



Microbial Identification in Pharmaceutical Compounding



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ABSTRACT

Compounding pharmacies and contract testing laboratories can readily utilize critical information that microbial identification methods provide. Rapidly identifying the genus and species of environmental isolates and sample contaminates provides pharmacies and laboratories the opportunity to determine the possible source and implement corrective actions to improve compounding and testing processes. The microbial identification data collected from a compounding environment is critical. It is important to have accurate and specific microbial information to guide environmental collection practices, validation studies, and troubleshooting initiatives. The different technologies available provide varying levels of identification. They range from phenotypic assays to more accurate molecular-based techniques, including macromolecular methods and whole genome sequencing. Selecting the appropriate identification methodology requires evaluating multiple factors including the level of information required (genus only, genus and species, etc.) and the pharmacy's tolerance for unidentified or incorrectly identified isolates.

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In response to increasing regulatory involvement by the U.S. Food and Drug Administration and other governing agencies, including state boards of pharmacy, pharmacists are making changes to their compounding practices. Recently, a heightened focus on laboratory quality-control and quality-assurance practices reveals a need for accurate identification of isolates found in compounding environments. As a result, more pharmacy and health-system laboratories are developing and implementing improvements to their existing environmental monitoring programs, as well as validating more stringent processes and procedures. Adding microbial identification testing to pharmacy and health-system laboratory standard operating procedures results in improved routine environmental data collection, facilitates more robust validation studies, and assists in troubleshooting product-testing failures.

IDENTIFICATION OF ENVIRONMENTAL ISOLATES

From a regulatory standpoint, the identification of microorganisms recovered from routine monitoring of controlled environments in a compounding facility is critical. The value of microbial monitoring of the compounding environment is realized when the data is used to identify and correct an unacceptable work practice.¹ Improved environmental monitoring programs represent an opportunity for compounding and health-system pharmacies to identify the organisms present and to determine trends in the types of organisms that represent

potential contaminants to their sterile preparations. Regardless of the number of colony forming units identified in the compounding facility, further corrective actions will be dictated by the identification of microorganisms recovered.¹ Potential routes of contamination, related to personnel, materials, and/or processes, can be identified and actions can be taken to reduce the risk of recurrence. Compiling historical data of the identification of common environmental isolates provides useful information regarding the source of the contamination² and aids in the timeliness of its correction. In contrast, misidentification could lead to inappropriate corrective actions.²

CLEANING EFFICACY STUDIES

The importance of developing a well-designed procedure for ensuring the sanitiza-



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tion of a controlled environment cannot be overstated. Environmental contact is a major source of microbial contamination of compounded pharmaceuticals. Consequently, scrupulous attention to cleaning and disinfecting the sterile compounding areas is required to minimize this as a source of compounded sterile preparation contamination.¹

The specific identity of the microorganisms in an area can greatly assist in the development, utilization, and continuous improvement of a cleaning and sanitization plan.³ Pharmacists and laboratory professionals can target those organisms that appear in the data collected from the environmental monitoring program.⁴ For example, if common environmental microorganisms such as *Bacillus subtilis*, *Clostridium sporogenes*, or *Streptomyces griseus* are isolated from the environment, a sporicidal agent should be added to the cleaning procedure. If molds or yeasts commonly appear in trending data, an antifungal-specific agent should be added. Microbial-identification testing aids in developing a custom cleaning strategy that targets known contaminants, providing a greater level of assurance for the quality of the finished preparations. Pharmacists and laboratory professionals can use the specific

microbial identification data collected from the facility to qualify a sanitization procedure with disinfectant efficacy studies and cleaning validations. In addition, it is recommended to periodically conduct disinfectant efficacy testing of the selected sanitizers, disinfectants, and sporicides if representative new isolates are routinely recovered in the environmental monitoring program.⁵

