WHITEPAPER

The Benefits of Using a DNA Sequencing Method for Microbial Identification

Mitigating Risk with the MicroSEQ™ Rapid Microbial Identification System

Introduction

Given the highly regulated nature of the pharmaceutical and medical device industries, quality control of processes and products requires the rapid and accurate identification of microbial contamination.

Until recently, testing laboratories relied on phenotypic identification of microbes. The phenotypic method is a time-consuming process of isolating samples, culturing cells, gram staining, and visual identification. It depends on the expertise of microbiologists trained to recognize a wide range of bacterial and fungal species.

With advances in DNA technology—notably PCR, DNA sequencing, and comparative sequence analysis—it has become faster and more accurate to identify microbes by comparing DNA sequences from small samples of isolated cells with comprehensive databases of bacterial and fungal DNA sequences. As a laboratory providing microbial testing services, Infinity Laboratories takes advantage of the speed and accuracy of this genotypic method of microbial identification using the Thermo Fisher Scientific MicroSEQ Rapid Microbial Identification System.

This white paper will review current methods of microbial monitoring, discuss when it's appropriate to identify organisms to help with risk mitigation, evaluate methods of microbial characterization, and detail the MicroSEQ™ ID System used in the genotypic method.

Methods of Microbial Monitoring

There are many methods for testing for microbial contamination throughout the process of manufacturing and packaging pharmaceuticals.

Environmental monitoring

Environmental monitoring is widely used for controlled environments within a manufacturing facility.[1] Controlled environments require sampling to determine the amount of microbial growth, level of risk, the need for investigation into the root cause, and the required corrective action.

Bioburden testing

Bioburden testing is used to measure the level of microbial contamination in raw materials and in non-sterile products. Spikes in otherwise consistent levels of microbial levels can affect sterility. For example, medical device manufacturers need to perform quarterly dose audits to ensure levels aren't spiking. Bioburden testing is also used to calculate the amount of radiation required to terminally sterilize ointments and creams.

Sterility testing

This is used to verify product sterility and, in the case of contamination, what species of microbe is involved. This can help lead to an investigation of the root cause of product failure.

Aseptic process simulations

Simulations are used to validate that a process, such as a media fill, is aseptic. This means that no microbial contamination is introduced to the process.

Water testing

The allowable microbial limits that are tested will depend on the end use of water.

When to perform microbial identification?

The stage at which microbial identification is performed depends on risk assessment. Depending on the risk, it can be performed during research and development, manufacturing, and/or for finished products. For example, in low-criticality, low-risk areas, it might be enough to characterize whether a sample contains a fungus or a bacterium as assessed by colony morphology. In highly critical or high-risk areas, identification of the species must be done.

1. See **ISO 14698-1 - Cleanrooms and associated controlled environments—Biocontamination control - Part 1: General principles and methods** and USP <1116> Microbiological Control and Monitoring of Aseptic Processing Environments.

Validation

When validating a controlled environment, knowing the ISO level allows you to determine which procedures to use to identify microorganisms. Following a defined process allows you to create a library of microbes that are commonly seen in higher-risk environments. For example, if routine testing turns up *Micrococcus luteus*, or *Staphylococcus epidermidis* - common skin bacteria - the source is likely personnel and better processes can be implemented. But if an uncommon pathogenic organism appears, it indicates the need to investigate further, and, perhaps, adjust cleaning techniques or disinfectants.

Abnormal trends

Assessing trending data during routine work and validations within a facility is important, especially within controlled environments. Knowing organisms that are normally seen within the environment can aid in future investigations, as well as monitoring to make sure organisms are within safe levels for the use of that space. An abnormal trend—a sudden increase in microbes that are not normally seen—on medical devices or a pre-sterilized pharmaceutical product indicates a problem and may lead to the root cause.

Sterility tests

A simultaneous spike in bioburden and a failure of sterility may help identify a root cause.

Gram stain

Gram stains can identify a specific organism, such as a gram-negative bacterium or a fungus, that should not be in a critical area. This test might trigger the need to do a complete microbial identification.

