

One Laboratory's View of Microbial Identification and the State-of-the-Art

Authors : N. Berthoumieu*, D. Pincus⁺, C. Somogye⁺

(*) Leo Pharmaceuticals

(+) BioMérieux

The decision process for how to identify microbes typically leads to a review of available commercial systems and planning for the validation and implementation of new SOPs. At Leo Pharmaceuticals, an objective approach was used, keeping the mission of the laboratory first, to provide accurate and efficient identification of bacteria and yeast. Leo Pharmaceutical is a sterile manufacturing site producing filled syringes for human therapy. At Leo Pharmaceuticals, we incorporated a review of the history of microbiology, which helped to determine what changes could be pragmatic and implemented in the routine microbiology laboratory, profiting from advances that impact microbiology.

One of the most significant contributions to the field of microbial ID and the early development of phenotypic characterization was the Gram stain developed in 1884 by Hans Christian Gram, still used today as the starting point to determine the correct choice for subsequent phenotypic test methods^{1,2}.

Up until the late 1960s, scientists were restricted to using conventional tube or plate media in order to perform the characteristic tests useful in microbial identification. This time marked the introduction of commercial methods, which began as simple combinations of key tests (e.g., triple sugar iron agar) and progressed to sophisticated methods employing a multitude of biochemical substrates and innovative data analyses.

With the availability of many more molecular data, the 2nd edition of Bergey's Manual of Systematic Bacteriology began publication in a phylogenetic format. Today's taxonomists unanimously require polyphasic approach to name new species (i.e. chemical, phenotypic and molecular data). All taxa are thus described with the inclusion of phenotypic data, which are the most relevant and practical for microbial identification of most unknown isolates in routine microbiology laboratories³.

We have always been attracted to the history of microbial identification and expertise provided to laboratories with early innovations and how these trends led to the development and introduction of commercialized, automated methods.

During our system selection process, we acknowledged that rapid microbiology methods offered a significant improvement over traditional manual methods. We considered various alternative technologies including nucleic acid based techniques. We decided to use the VITEK[®] 2 Compact rapid microbiology method over other methods as it fully meets the needs of our routine laboratory. Colleagues share this opinion, as evidenced by early and recent publications⁴. We decided that the most practical approach for the routine workload in a

microbiology laboratory is use of phenotypic methods, in order to meet the needs of today's microbiology laboratory.

VITEK 2 compact

The VITEK[®] 2 Compact was launched in 2005 and is a state of the art identification system. This system benefits from development of new chromogenic substrates, proprietary probabilistic algorithms, novel optical systems, advanced levels of automation, one of the world's largest collections of well-characterized microorganisms, and an expanded database focusing on pharmaceutically relevant microorganisms. The extensive engineering background of bioMérieux enabled introduction of robust automation.

The this system meets all regulatory requirements for automated identification of microorganisms found in the pharmaceutical industry environment and employs reagent 'cards' allowing for detection of various metabolic processes from an unknown microbial isolate. When these metabolic activities are assessed and compared to knowledge bases containing strain data, the microbiologist is given the final identification.

Reagent Cards

There are currently six reagent cards available for the identification of different organism classes as follows:

1. GN - Gram-negative fermenting and non-fermenting bacilli
2. GP - Gram-positive cocci and non-spore-forming bacilli
3. YST - yeasts and yeast-like organisms
4. BCL - Gram-positive spore-forming bacilli
5. NH - fastidious Gram-negative bacilli
6. ANC - anaerobic & coryneform bacteria

Product-specific details for each of the identification cards are shown below in Table 1.

Selection of the Appropriate Microbial Identification System

The goal of this summary is twofold: 1) to illustrate how microbial identification with the VITEK[®] 2 Compact meets our needs, and possibly the needs of other laboratories for pharmaceutical regulations, guidances, and related recommendations and 2) to show that the the system provides reliable identifications. While it is neither the intent nor the goal to show deficiencies or positive attributes of other systems considered, references are listed for the reader to facilitate literature review.

It will be the reader's responsibility to identify which regulations or recommendations impact the laboratory, review references for identification systems under consideration, and reach the appropriate conclusion. In such a

literature review, one should take note of several points that can help in the best system selection. At Leo Pharmaceutical, a carefully constructed URS, user requirements specification, clearly identified our needs and kept the selection team objective. The selection team was multi-functional, also assuring objectivity, and had participation from the microbiology laboratory, quality management and our financial department.