MICROBIOLOGY DATA INVESTIGATIONS

Microbial identification is used in support of all pharmaceutical microbial-testing and quality-control programs. Several *United States Pharmacopeia (USP)* general chapters specifically cite the requirement of microbial identification for varying purposes.² These purposes include confirming microbial test

results, invalidating microbial test results, and supporting quality-control data for both the compounding and the testing facility. If a contamination event occurs or a product fails microbial testing, the source can be more accurately determined if the identity of the contaminating microorganism can be compared to the environmental monitoring data.³ For this reason, compounding pharmacies and testing laboratories should have an up-to-date and thorough catalog of organisms identified in their controlled areas. The organism catalog of both the testing area and the compounding area can be compared to help determine whether a systemic issue exists with the compounding process, or to determine if contamination originated from the testing process itself.⁶ If a laboratory is contracted for sterility testing, it is recommended that a thorough environmental moni-

toring program be in place. The laboratory performing the test should also have investigational procedures in place where environmental isolates are identified and compared to the preparation test-failure isolates. The identification of isolates collected during and around the time of testing, as well as isolates known to be routinely recovered in the testing environment, provides critical information that aid in the investigation and any resultant corrective action.

METHODOLOGY OPTIONS FOR MICROBIAL IDENTIFICATION

The availability of multiple methodology platforms for selecting and implementing microbial-identification testing requires evaluation of multiple variables, including tolerance of the occurrence of contamination, level

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of strain identification, and costs. Each method has certain benefits as well as limitations that compounding pharmacies should consider before selecting and implementing into their quality operations.

PHENOTYPIC ANALYSIS

Simple phenotypic analyses can be used to differentiate between bacterial and fungal genera and species. Analysis can be conducted as individual tests or in a more comprehensive automated platform such as that of the Biolog Omnilog or Vitek 2 identification systems. Automated platforms that provide multiple phenotypic assays, typically based on a colorimetric change, are generated simultaneously, and the results are compared to a library to provide a classification or identification of genus and species. This method requires less interpretive expertise and is relatively inexpensive; however, it is considered less likely to produce species level data⁷ and can be confounded by differences between strains of the same species. Historically, phenotypic analysis has been considered a less-specific assay for obtaining the identification of microorganisms. Advancements over the last several years have produced improvements in design and library depth that allow closer accuracy to the macromolecular analysis.⁸

MACROMOLECULAR OR GENOTYPIC ANALYSIS

Macromolecular microbial identification methods may be less subjective, less dependent on the culture method, and theoretically more reliable than phenotypic methods.⁹ The two most common techniques for identification rely on analyzing either proteins (MALDI-TOF) or DNA (sequencing). Genotypic microbial identification methods are theoretically more reliable because nucleic acid sequences are highly conserved in most microbial species. Applicable genotypic methods include:

- DNA-DNA hybridization
- PCR
- 16S
- 23S rRNA sequencing
- Multilocus sequence typing (MLST)
- Pyrosequencing
- DNA probes
- Analytical ribotyping

These methods can be technically challenging for microbiologists. They also require more expensive analytical equipment and supplies.

Often these analyses are conducted by contract laboratories, government laboratories, universities, research institutes, or specialized laboratories within industrial firms. Therefore, the use of genotypic identification methods is typically limited to critical microbiological investigations such as product-failure investigations. Further, if strain-level identification is done in the course of an investigation, analysts must ensure that the method is appropriate.²

MALDI-TOF

In MALDI-TOF-based analysis, proteins from a microbial colony are ionized with a laser and then analyzed by a time-of-flight mass spectrometer. A spectrum is produced, which is compared to a library of spectra emitted by different known bacterial and fungal species. The main advantages of the MALDI-TOF approach are the rapid turnaround time, the lower cost compared to sequencing approaches, and improved specificity when compared to phenotypic analysis. MALDI-TOF has been shown to outperform phenotypic assays.¹⁰ Libraries are required for bacterial or fungal identification, and a number of companies have developed validated libraries for common species. These perform well on clinical samples. However,

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Sequencing remains the most accurate and gold standard for bacterial and fungal identification with most bacterial ID relying on sequencing all or part of the 16S ribosomal gene, and fungal identification relying on the ITS or D2 region.

because protein expression can differ between strains and greater strain coverage is needed for environmental samples, MALDI-TOF generally performs less well on the identification of environmental isolates. Protein expression also varies according to growth conditions and media; consequently, samples must be grown in very similar conditions, which limit the range of isolates that can be tested.