Regulatory requirement

There are times when microbial identification is a regulatory requirement. An example is USP **<62> Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms.**

Methods of Microbial Characterization

There are different methods to characterize microbes, the choice of which depends on the risk or criticality of the area tested.

Colony morphology

A trained microbiologist can glean a lot of general information from this simple method. If colony shape and color suggest a pathogenic microbe, more detailed tests would be needed.

Cellular morphology and gram reaction

Cellular morphology and a gram stain could identify, for example, a gram-positive rod such as a coccus presenting a problem in a high-risk or highly critical area.

Identification to genus and species level

For areas with the highest risk and criticality, the gold standard is the identification of the genus and species of the microbe. This can be accomplished using either a phenotypic or genotypic (i.e., DNA sequencing) method.

Recognizing the superiority of the genotypic method, Infinity Laboratories made the switch in 2015 and now only uses the DNA sequencing method of microbial identification.

The Genotypic Method of Microbial Identification

The genotypic microbial identification method is more reliable because of the highly conserved nature of nucleic acid sequences in most species. It can be used with QC organisms, as well as environmental monitoring samples that might be stressed. This is the most important reason that Infinity Laboratories switched to the genotypic method. It is the preferred method in GMP environments and many customers were insisting that they needed a genotypic identification of microbial contamination.

The inclusion of a large library of DNA sequences and a phylogenetic tree tool improves the confidence in species identifications. The phylogenetic tree tool can be used to compare the sequence of an identified microbe to other organisms. The genotypic method has the benefit that it can be performed on dead organisms, which is not the case for the phenotypic method.

Benefits of the Genotypic Method

The genotypic method for microbial identification has the following benefits when compared to the phenotypic method (Figure 1).

- **Lower costs** - While phenotypic reagents are less expensive, having to repeat tests often drives up the cost. The genotypic method gives an answer on the first attempt, meaning less time and reduced costs.
- **Accurate and reliable results** - The phenotypic method works well with QC microorganisms or those that are not too far removed from the original ATCC number. However, an identification often must be confirmed with a gram stain. There is no need to do a gram stain with the more accurate and reliable genotypic method, although some customers may request it.
- **Testing non-viable samples** - DNA can still be extracted and sequenced from dead cells.
- **Results within 24 hours** - The phenotypic method requires incubation of a swab for at least 24 hours on PSA, then another 24-hour incubation on blood agar before identification. The genotypic method can identify bacterial and fungal species in less than 5 hours.
- **Small sample size** - Small samples can come directly from the original plate, broth, or slant cultures. (Subculturing is necessary to isolate a single colony if the contamination is suspected to be a mixed colony.)
- **Time-use efficiency** - Quick turnaround means more IDs in a week. Increased throughput allowed Infinity's batching capabilities to rise to 92 samples within 2–3 days compared to 40 samples per week.

