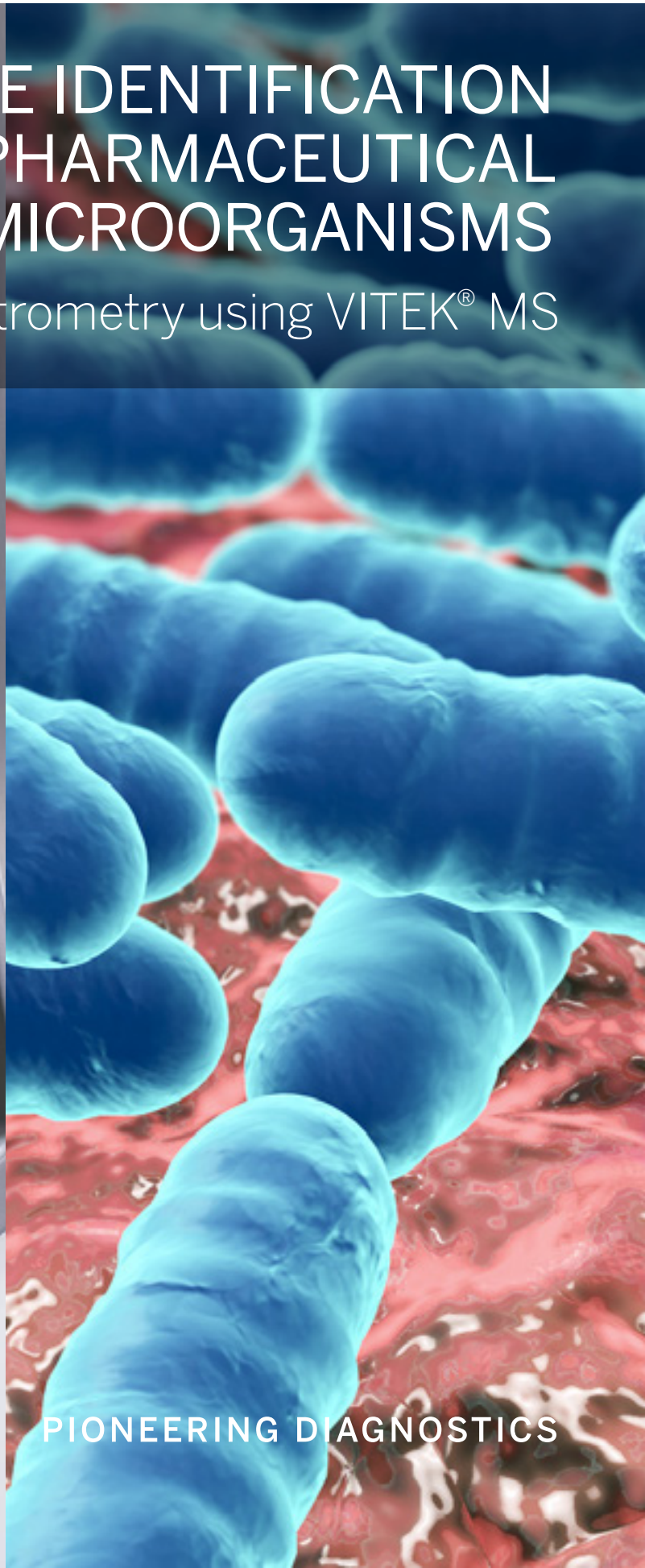
BIOMÉRIEUX

ACCURATE IDENTIFICATION OF PHARMACEUTICAL ENVIRONMENTAL MICROORGANISMS

By MALDI-TOF Mass Spectrometry using VITEK® MS



PIONEERING DIAGNOSTICS

When the knowledge base quality matters!

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BACKGROUND

Microbial contamination of pharmaceuticals poses a great problem to the pharmaceutical manufacturing process from both a safety as well as an economic point of view. Rapid and accurate identification of microorganisms may contribute to reduce cost and time linked to investigations and corrective actions. Clearly, sterile as well as non-sterile pharmaceutical products should be free of harmful microbial species. What's more, the financial impact of microbial contamination on the manufacturing process can be significant; from scrapping large amounts of high value finished product to all the costs and brand damage associated with a product recall.