One must first identify regulatory-related needs (see Table 1):

- Which regulations impact your laboratory? USP, FDA, EP, JP, PIC\S?
- How many people (if any) in the lab can be fully dedicated to handling the ID instrument
- What is the ratio between routine identification or investigation?

Table 1. Regulatory Guidelines for Identification Practices

Norm	Recommendation	VITEK® 2 Compact Answer
EC Guide to GMP, Revision to Annex 1, Manufacture of Sterile Medicinal Products ⁵	➤ Advises checking background environments, which are indicators of Grade A trends.	➤ Cost effective and ease of use for building a database with frequent analysis
EP 2.6.1 Sterility Test (harmonized with USP & JP) ⁶	➤ Conventional microbiological/biochemical techniques(...) are satisfactory for (...) a sterility test.	➤ Method giving identification to species or subspecies is adapted for initiation of investigation
FDA Guidance for Industry – Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice, Sept 2004 ⁷	<ul style="list-style-type: none"> ➤ Appropriate biochemical (...) methods can be used for the routine identification of isolates. ➤ Monitoring (...) clean areas as well as personnel should include routine identification(...) ➤ When comparing results from environmental monitoring and sterility positives, both identifications should be performed using the same methodology. 	<ul style="list-style-type: none"> ➤ Method giving identification down to species or subspecies adapted for initiation of investigation ➤ Cost effective and ease of use for building a database with frequent analysis ➤ Routine use and customer database expansion feature allows comparison of results throughout the entire plant.
PIC/S (Pharmaceutical Inspection Co-operation Scheme) - Recommendation on Sterility Testing ⁸	➤ Routine identification (...) to at least the genus level should assist in detecting trends.	➤ Standardized & adapted for routine use; reproducible to at least the genus level.
USP 1116 - Microbiological Evaluation of Clean Rooms and Other Controlled Environments ⁹	➤ Routine identification needed for tracking contaminants, trending OOS and validation of	➤ Identification followed by typing investigation.

	cleaning procedures.	
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Literature review for identification system selection:

- When choosing an identification system, a detailed comparison of the URS, and the described features in the literature should be done. The objectivity of the study should be considered, e.g., was there involvement of the manufacturer in the performance evaluation?
- The second point to consider is how the experimental design was set, e.g., number of species tested, number of isolates representing each species, statistical significance of numbers used in the assays. Also, one should consider the presence of negative controls (i.e., did testing include species not identified by the product and if so, were these included in the data tabulation?). What was the reference method employed? Was the scheme for retesting reasonable and what one would expect in routine use? Were the results evaluated fairly in the same manner as you would?
- Was the product used correctly as specified by the manufacturer or did the evaluator modify the procedure and thereby, impact the results? For example, were the isolation medium, culture incubation conditions, suspension turbidity, etc. all as specified in the manufacturer's instructions.
- Is the product and/or software (if applicable) the most current available or is this an outdated evaluation?

Often, one can find these evaluations online without needing a subscription to the scientific journal. A useful link that will lead to the abstract and may also lead to the respective publication is:

<http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed>

Given the organizational constraints in the lab and hands on time for sample preparation associated with genotypic testing, a careful review of the literature is needed in order to select the most reliable identification method. Selection criteria, should be established in advance and should include accuracy, reliability, ease of use, and cost with emphasis on which method fulfills your regulatory needs for routine identification. These factors should all be taken into account to select the best method to incorporate for routine microbial identification. For high volume microbiology laboratories, automated systems are invaluable. As with any decision, take care not to seek the information you want to find, creating a biased literature review. It is well worth the time and effort to search a variety of published sources and discuss with other laboratories using the systems available. Document the results of a literature search or colleague interviews, sticking to facts. Subsequent review will aid in your team's decisions making.

Validation for Routine Use: a customer perspective (Leo Pharmaceutical Laboratories)

The VITEK[®] 2 compact was installed in Leo Pharmaceutical laboratories in 2005, making it one of the first laboratories to validate the system for routine use. A trainee was dedicated to this task for 6 months to do all the qualifications necessary.

The bioMérieux package accelerated the validation process as it provided templates for IQ, OQ, and PQ. However, advanced planning for procurement of Quality Control organisms may be required depending on one's location. Unfortunately, since this instrument was one of the first installed, it was not until the end of the qualification that a local company providing QC strains began to market a package with all the organisms. Other multinational organizations offer similar QC organism packages.

