

Accurate classification of unknown bacterial isolates is an essential first step in understanding the impact these organisms have on an environmental monitoring program. Accugenix offers various methods for bacterial identification including our proprietary AccuGENX-ID™ and AccuPRO-ID® services, as well as MicroSEQ®, BioTyper and VITEK®. The Accugenix brand includes DNA sequence-based and ribosomal protein-based methods, backed by our relevant, validated, up-to-date libraries. We now offer out-of-the-box, commercially available methods to give clients an opportunity to select microbial identifications based on price point, accuracy and quality of the result.

Our most recent proprietary method, AccuPRO-ID®, the first practical proteotypic identification method, utilizes matrix-assisted laser desorption/ionization – time of flight (MALDI-TOF) mass spectrometry. For industries that are required to identify microorganisms on a regular basis, the ideal technology is one that is accurate, reproducible, fast and inexpensive. MALDI-TOF technology is less dependent on growth conditions than phenotypic methods, because it analyzes primarily ribosomal proteins, which are not differentially expressed. Accugenix optimized MALDI-TOF for use in bacterial identification and backs the method with our 16S sequencing technology, providing clients with results 98% of the time – an increase of 26% over the commercially available BioTyper®. Accugenix conducted two different studies to evaluate MALDI-TOF as a suitable identification method and to compare it to commercially-available methods of identification.

STUDY 1: Evaluation of MALDI-TOF as a Method for Bacterial Identification

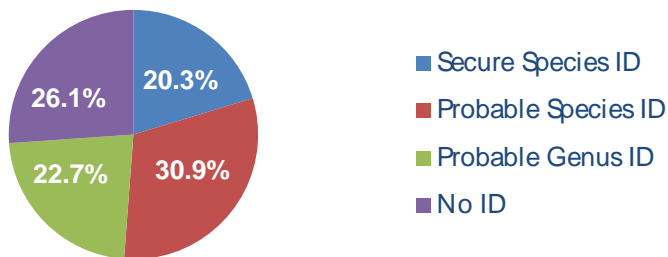
The first study used a diversity panel of 31 known bacterial strains from culture collections and 146 different strains representing the 43 most frequently identified species that are known to occur in pharmaceutical manufacturing environments. Identities of all organisms and strains were verified using the Accugenix 16S sequencing method. Results demonstrated that MALDI-TOF resulted in accurate species level identifications most of the time, with a very low rate of species being misidentified

Bacteria used in each experiment were tested using both the direct spotting method and extract method (ethanol-formic acid-TFA) per the manufacturer's recommendations. The following variables were tested:

- 24 hour cultures on three different days to investigate overall variability
- 24, 48 and 72-hour old cultures to determine if the age of the culture affected the identification
- Different media types to determine if growth conditions affected the ability of the organisms to be identified.

A total of 1,873 sample spectra were obtained. The overall variability of MALDI-TOF processing is higher than genotypic testing, but there were no significant trends associated with processing samples from different media types. Cultures greater than 48 hours old had lower match factors overall but still correctly identified the majority of the time. Less than 2% of the organisms were misidentified. When observing the average match factors of organisms correctly identified by the BioTyper® software, it was found that each environmental species tested could confidently be identified with a match factor cut-off of 1.75, which is lower than the software's default recommendation, with very little risk of misidentifying organisms (Figure 1).

Data Set with Manufacturer's Software Recommended Interpretation (n=1873)



Data Set with Optimized Accugenix Interpretation (n=1873)

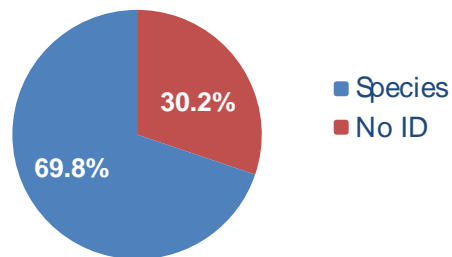
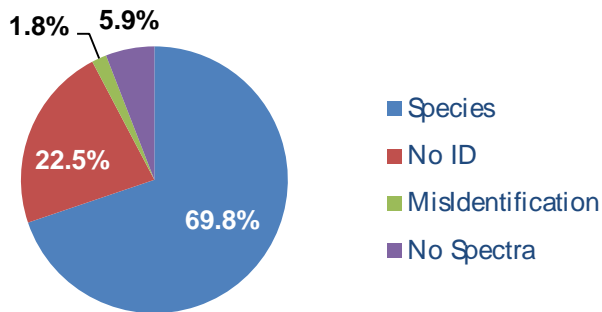


Figure 1: (A) Distribution of sample identifications with BioTyper software recommended interpretation. (B) Distribution of sample identifications with the match factor cut off set at 1.75 for a species level identification.