IDENTIFICATION OF PHARMACEUTICAL ENVIRONMENTAL MICROORGANISMS: FUNDAMENTAL FOR QUALITY

The importance of microbial identification, has been stressed by regulators, thus, the new EU GMP Annex 1 (Version 12) for the Manufacturing of Sterile Products stipulates that:

"9.31 Microorganisms detected in Grade A zone and Grade B area should be identified to species level and the potential impact of such microorganisms on product quality (for each batch implicated) and overall state of control should be evaluated. Consideration should also be given to the identification of microorganisms detected in Grade C and D areas (for example where action limits or alert levels are exceeded or where atypical or potentially objectionable microorganisms are recovered). The approach to organism identification and investigation should be documented."

The guidance for industry published by the FDA (Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice), specifies that the *"characterization of recovered microorganisms provides vital information for the environmental monitoring program. Environmental isolates often correlate with the contaminants found in a media fill or product sterility testing failure, and the overall environmental picture provides valuable information for an investigation."*

The USP Chapter <1116> Microbiological Control and Monitoring of Aseptic Processing Environments, also mentions that *"a successful environmental program includes an appropriate level of identification of the flora obtained by sampling. A knowledge of the flora in controlled environments aids in determining the usual microbial flora anticipated for the facility and in evaluating the effectiveness of the cleaning and sanitization procedures, methods, agents, and recovery methods. The information gathered by an identification program can be useful in the investigation of the source of contamination, especially when recommended detection frequencies are exceeded. Identification of isolates from critical and immediately adjacent areas should take precedence over identification of microorganisms from noncritical areas. Identification methods should be verified, and ready-to-use kits should be qualified for their intended purpose"*

Monitoring critical and immediate surroundings of clean areas as well as personnel should include routine identification of microorganisms to the species (or, where appropriate, genus) level.

Thus, microbial identification is extremely important to control the manufacturing area and the process. In some cases, environmental trending data have revealed migration of microorganisms into the aseptic processing room from either uncontrolled or less controlled areas.

A WIDELY USED TECHNOLOGY: MALDI-TOF MASS SPECTROMETRY

A technology becoming more widely used in the pharmaceutical industry for microbial identification is Matrix-Assisted Laser Desorption Ionization–Time of Flight (MALDI-TOF) Mass Spectrometry. This technology is recognized as a comprehensive and efficient tool for the identification of microorganisms in both clinical and pharmaceutical environments. The principles of the technology are illustrated in **Figure 1.**

