

Qualification of a Microbial Identification System

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"Microbiology Topics" discusses various topics in microbiology of practical use in validation and compliance. We intend this column to be a useful resource for daily work applications.

Reader comments, questions, and suggestions are needed to help us fulfill our objective for this column. Please send your comments and suggestions to column coordinator Scott Sutton at scott.sutton@microbiol.org.

IMPORTANT POINTS

The following important points are discussed:

- There are a variety of microbial identification technologies on the marketplace available to the quality control laboratory.
- The choice of which technology to use is one that must be approached with a firm grounding in the requirements of the facility for cost, turnaround time, capacity, level of identification, and many other considerations.
- Development of a user requirements specification document should be a priority to gain clarity in requirements and to build consensus among management.
- Once the appropriate technology is determined, qualification of that technology should ensure that it meets user requirements.

INTRODUCTION

Microbial identification plays a central role in the quality control (QC) microbiology lab, both in support of product testing as well as support of the cleanroom control program (1). The method of identification, however, must be wedded to the need. For example, any organism isolated from the critical aseptic processing area must be

identified to great detail, while those from class D/ISO 8/100,000 areas might only be characterized to the genus level. In addition, current regulatory expectations require that identifications used in investigations of microbial data deviations (MDD) will be accurate to the strain level.

Most identification schemes still rely on the Gram stain, a differential staining technique developed in the late 1800s by Christian Gram (2). This differential counterstaining technique is good at distinguishing a real difference in cellular morphology. Unfortunately, this method is prone to a significant level of operator error, which has encouraged the development of alternate methods for showing the difference in cell structure (3, 4). Traditional methods of identification also consider a variety of phenotypic characteristics.

The following discusses phenotypic methods, genotypic methods, proteotypic methods, considerations for selection, and qualification (accuracy and reproducibility) of methods.

PHENOTYPIC METHODS

Phenotypic methods typically incorporate reactions to different chemicals or different biochemical markers. The active pharmaceutical ingredient (API) strip is basically a prepackaging of the standard method that required racks of test tubes into a convenient bubble-wrap. This method was further refined in the Vitek automated system that miniaturized the process (5, 6). This system has been enhanced to provide greater resolution of microorganisms (7, 8).

Biolog, Inc. offers a second phenotypic system. The fundamental unit in this system is a 96-well plate that has different carbohydrate sources in each well, with a tetrazolium redox dye. If the microorganism is capable of

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utilizing the carbohydrate, the well turns dark indicating reduction of the dye (9, 10). The end result is a pattern of wells (a “metabolic fingerprint”) that allows the user to identify the unknown microorganism. This method has been extended to include the identification of molds and filamentous fungi with a proprietary software package.

The use of cellular fatty acid (FA) composition to identify the genus and species has been popular for several years (11, 12). The fatty acids are extracted from the cell cultures and then the patterns of fatty acid esters are determined by gas chromatography (13).

There are some new methods under development for the pharmaceutical QC lab. These include Fourier-Transform Infrared (FTIR) microscopy (14) and Matrix-Assisted Laser Desorption Ionization–Time of Flight (MALDI-TOF) mass spectroscopy (15, 16). However, these have not seen widespread use in the QC lab as of yet. As they share some common analytical characteristics, the current convention is to refer to these methods by the alternate description of “Proteotypic” methods.

GENOTYPIC METHODS

FDA has elevated the use of genotypic identification methods with the release of the revised aseptic processing guidance document late in 2004 (17).

The Riboprinter is fundamentally an automated Southern Blot apparatus using labeled ssDNA probe from the 16sRNA codon. The resulting pattern is then used to identify the unknown microorganism (18, 19). If the initial banding pattern is inconclusive, then the restriction endonuclease can be changed to provide an extraordinary level of strain discrimination (20).

Another genotypic identification system on the market is the MicroSeq 500 16S rDNA Bacterial Sequencing Kit that is offered by Applied Biosystems. As the name implies, it provides the materials needed to sequence the first 500 basepairs of the unknown microorganism’s 16s ribosomal RNA codon (21). The technology involves amplification of the 16S codon by polymerase chain reaction (PCR), followed by automated sequencing.

A final genotypic method that is being marketed into the QC pharmaceutical laboratory is the Bacterial Barcodes system (22). This system is also based on PCR technology, using as a primer a sequence homologous to a repetitive sequence in the bacterial genome. The amplified sequence is then separated by gel electrophoresis and visualized to give the “barcode” specific to that strain.

Qualicon markets the BAX system to the food industry that contains primers for *Salmonella*, *Listeria*, or *E. coli* O157:H7 (23). This system has promise for determination of the absence of specified organisms in the product.

Other genetic methods have been published in the

literature, although few are available to the pharmaceutical market (24).

PROTEOTYPIC METHODS

There has been a good deal of activity in a new area of microbial identification technology—Proteotypic methods (25). These methods use chemical and physical means to elicit emissions from the unknown microorganism and base the identification on the analysis of the emission spectra from that particular sample. Current technology in this area includes Fourier-Transform (FTIR) spectroscopy, MALDI-TOF, and Raman spectroscopy. FTIR and Raman spectroscopy are still a bit out of reach in terms of the QC lab, with little or no support in terms of validated identification databases and support for good manufacturing practice (GMP) qualification procedures. All of these technologies should be evaluated in terms of the relevance and breadth of their supporting databases to microorganisms found in a facility.

