ACCURATE IDENTIFICATION OF ENVIRONMENTAL BACTERIA BY MALDI-TOF MASS SPECTROMETRY USING VITEK® MS

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BIOMÉRIEUX

The relevance of a robust knowledge base for routine environmental monitoring

ABSTRACT

Used in contamination investigations and to understand the microbial ecology of a manufacturing facility, accurate microbial identification (ID) is an essential part of pharmaceutical quality control testing. In this study we demonstrate the accuracy of bioMérieux's latest VITEK® MS knowledge base (KB) which uses Matrix-Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry (MALDI-TOF) for ID of microorganisms in pharmaceutical environments.

Data presented in this poster utilizes the latest VITEK MS KB v3.2 that was built with multiple well-characterized environmental strains, tested using various media and incubation times and coming from different regions of the world. In this study, we assess the performance of this new knowledge base using 270 isolates representing 47 genera and 142 species including organisms frequently observed in pharmaceutical environments.

The VITEK MS results were in concordance with gene sequencing for 246 isolates (91, 4%) to the genus level and for 236 isolates (88,1%) to the species level. 23 isolates (8,6%) were not identified and 9 isolates (3,4%) were misidentified. Among the 23 not identified isolates, 15 isolates (5.5% of all isolates) were not claimed in the database.

These data demonstrate that the VITEK MS provides reliable identification of routine pharmaceutical isolates using the latest robust knowledge base v3.2 and can therefore be used as an accurate identification platform in pharmaceutical companies.
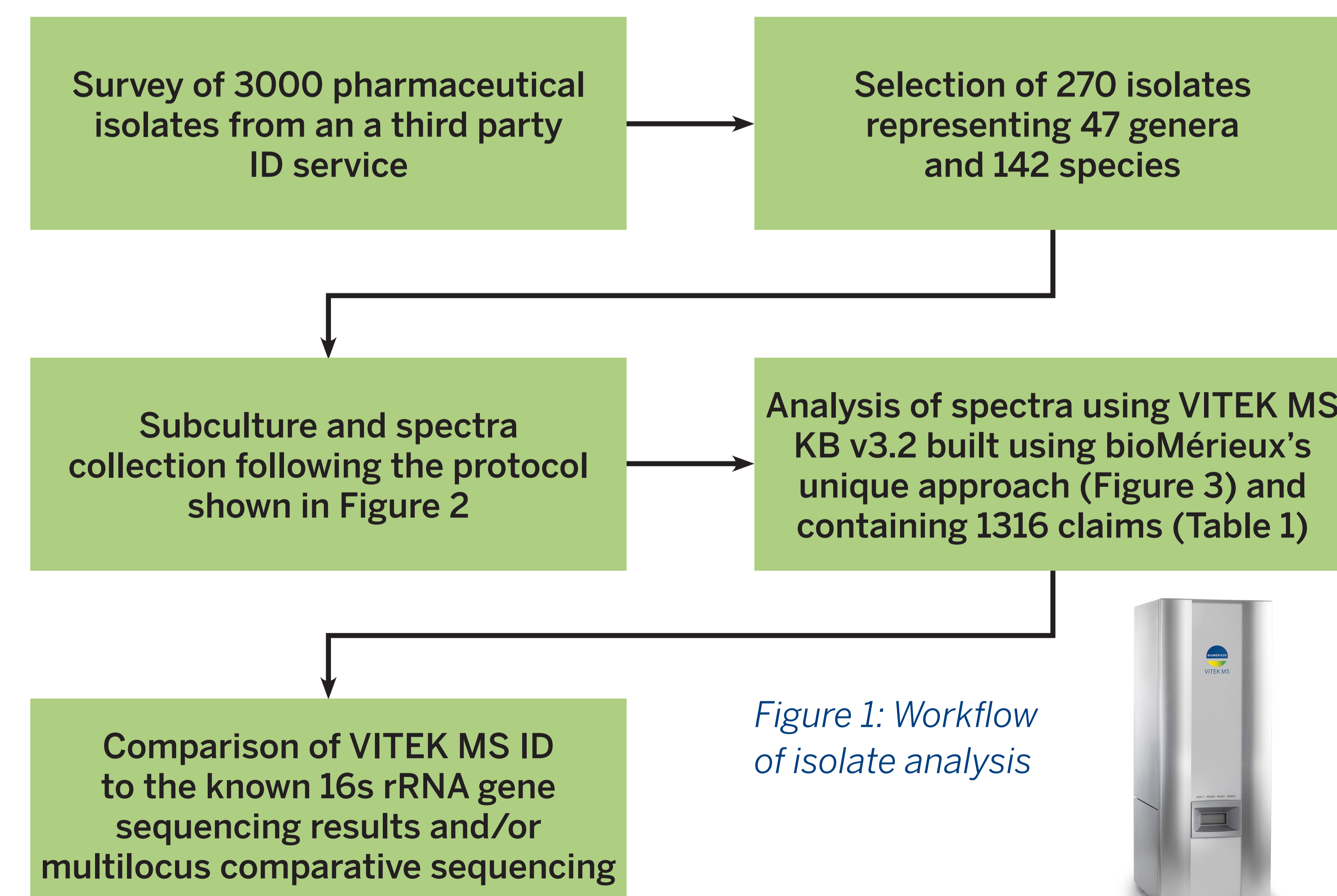
MATERIALS AND METHODS