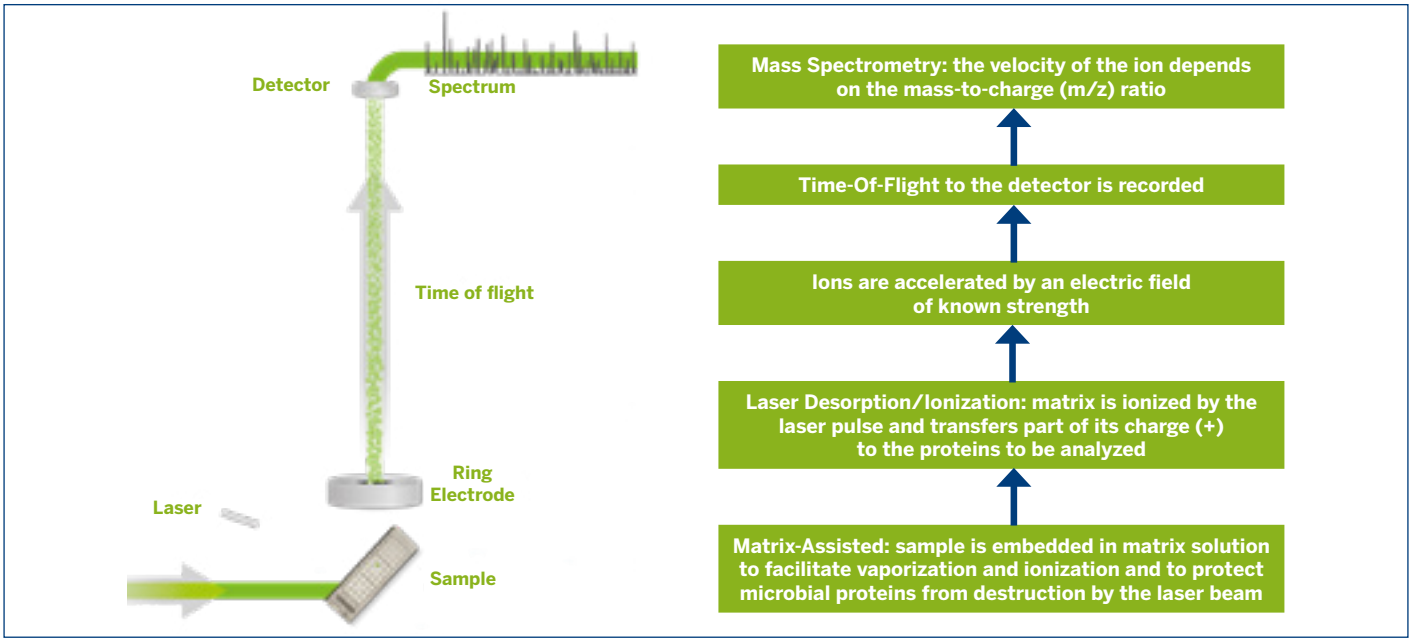

Figure 1. Principle of MALDI-TOF technology

However, to ensure accurate identification, it should be accompanied by a robust knowledge base that incorporates natural microbial variability (geography, source, etc.) and technical variability linked to the test itself (media, operator, incubation time, etc.). **(Figure 2)**

Using a database with multiple well-characterized strains from both clinical and environmental sources will ensure a broad coverage of needs, especially for pharmaceutical manufacturers who typically test many isolates from environmental samples.

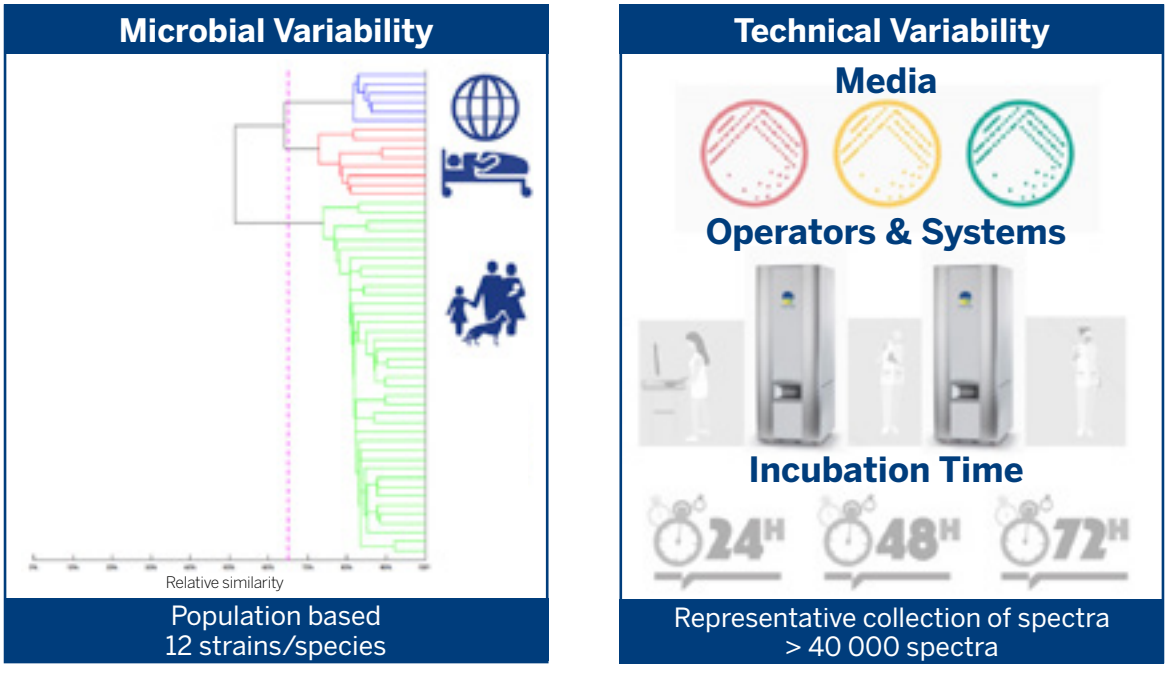

Figure 2. VITEK® MS Knowledge Base building strategy including microbial and technical variability.

ACCURATE IDENTIFICATION USING A ROBUST VITEK® MS KNOWLEDGE BASE

In this white paper, we assess the performance of a new update to the VITEK® MS Knowledge Base (VITEK® MS Knowledge Base v3.2) which has been designed to incorporate intra-species and technical variability encountered in the field. VITEK® MS Knowledge Base v3.2 has been built using a variety of organisms from both clinical and industrial environments.