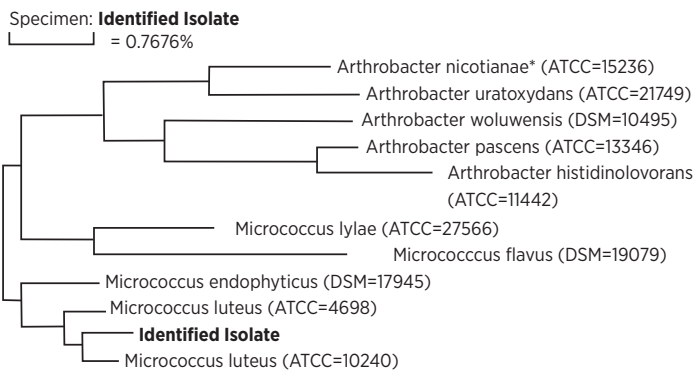
DNA Sequencing

Sequencing remains the most accurate and gold standard for bacterial and fungal identification,¹¹ with most bacterial identification relying on sequencing all or part of the 16S ribosomal gene, and fungal identifica-

tion relying on the ITS or D2 region. Sequencing is more specific and identifies more samples to species and strain than phenotypic based methods or MALDI-TOF.^{10,12} Because the DNA sequence of an organism is not dependent on growth stage, conditions, or media, sequencing approaches can be more robustly applied, even enabling identification of isolates that have ceased growth. The cost of identification can be considerably higher than that of other approaches. However, DNA analysis remains the approach that is most likely to produce identification to the species level and the least likely to produce an incorrect identification. Table 1 shows the colony morphology of *Micrococcus luteus* in comparison to the phylogenetic tree produced by DNA sequencing.

In all cases, the identification assigned is dependent on the reference library to which assay results are compared. Several validated libraries are commercially available and can be supplemented with internally validated libraries for isolates of specific interest. A significant advantage with sequencing approaches is the existence of the large nucleotide sequence collection at the National Center for Biotechnology Information (NCBI). This searchable database contains 30-million nucleotide sequences, including eighteen thousand that are specifically identified as 16S ribosomal species.

TABLE 1. Environmental Isolate Plate Streak and DNA Sequencing Results.



The identified isolate is 99.45% similar to *Micrococcus luteus* (ATCC 10240) and therefore identified as such.

TABLE 2. Recommended Scheme for Microbial Identification.

EXTENT OF IDENTIFICATION/ CHARACTERIZATION (MINIMUM EXPECTATIONS)	ISOLATE AND ORIGIN
Characterization (Gram stain reaction and morphology) only	Environmental monitoring ISO 7 and 8 classification areas, for alert-level excursions
Identification to genus (and species when possible)	Environmental monitoring for action-level excursions for ISO 7 and 8 classification areas
Identification to species	ISO 5 and 6 classification areas alert- and/or action-level isolates from excipient, finished preparation, environmental, and water samples
Strain typing or molecular fingerprinting	Significant preparation contamination failure (e.g., media fills, sterility test) and significant adverse trends in environmental and water monitoring

ISO = International Organization for Standardization

This resource can be effectively used to supplement a validated library, however, caution should be exercised as many entries are not identified and some are incorrectly identified. Consequently, a relatively high-level of expertise is required to effectively assess and compare validated and un-validated libraries to avoid generating inaccurate conclusions.

CONCLUSION

All of the data gained by extensive identification of isolates from environmental monitoring, personnel monitoring, and preparation testing allows a facility to obtain a detailed picture of the microbiological state of control of its operation.³ Table 2 illustrates an example of a scheme for the extent of characterization that is recommended for the recovered microbial isolates.⁵ In many cases, phenotypic analysis can provide sufficient information for ongoing evaluation. However, when circumstances dictate greater in-depth assessment and specificity, identification to the genus, species, or strain level can yield valuable insights. In addition, microbial identification to the species and even strain level can be critical in assessing

and mitigating risk from microbial contamination.² The extent of identification and rationale must be documented and determined based on risk assessment, facility validation, and appropriate trend analysis.⁵ The information gained from accurate, macromolecular-based microbial-identification methods provides information that is used to improve the quality in every aspect of the compounding pharmacy and laboratory operations. Facility design, sanitization procedures, compounding operations, personnel qualifications, microbial testing, and data investigations all benefit from the data generated from quality microbial-identifications methods.

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