Almost 30% of the samples gave no identification with MALDI-TOF or did not produce analyzable spectra. These samples must be reprocessed with 16S DNA sequencing for identification. Overall performance of MALDI-TOF spectrometry for identifying environmental isolates resulted in accurate species level identifications nearly 70% of the time (Figure 2). The error rate was very low with only 1.8% of species being misidentified. A small percentage of samples did not produce analyzable spectra, which is an indication of processing variability. One of the factors contributing to about 30% of samples not being successfully identified with the Bruker BioTyper® is that some species frequently seen in environmental monitoring programs are missing from the database of known spectra. Adding relevant species and sub-species to the AccuPRO-ID® database increases the number of isolates identified (Figure 3) when compared to the Bruker BioTyper®. The low cost and rapid turnaround time of MALDI-TOF analysis remain strong advantages for this technology, but a growing, curated database will be required to optimize this technology for use in the pharmaceutical and sterile manufacturing industries. The AccuPRO-ID® service from Accugenix is an attractive alternative, which uses a combination of MALDI-TOF and 16S DNA sequencing to successfully identify 98% of environmental isolates.

Overall Performance of MALDI-TOF (n=1873)



Identification Rate with Updated Library

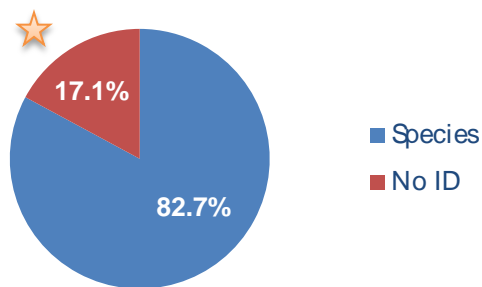


Figure 2: Performance of sample identifications with the match factor cut-off set at 1.75 for a species level identification using DNA sequencing as a reference.

Figure 3: Percentage of sample identifications with the addition of 15 relevant species to the spectra database (match factor cut-off set at 1.75 for a species level identification).

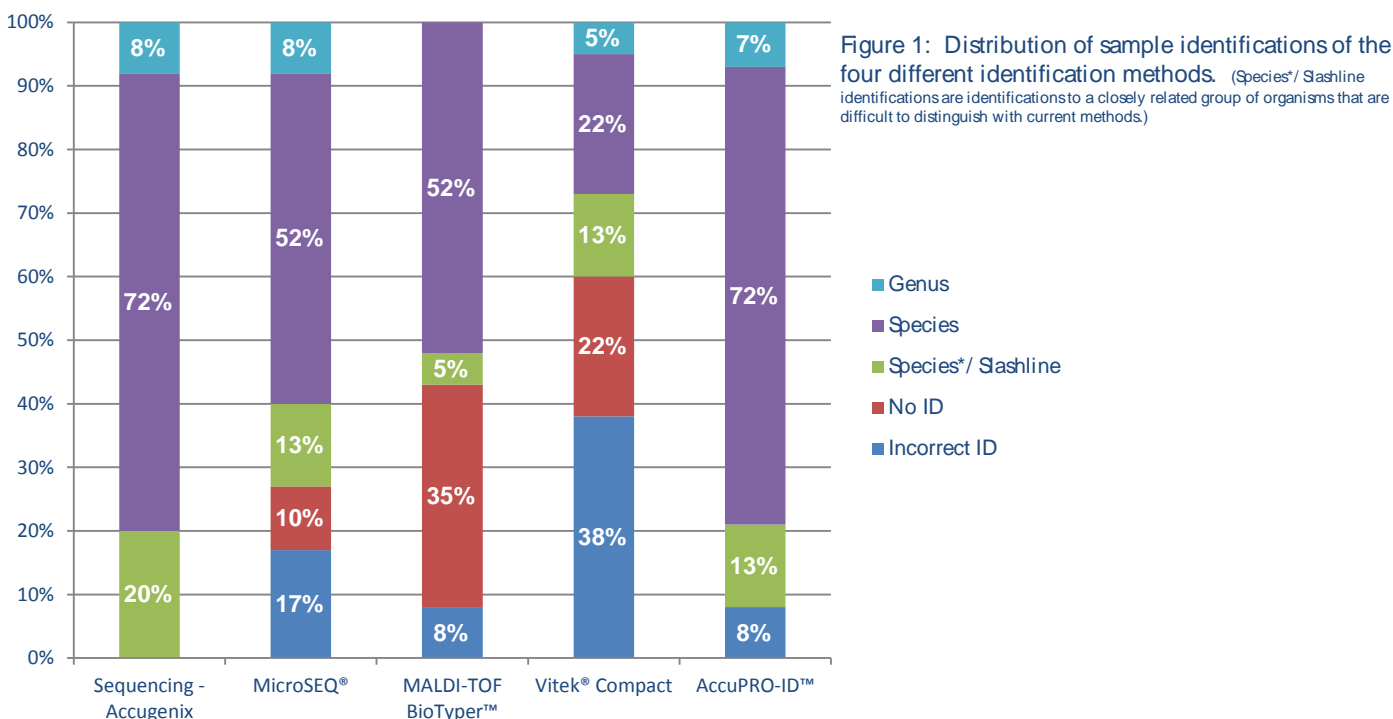


AccuPRO-ID® process offered by Accugenix, Inc. had a 17.1% ID improvement over the current BioTyper® performance.