Survey of 3000 pharmaceutical isolates from an a third party ID service

→ Selection of 270 isolates representing 47 genera and 142 species

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Subculture and spectra collection following the protocol shown in Figure 2

→ Analysis of spectra using VITEK MS KB v3.2 built using bioMérieux's unique approach (Figure 3) and containing 1316 claims (Table 1)

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Comparison of VITEK MS ID to the known 16s rRNA gene sequencing results and/or multilocus comparative sequencing

Figure 1: Workflow of isolate analysis

Figure 2: Spectra acquisition workflow with VITEK MS

Pick Colony | Smear on the target slide | Add Matrix and dry | Load into the VITEK® MS

Figure 3: Unique approach to building a robust knowledge base.
VITEK® MS v3.2 database used in this study was designed to incorporate intra-species and technical variability encountered in the field. Development used well-characterized strains coming from different regions of the world, different samples, including environmental strains which were tested using various media and incubation times, in order to make a robust knowledge database.

Microbial Variability

Intra species variability covered by multiple strains from different sources

Relative similarity

Population based 12 strains/species

Technical Variability

Media

Operators & Systems

Incubation Time
24H 48H 72H
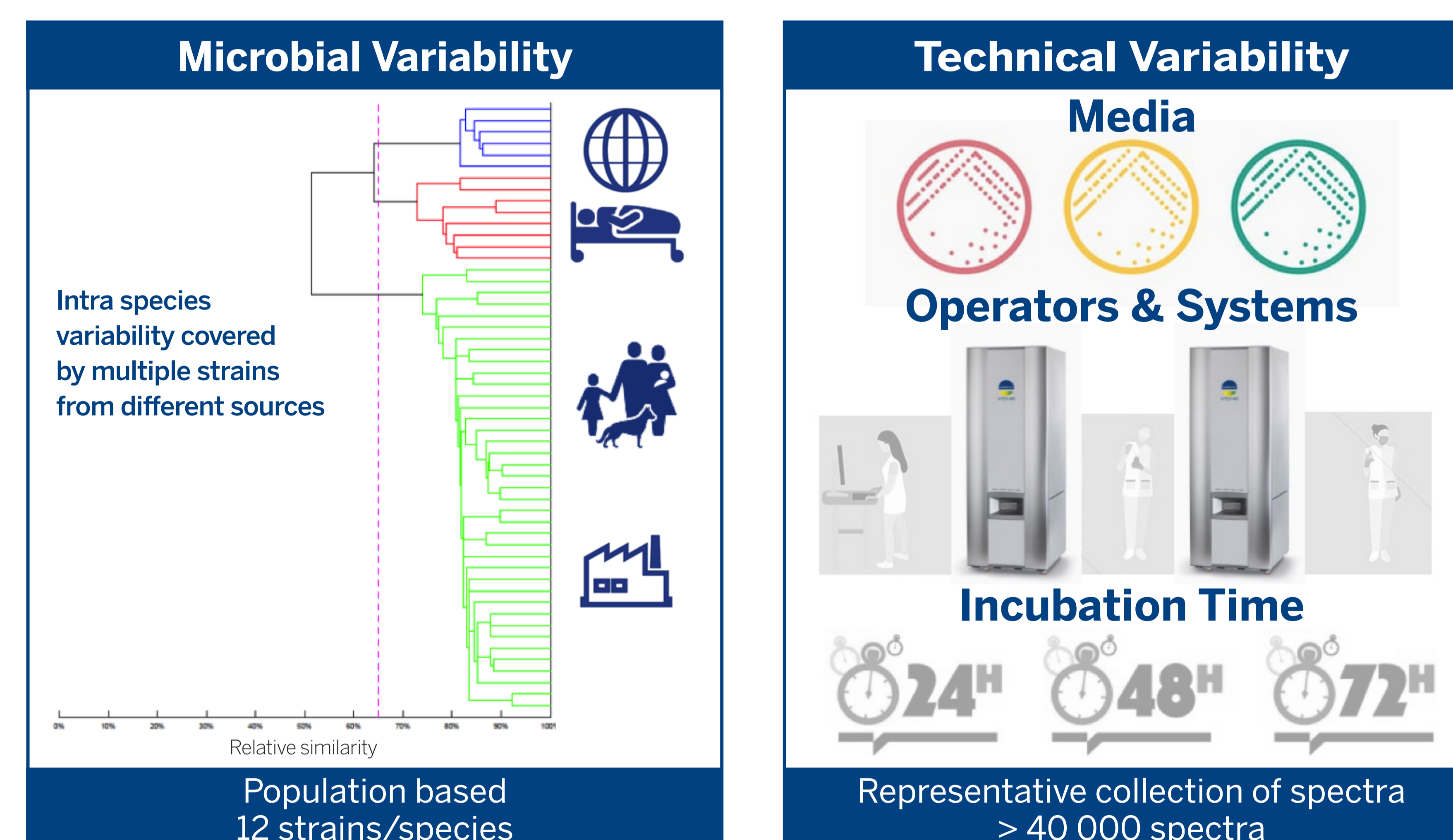
Representative collection of spectra > 40 000 spectra