VITEK® MS Knowledge Base v3.2	Claimed
Total species bacteria + fungi (including groups)	1,316
Bacteria	1,095
Fungi	221

Table 1. Content of VITEK® MS Knowledge Base v3.2

From a survey of 3000 isolates, 270 isolates representing 47 genera and 142 species were selected from an ID service lab performing microbial identification for pharmaceutical manufacturing companies. These isolates were sub cultured and spectra were collected as described in **Figure 3.**

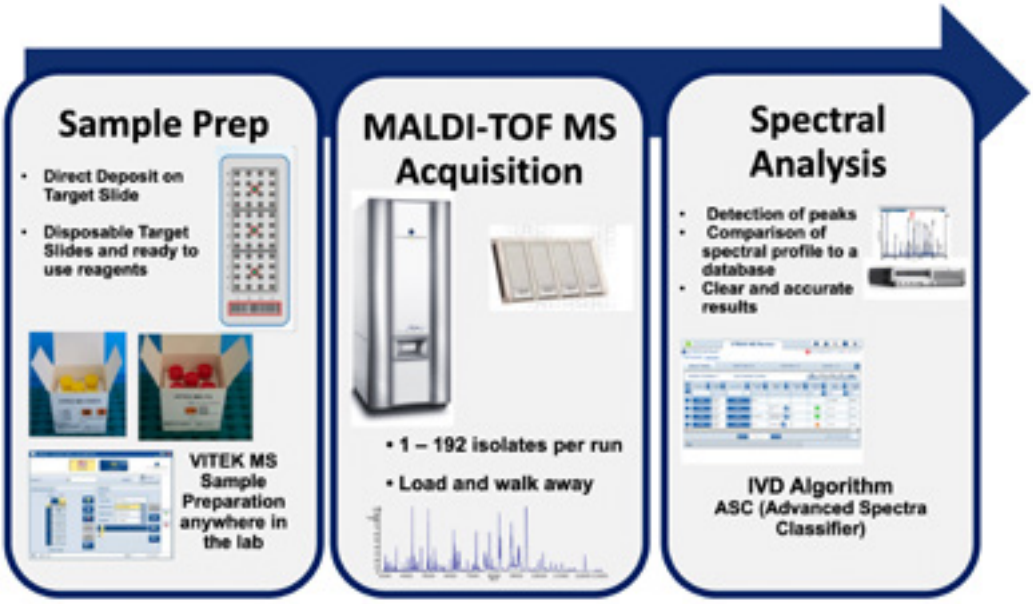

Figure 3. VITEK® MS workflow for Identification

The spectra were analyzed against the VITEK® MS Knowledge Base v3.2. The VITEK® MS ID obtained from the workflow indicated in **Figure 3** was compared in each case to 16s RNA gene sequencing following the workflow described in **Figure 4.**

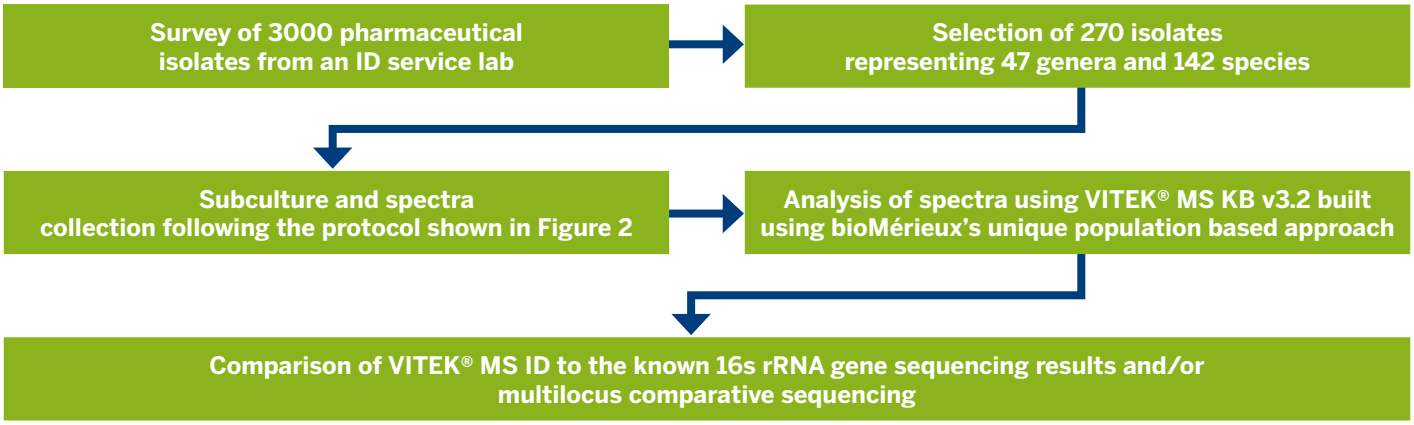

Figure 4. Workflow of the study

The **Table 2** below shows the most frequently observed species found by this study.