The IQ and OQ were successful without incident. Despite the availability of a built-in thermometer, a temperature sensor was added to the the system in order to have temperature verification at all times. During the PQ, typical troubleshooting identified an issue with a QC organism that was too old. Once a fresh subculture was prepared, performance was acceptable.

One of the organisms used for PQ, *Geobacillus stearothermophilus*, initially had an AGAL test, which was negative instead of the expected positive. As an early user, Leo Pharmaceutical helped bioMérieux to identify the need to change this test to "variable". This aspect has been corrected for improved identification in a subsequent software update through collaboration with bioMérieux. One difficulty that all laboratories may face during qualification is comparing the new method to a previous method. If the results are different, which are the good results? Many publications still compare 16S 500 bp to another method having in mind that this is the "reference" whereas this technology does not provide more accurate results and there can be discordances with both techniques. This is especially true in the case of our example where Bacillus species are extremely difficult to separate by 500bp sequencing. Laboratories should exercise caution to not assume additional work (e.g., performance comparison to predicate device) that will not be value-added. Without an appropriate reference method, it is impossible to know which system gives the correct result in case of discrepancy.

The QC organisms recommended by bioMérieux were a good choice for reagent and reproducibility control. However, the occurrence of other species in Leo Pharmaceutical applications, made it absolutely necessary to validate the performance of the system with isolates from the plant.

The integration of VITEK 2 Compact in the laboratory was accepted very easily. During qualification activities, new procedures were written, all the microbiology technicians were trained, and no problems were encountered with

implementation of the system. It was accepted as a standardized process for identifying organisms. The old problems of reading the manual galleries were obsolete. The technicians no longer questioned “what is the color of this well?”

In a sterile plant such as the one of Leo Pharmaceuticals, several sterile areas are controlled and if an organism is found in a class A area, the identification must be made as quickly as possible. The VITEK 2 Compact is the only way to work quickly. In contrast to other techniques the preparation time for the inoculum is really minimal. It takes less than five minutes to start the analysis. No centrifugation, extraction, controls or purification are required before launching the test in the instrument.

In case of detecting a new organism, it is possible to include this organism in a customizable database SRF (supplemental react file). It is recommended to have several occurrences of such an organism, before entering it in the SRF. Also, it can be sent to a microbiology expert laboratory for polyphasic identification so that the SRF file is generated with the most accurate data. A similar approach can be taken in the rare case where VITEK 2 Compact identifies an organism with low accuracy. But it is evident that the requirement for polyphasic identification is infrequent. A polyphasic approach using complex chemical, molecular and phenotypic methods cannot be used routinely in the lab. Leo Pharmaceuticals chose the technology of VITEK 2 Compact as the best way of working in the laboratory.

Culture Requirements

The technical product information lists a range of flexible parameters for appropriate culture and inoculum preparation, which include the acceptable ranges for isolation media, temperature, atmosphere, age of culture incubation, and organism suspension turbidity. The inoculum turbidity is adjusted accordingly (see Table 2) and measured using a hand-sized instrument called the DensiChek™.

Table 2. VITEK® 2 Compact Product Features

Product	Number of Substrates	Number of Taxa Claimed	Inoculation Turbidity (McFarland Range)	Incubation Time (h)
GN	47	165	0.50 – 0.63	3 – 10
GP	43	119	0.50 – 0.63	2 – 8
YST	46	54	1.80 – 2.20	18
BCL	46	46	1.80 – 2.20	14
NH	30	27	2.70 – 3.30	6
ANC	36	63	2.70 – 3.30	6

Inoculation, incubation and interpretation

Reagent cards are inoculated automatically with organism suspensions using integrated vacuum fillers. Incubation of cards, reading and interpreting results are also functions integrated in the system.

The bioMérieux product information was very clear and thorough and additionally, the technical support team was easily accessible for all of our questions. This was instrumental for implementation into routine use and a smooth transition for the staff.

Summary

The VITEK[®] 2 Compact system has been evaluated against various reference methods including molecular characterization and the data show that VITEK 2 Compact provides an excellent solution for accurate microbial identification in a routine environment.

The VITEK[®] 2 Compact is at the state-of-the-art for automated identification of quality at the species level¹⁰⁻²³.

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