STUDY 2: Comparison of Genotypic, Proteotypic and Phenotypic Methods for the Identification of Bacteria

There are many different methods designed to differentiate and identify microorganisms, ranging from the Gram stain to genome sequencing. The microbial identification strategies employed by the environmental monitoring programs of pharmaceutical and sterile manufacturing industries are influenced by accuracy and repeatability but also to a large extent by cost and the amount of time it takes to get an identification. One challenge with many identification systems is that the developers focused their technology on identifying clinical isolates. When applying these technologies to environmental isolates the methods underperform due to the diversity of organisms found in EM programs.

The bacteria used in this study were a group of 60 unknowns from the pharmaceutical and sterile manufacturing environments that represented 45 different species. Five methods were evaluated for performance with respect to their ability to identify environmental isolates (VITEK® 2 Compact, MALDI-TOF BioTyper®, MicroSEQ® 2.0, AccuGENX-ID™ and AccuPRO-ID®).



Overall the MALDI BioTyper® reported fewer incorrect identifications than other methods compared to the Accugenix sequencing method. MicroSEQ® 2.1 and MALDI-TOF identified more organisms correctly to the species level than the VITEK® 2 Compact method (Figure 1). Limitations of these technologies are attributed in part to the underrepresentation of environmental species in their databases. Accugenix utilizes proprietary, updated libraries, and a comparison between four different identification databases based on the inclusion of 1183 unique species encountered by Accugenix demonstrates that other systems lack species relevant to the pharmaceutical and sterile manufacturing environments. Most of the complexity for MALDI-TOF is in the database, and will require a custom database or supplementation with a method such as 16S sequencing, if it is to be used for identifying environmental isolates. Based on this study, MALDI-TOF has a high level of accuracy, produces results rapidly and is affordable enough to screen routine samples. A polyphasic approach such as AccuPRO-ID® that is offered by Accugenix and involves using MALDI-TOF for initial identifications and using 16S sequencing for organisms not identified is a cost effective, highly accurate alternative for routine monitoring.

About Us

Accugenix, Inc. provides leading-edge technology in microbial identification and characterization services. Our FDA-registered lab is cGMP compliant and maintains rigorous standards competitive at the global level. We specialize in testing, analyzing and interpreting data from environmental isolates commonly found in pharmaceutical, biotechnology, medical device, nutraceutical, personal care and other manufacturing industries.

For more than 20 years, Accugenix has provided the fastest, most accurate and reliable microbial identification services to over 400 facilities around the world. Accugenix updates its validated, proprietary DNA sequence libraries annually to reflect current taxonomy and newly described relevant species. We have the industry's first Fungal Library based on the ITS region. Since inception, we have tested more than 400,000 microorganisms – more than any other service laboratory in the industry, while maintaining an on-time delivery of over 99%.

History of Accugenix

1990.

Accugenix, Inc. began as Acculab, Inc., a reference laboratory specializing in microbial identification for industry and research clients. At the time we were one of only a few service laboratories in the world offering cellular fatty acid analysis, beginning a tradition of bringing cutting-edge microbiology methods to full commercial potential and utilization.

1999.

To reflect the addition of comparative DNA sequencing to our menu of validated methods, we created Accugenix, A Division of Acculab, Inc. Since then we have sequenced hundreds of thousands of environmental isolates from over 1000 pharmaceutical and biotechnology production facilities around the world, allowing us to build the largest and most unique industry database for bacteria and fungi that often occur in cleanroom manufacturing environments.

2005.

Our official name changed to Accugenix, Inc. on February 25, 2005.

2008.

Accugenix GmbH, our European subsidiary, was launched in Spring 2008.

Today.

Dedicated to being the industry leader for providing the most progressive microbiology methods available, Accugenix has invested in the technology, instrumentation and expertise to conquer genetic-based testing methods, their process validation, cGMP compliance, and other rigorous regulatory standards at the global level. Accugenix continues to staff its ranks with scientists and experts to guide and/or fast-forward your transition to genotypic microbial identification.



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