Frequently Identified Genera	Species identified within the genus
Micrococcus	*M. luteus*
Bacillus	*B. cereus group, B. licheniformis, B. subtilis, B. atrophaeus, B. simplex, B. circulans, B. firmus, B. megaterium, B. mycoides*
Staphylococcus	*S. haemolyticus, S. cohnii, S. aureus, S. epidermidis, S. hominis, S. warneri, S. capitis, S. lugdunensis, S. saprophyticus*
Paracoccus	*P. yeei*
Pseudomonas	*P. aeruginosa, P. stutzeri*
Stenotrophomonas	*S. maltophilia,*
Burkholderia	*B. cepacia group*
Corynebacterium	*C. amycolatum, C. diphtheriae, C. striatum, C. jeikeium, C. mucifaciens, C. ureicelerivorans*
Ralstonia	*R. pickettii, R. insidiosa*
Sphingomonas	*S. paucimobilis*

Table 2. Most frequently observed species

The VITEK® MS results were in concordance with the reference identification for 246 isolates to the genus level (91,4%) and for 236 isolates to the species level (88,1%). 23 isolates (8,6%) were not identified and 9 isolates (3,4%) were misidentified **(Figure 5).** Among the 23 not identified isolates, 15 isolates were not claimed in the database. **Table 2** illustrates the most frequently identified genera in the set of samples analyzed. Of all isolates collected during the study period, 94.5% are present in VITEK® MS Knowledge Base v3.2 **(Figure 6).**

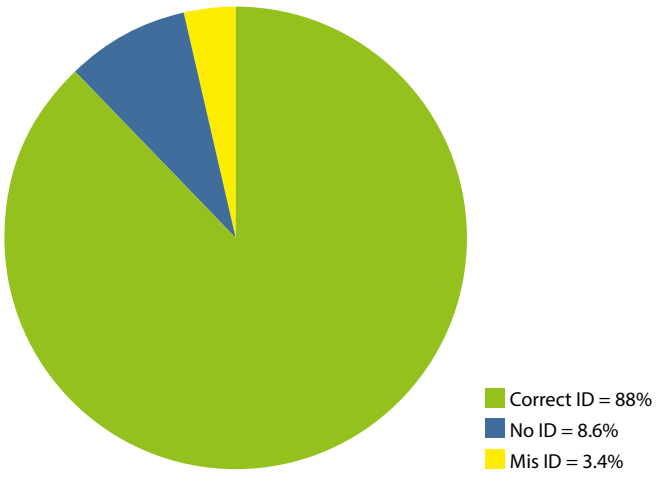

Figure 5. Final ID results

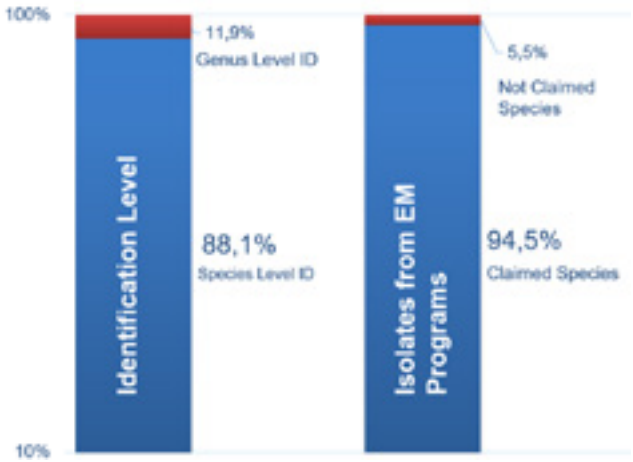

Figure 6. Summary of the accuracy of VITEK® MS Knowledge Base v3.2

CONCLUSION

All together, these data demonstrate the accuracy of bioMérieux's latest VITEK® MS Knowledge Base which uses Matrix-Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry (MALDI-TOF) technology. bioMérieux's solution provides reliable identification of routine pharmaceutical isolates and can therefore be used for rapid high-throughput identification of microorganisms from pharmaceutical manufacturing environments.

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