The most widely used of these methods at the present time is MALDI-TOF, with two separate vendors introducing products into the pharmaceutical marketplace. This method has some significant potential advantages, requiring little in terms of sample volume, low consumable costs, and providing immediate results. It suffers from the potential disadvantages of any leading edge technology in the QC lab.

HOW TO CHOOSE?

There are a variety of identification technologies available. When choosing one for the lab you must bear in mind the strengths, and weaknesses, of the various methodologies. For example, the FDA aseptic processing guidance document strongly recommends the use of genotypically-based methods (17). However, if a PCR-based method or DNA sequencing is chosen, there is potentially an associated cost in facilities, labor (i.e., highly-skilled technicians), and maintenance that is not present with the more traditional methods.

The most direct approach to deciding the appropriate technology is to research the choices fully based on an understanding of what the requirements may be. The development of a user requirements specification (URS) document is recommended to drive this process. This is a formal quality document, similar in concept to a design qualification document. Different companies will have different formats for these documents. The primary essential features of the document will be that it has the essential requirements and that it has upper management sign-off. For a variety of reasons it is a good idea to document upper-management commitment.

A partial list of topics to be covered in any URS designed for an identification system should include the following:

- Assay throughput. How many samples a day?
- Assay time-to-completion. How quickly?
- Cost of consumables. How much? Frequently the cost of consumables can soon dwarf the capital expense.
- Labor requirements. Including the technological sophistication of the operators—can the technicians actually operate the equipment reliably?
- Size of microorganism identification database. This is a major consideration. If you purchase two systems to cover identifications of unknowns, it is imperative to ensure that the databases are large and complementary; that is they both don't have the same organisms in them, but that they include many different ones as well.
- Facility requirements (e.g., electrical and plumbing, but also less obvious concerns about RNA/DNA contamination and cleanroom issues).
- Compatibility with existing systems (e.g., LIMS, workflow).
- Need for physiological information. Do you need to know if the organisms are capable of degrading your product components? You may want to use a system that will help determine this.
- Purpose—Routine ID or investigations. The use of the system may be different for different objectives. A good system for routine work may not be the best for investigations, and vice versa.

In short, there are a wide variety of choices available to help with the identification of unknown organisms. It is important to define the specific requirements and to purchase the appropriate system to meet those needs. This is also the first step in qualifying the microbial identification technology for use in the lab.

QUALIFICATION OF THE TECHNOLOGY

The United States Pharmacopeia (USP) published a draft informational chapter to assist in designing a qualification for identification technologies (26). USP chapter <1113> describes, in some detail, the different technologies and then moves into "Verification" of the methods. In this description, after the expected requirements of installation qualification (IQ) and operational qualification (OQ), the parameters of "accuracy" and "reproducibility" are highlighted as the most important of the verification tests.

Accuracy and Reproducibility

One of the challenges in microbial identification is that all names are, by their nature, arbitrary. This is

true even in the genus-species naming conventions of biology. All identification systems measure some aspect of the microorganism, compare the response to a database of similar responses, and then provide the user with the best match to the observed results. This may, or may not, result in two different technologies providing the same name for a particular unknown microorganism. Therefore, it is ill advised to require a new system to meet the results of a "gold-standard" method. There are no gold standards in common use. Having said that, there still needs to be a measure of accuracy. The draft USP chapter defines % accuracy as $(\text{Number of correct results}/\text{total number of results}) * 100$. In this case, the "correctness" of the result can be defined by authority (i.e., use of ATCC-type strains or in-house strains identified by several different methods consistently). Reproducibility relies on the work done for accuracy and is defined in the draft chapter as $(\text{number of correct results in agreement}/\text{total number of results}) * 100$. The user is expected to achieve numbers of >90% for both measures.

There are additional qualification (i.e., verification) measures suggested in the draft informational chapter. However, these are of value mainly to a comparison of two methods, one of which is expected to be significantly superior to the other. While these measures are of interest, they may not be appropriate in all studies.

One aspect of method qualification that cannot be overlooked, however, is performance qualification (PQ). This step should be devoted to establishing whether or not the user requirements established in the beginning of the process were met. An excellent description of the purpose and design of the PQ can be found in another USP informational chapter devoted to analytical instrument qualification (27). The importance of this step cannot be overstated, as it is the only real opportunity to confirm the desired functionality of the equipment as part of the qualification protocol.

CONCLUSIONS

There are a variety of microbial identification technologies on the marketplace available to the QC laboratory. The choice of which to use is one that must be approached with a firm grounding in the requirements of the facility for cost, turnaround time, capacity, level of identification, and many other considerations. Development of a user requirements specification document should be a priority to gain clarity in requirements and to build consensus among management. Once the appropriate technology is determined, qualification of that technology should ensure not only that it works, but that it also meets user requirements.

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ARTICLE ACRONYM LISTING

API	Active Pharmaceutical Ingredient
FA	Fatty Acid
FDA	US Food and Drug Administration
FTIR	Fourier-Transform Spectroscopy
GMP	Good Manufacturing Practice
IQ	Installation Qualification
MALDI-TOF	Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry
MDD	Microbiological Data Deviation
OQ	Operational Qualification
PCR	Polymerase Chain Reaction
PQ	Performance Qualification
QC	Quality Control
URS	User Requirements Specifications
USP	United States Pharmacopeia