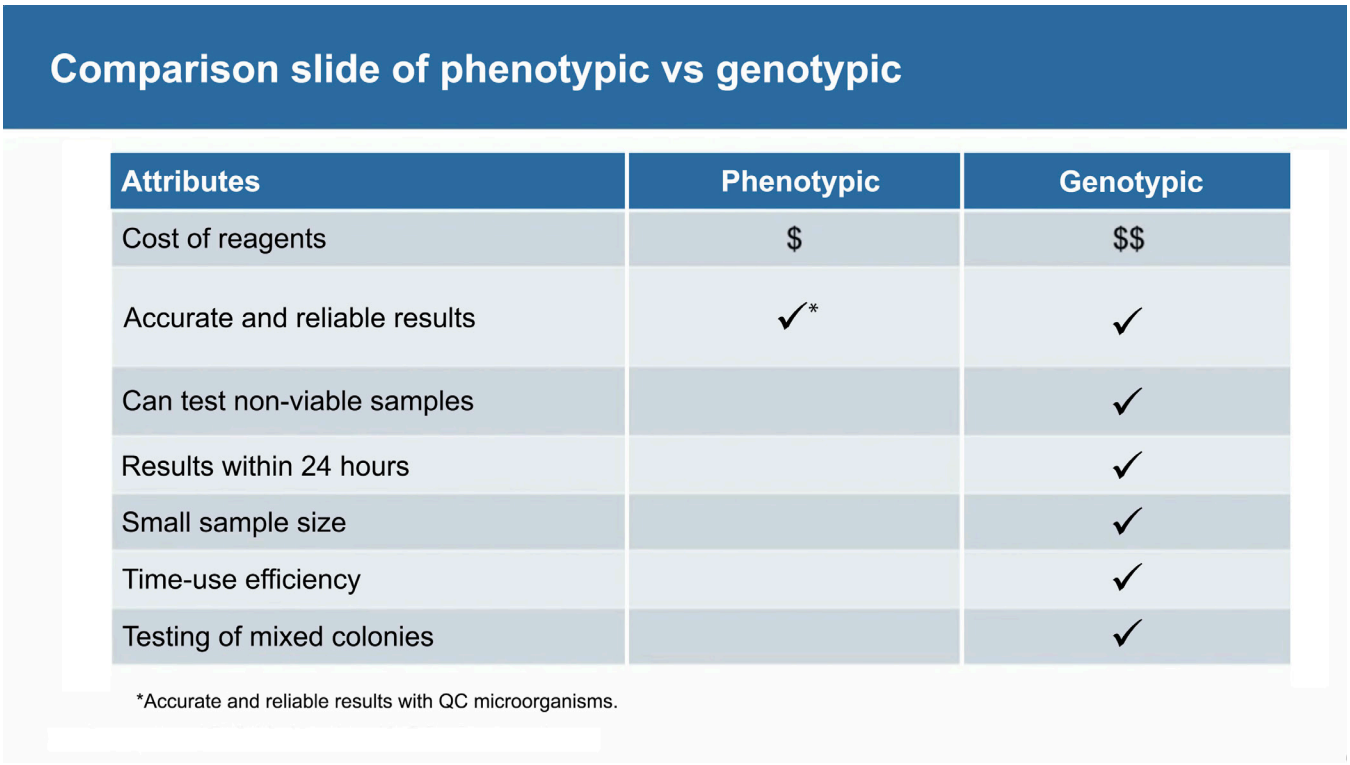

Figure 1. Comparison of the benefits of the phenotypic and genotypic methods of microbial identification.

- **Testing of mixed colonies** - DNA can come from isolated colonies that are impure. Even with a bit of background noise (e.g., from DNA from another species), the genotypic method can still provide reliable identification of the main microbe.
- **Mold identification** - In addition to thousands of bacterial and fungal species, a good genotypic method can identify yeasts, molds, and mycoplasmas.

Implementing the genotypic method with the MicroSEQ ID System

Infinity Laboratories' GMP customers were pushing for the testing provider to get a robust genotypic testing system in place to support their microbial identification needs. The genotypic microbial identification system chosen was Thermo Fisher Scientific's MicroSEQ ID, which is known for its speed, reliability and vast identification libraries. It consists of the Applied Biosystems™ 3500/3500XL Genetic Analyzer, which sequences bacterial 16S ribosomal DNA and fungal D2 rDNA using PCR. Sequences are compared to extensive validated bacterial and fungal libraries more extensive than libraries used in phenotypic microbial identification systems.

MicroSEQ ID is a high-throughput system, capable of identifying fungi and bacteria in less than 5 hours and ideal for labs, like Infinity's, that do 2,000 or more tests per year.

Unlike the phenotypic method, which requires microscopic verification of spores or hyphae to identify yeasts and molds, genotyping can test for these organisms using fungal reagents. This makes it easy to run identifications of all organisms with one system and is one of the main reasons Infinity Laboratories switched to the genotypic method.

To get the MicroSEQ ID system fully implemented, including qualification, validation, training, and regulatory agency filing and acceptance, took the lab less than 4 months.

Qualification and validation

Infinity Laboratories is FDA registered and ISO accredited, which ensures a level of validated quality that customers can depend on. The company provides accurate results and maintains data integrity through adherence to regulations and standards. Its customers have access to raw data and any additional support they may need during an audit.

