta/vesicularis</i>	97%	No	<i>Cupriavidus metallidurans/pauculus</i>	Species
3	<i>Sphingomonas paucimobilis</i>	92%	No	<i>Paenibacillus motobuensis</i>	Genus
4	<i>Acinetobacter lwoffii</i>	99%	No	<i>Moraxella osloensis</i>	Species
5	Low Discrimination Organism	-	No	<i>Corynebacterium tuberculostearicum</i>	Species
6	<i>Staphylococcus auricularis</i>	99%	No	<i>Staphylococcus pettenkoferi</i>	Species
7	<i>Bacillus smithii</i>	94%	No	<i>Bacillus simplex/muralis</i>	Species*
8	<i>Bacillus lentus</i>	91%	No	<i>Bacillus clausii</i>	Species
9	<i>Streptococcus mitis/Streptococcus oralis</i>	91%	No	<i>Streptococcus pneumoniae</i>	Species*
10	<i>Staphylococcus saprophyticus</i>	99%	No	<i>Staphylococcus hominis</i>	Species
11	<i>Corynebacterium amycolatum</i>	90%	No	<i>Corynebacterium tuberculostearicum</i>	Species
12	<i>Corynebacterium minutissimum</i>	85%	No	<i>Corynebacterium singulare</i>	Species
13	<i>Corynebacterium amycolatum</i>	90%	No	<i>Corynebacterium simulans</i>	Species
14	<i>Corynebacterium jeikeium</i>	95%	No	<i>Corynebacterium mucifaciens</i>	Genus
15	<i>Kocuria varians</i>	97%	No	<i>Kocuria rhizophila</i>	Species

yeast **
← *

Conclusions

Current available methods of identification range from genotypic to phenotypic, with 16S sequencing being the gold standard for bacterial identification. Accugenix provides reference quality genotypic methods for identification and has maintained a bacterial library focusing on organisms relevant to all industries we serve. When identifications are based on phenotypic

characteristics, such as with the VITEK®2 Compact, the methods are highly error prone, variable and subjective. With the introduction of the proteotypic MALDI-TOF-based method of identification that is supported by sequencing, the AccuPRO-ID™ solution, Accugenix provides an additional highly accurate, lower risk and economical option for routine monitoring programs.

We know how important it is for you to get the right answer, not just any answer.

Reference:

Zbinden A, Böttger EC, Bosshard PP, Zbinden R. Evaluation of Colorimetric VITEK 2 card for Identification of Gram Negative Non-fermentive Rods: Comparison to 16S rRNA Gene Sequencing. Journal of Clinical Microbiology. 2007 Vol. 45 No. 7 p. 2270-2273.

Having an Identity Crisis?

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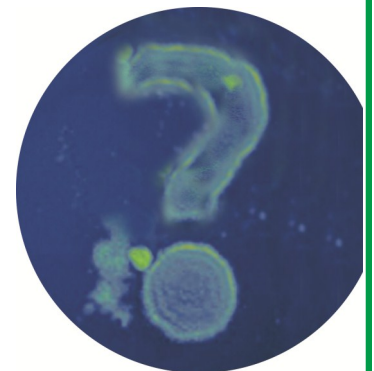
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