Table 1: KB V3.2 species claims. VITEK® MS v3.2 KB contains a variety of organisms from both clinical and industrial settings including more than 1300 species of bacteria and fungi. This study focused on isolates from environmental monitoring programs with frequently observed species included in the study.

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

RESULTS

Table 2: Species tested during the study.

Top 10 Most 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

The VITEK MS results were in concordance with gene sequencing for 246 isolates (91, 4%) to the genus level and for 236 isolates (88,1%) to the species level. 23 isolates (8,6%) were not identified and 9 isolates (3,4%) were misidentified.

Of the 100 most frequently occurring pharmaceutical isolates used in this study, VITEK MS KB v3.2 contains 95% as claims.
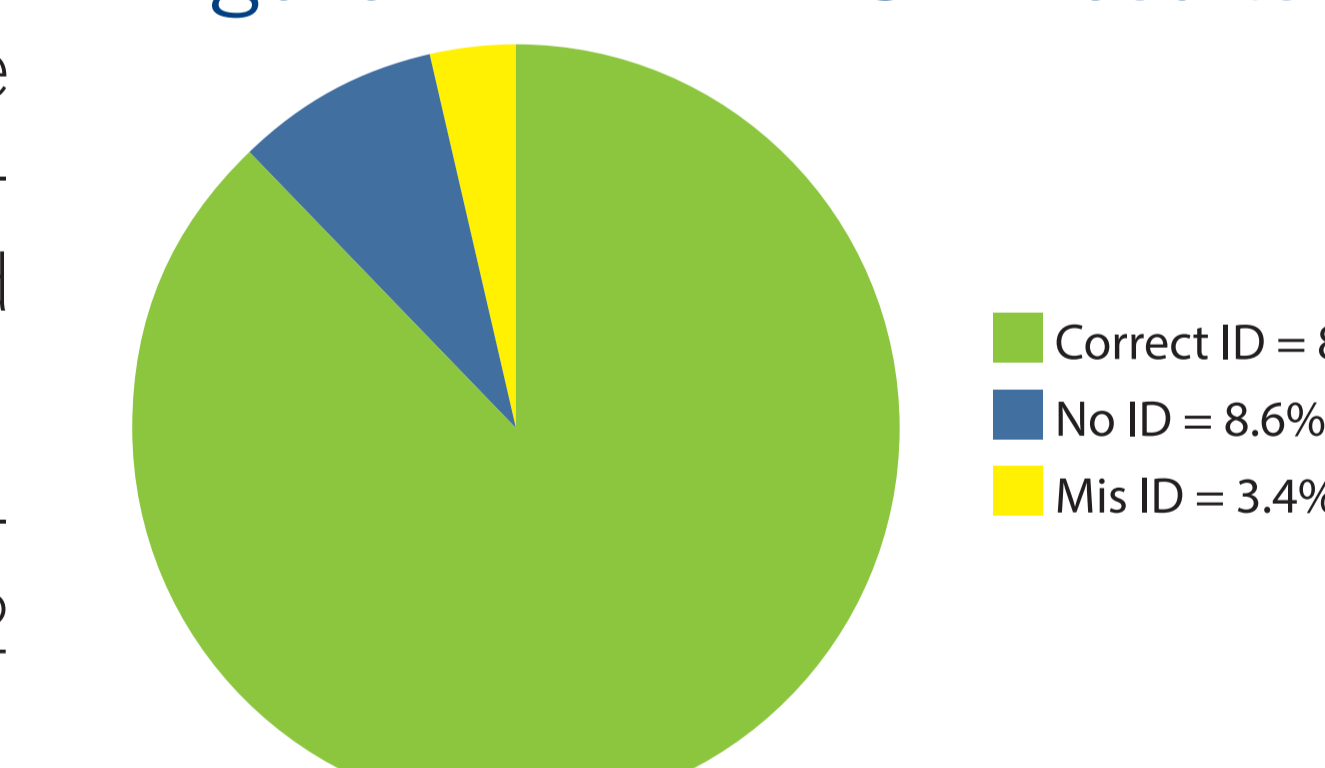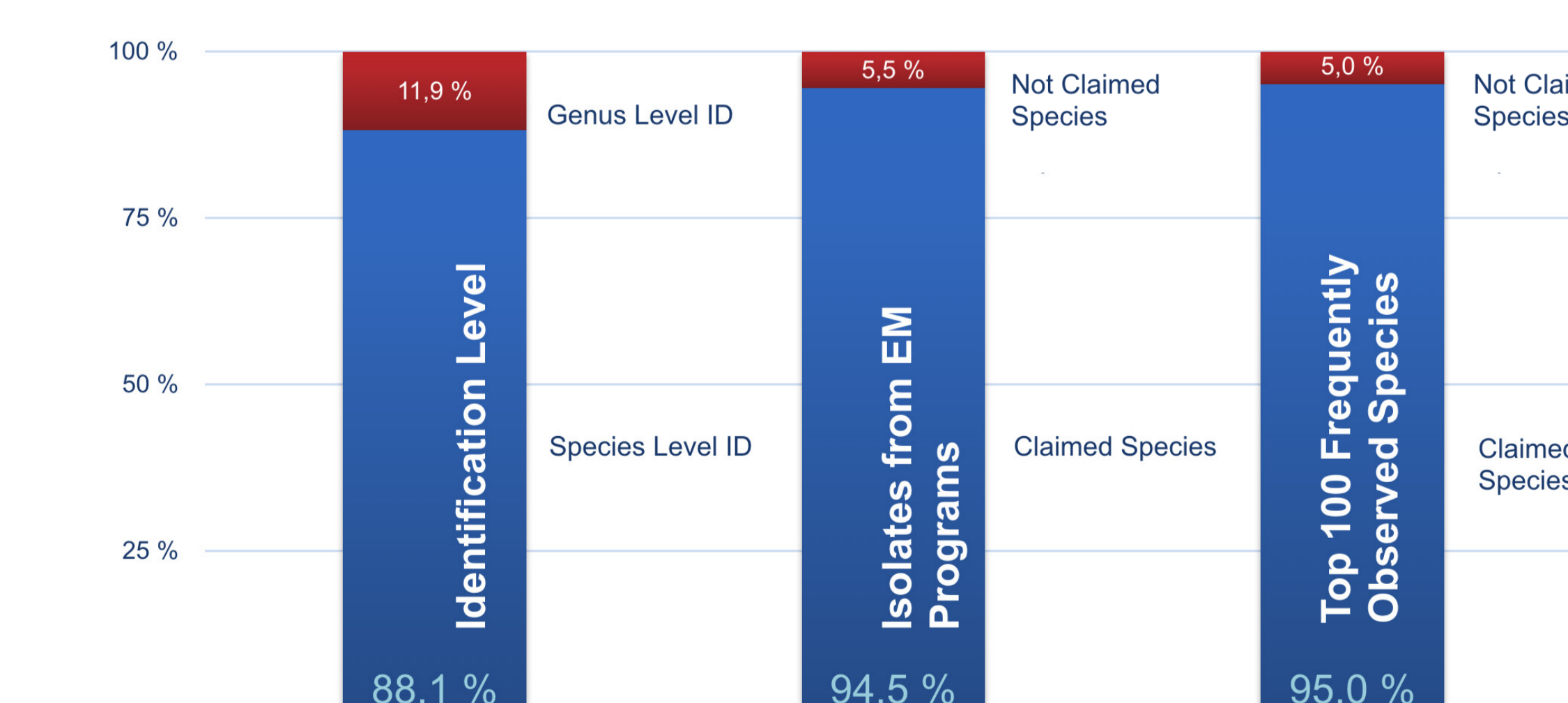
Figure 4: VITEK MS ID results

Correct ID = 88%
No ID = 8.6%
Mis ID = 3.4%

Figure5: VITEK MS Knowledge Base v3.2 Performance

Identification Level — Genus Level ID 11,9 % / Species Level ID 88,1 %
Isolates from EM Programs — Not Claimed Species 5.5 % / Claimed Species 94,5 %
Top 100 Frequently Observed Species — Not Claimed Species 5.0 % / Claimed Species 95,0 %

CONCLUSION

These data demonstrate that the VITEK MS v3.2 knowledge base provides reliable identification of routine pharmaceutical isolates and can therefore be used as a high-throughput MALDI-TOF identification platform in pharmaceutical companies' environmental monitoring programs.