Thermo Fisher Scientific field service experts provided the setup, guidance, and training needed to implement the MicroSEQ ID system. This included qualifications required by regulators:

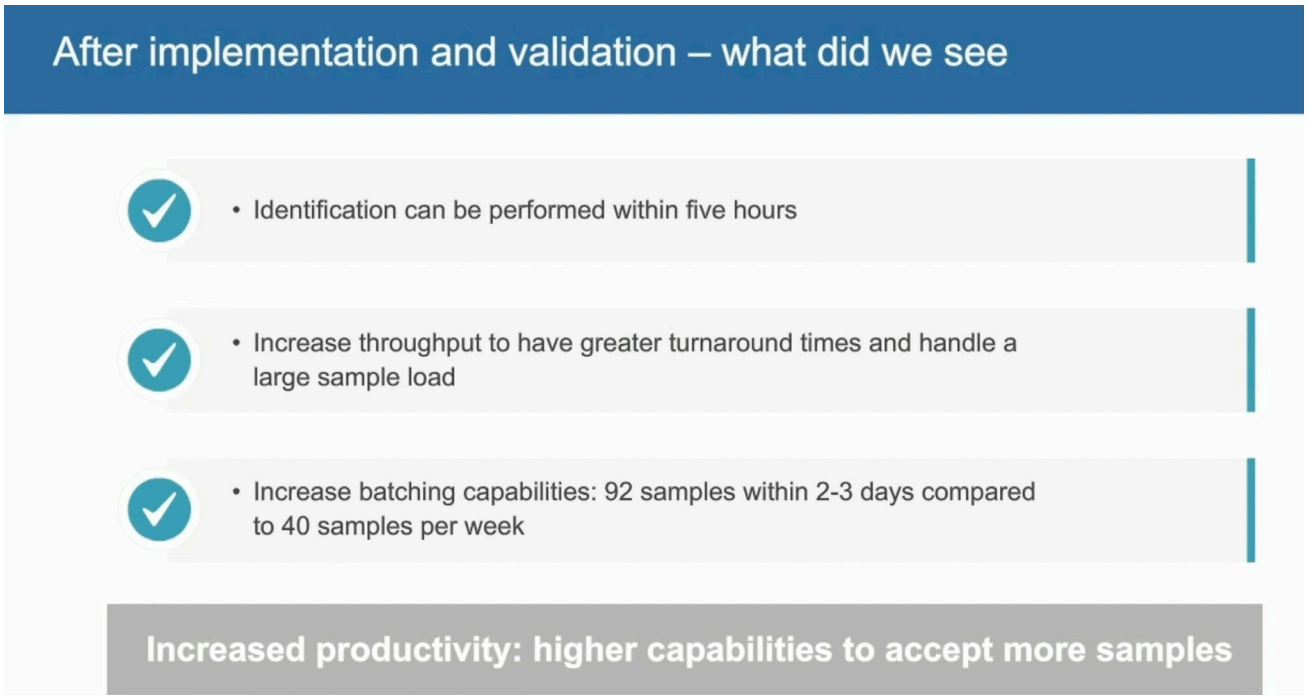

Figure 2. The benefits of implementing the MicroSEQ ID system.

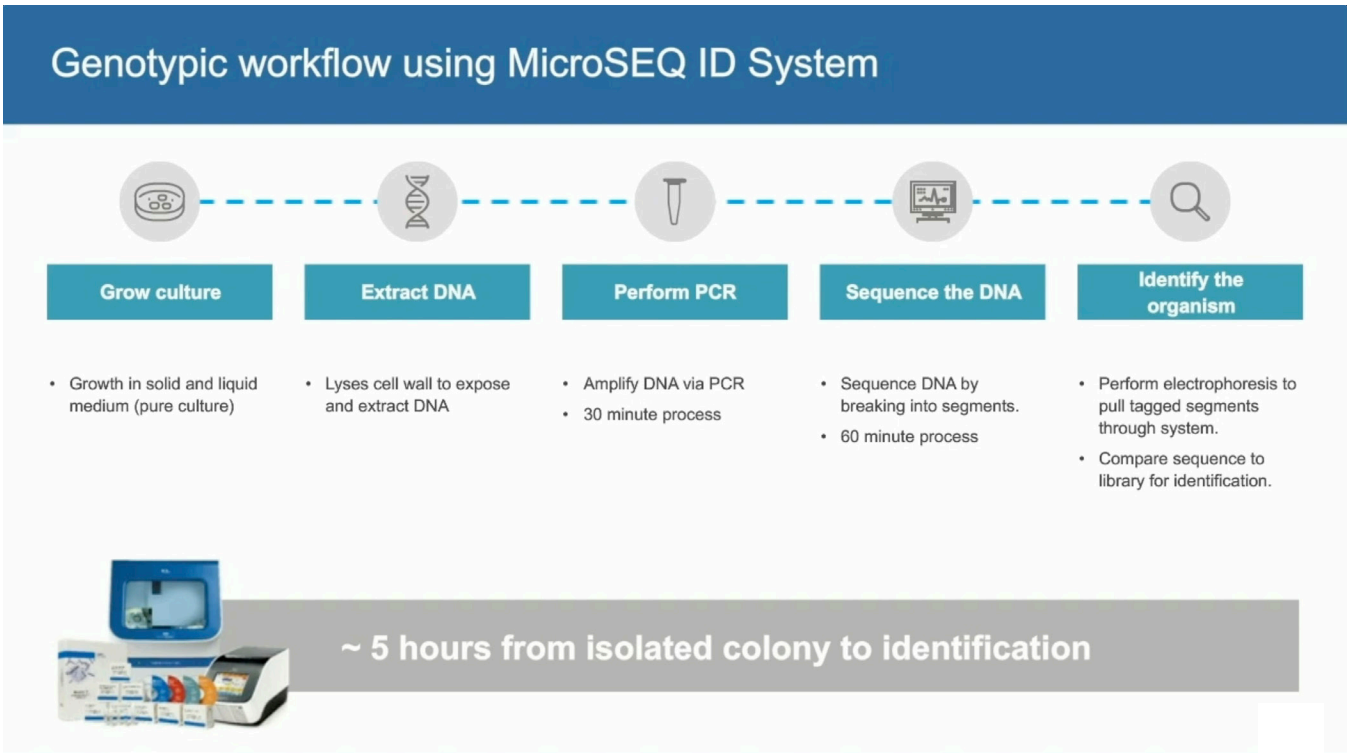

Figure 3. Workflow of the MicroSEQ ID system.

- Installation qualification (IQ) - instrumentation and software setup
- Operation qualification (OQ) - guidance and training while we performed the operation qualification
- Performance qualification (PQ) - this validates the entire process

Completion of the IQ, OQ, and PQ meant the entire process was tested and validated, running selected test microorganisms through the DNA extraction, PCR, sequencing, and data analysis process.

Ongoing training and support

Appropriate training extends to additional staff to ensure multiple people understand the workflow, as well as how to operate the system and the software. The ongoing maintenance plan ensures optimal performance and that the system is up-to-date.

Genotypic workflow using the MicroSEQ ID System

The rapid and reliable identification of both bacterial and fungal species using the MicroSEQ ID System can be accomplished in less than 5 hours following these steps (Figure 3).

1. Cell collection and culture
2. DNA extraction
3. PCR amplification of target sequences using an Applied Biosystems Thermal Cycler
4. Sequencing of DNA samples with the Applied Biosystems 3500/3500XL or new SEQStudio Genetic Analyzer
5. Comparison of sequenced DNA to extensive libraries

Conclusion

Technological innovations in the sequencing and analysis of DNA have led to faster and more reliable methods for the identification of microbes throughout pharmaceutical manufacturing. In particular, the evolution from visual identification using the phenotypic method to the more robust genotypic method using these technologies has lowered costs, increased throughput, and simplified testing for bacteria and fungi, including yeasts and molds.

To learn more about the MicroSEQ Microbial Identification System from Thermo Fisher Scientific, please visit thermofisher.com/microseq.

About Infinity Laboratories

Infinity Laboratories is a powerful network of state-of-the-art testing facilities that are FDA Registered, ISO 17025 Accredited, and DEA licensed. With an enhanced focus in the areas of chemical and microbiological sciences, we support pharmaceutical and medical device manufacturers. Our goal is to go Beyond Testing and become a partner in the success of our customers