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Fast, Advanced Culture Technology Holds Potential for Easing QA/QC Microbe Bottleneck

By Bret Barnhizer

In the summer of 2011, the US FDA released its strategic plan for the Advancement of Regulatory Science [1]. In it, the Agency emphasizes four areas to reduce the risk of microbial contamination in various manufacturing industries. One area was to reduce the risk of microbial contamination. Among its recommendations is the development of "sensitive, rapid, high-throughput methods to detect, identify, and enumerate microbial contaminants and validate their utility in assessing product sterility." For the pharmaceutical industry, this is a call to decrease the time required for microbial quality assurance associated with clean room validation, in-process testing, and final sterile product release.

A novel technology approach by the American biotech firm NanoLogix is answering this call by offering microbiological QA/QC the opportunity to see microorganism growth quickly though a significant enhancement in reliable Petri culture methods. This new nanopore membrane method shortens the time needed for detecting microbe contamination in all stages of pharmaceutical manufacturing. As an example, the NanoLogix technology shortens wait times for *Listeria* spp and E.*coli* from 18-24+ hours down to just 5 hours and *Salmonella* down to 4 hours.

Manufacturing Encumbrances from the Microbe's Timetable

During sterile product manufacturing, whether by aseptic processing or sterilization, sterility assurance verification is often necessary after key steps, such as solution preparation, bulk transfer, filtration, vial filling, etc. If multiple hold times are required for in-process microbiological testing, manufacturing can be extended for days, thus complicating the efficient utilization of equipment and personnel.

In-process microbial testing with alternative technologies that are more rapid than culturing, such as PCR and Flow Cytometry (discussed below) can be employed. But these generally lack the ability to provide expedient viable results, as as PCR assays alone can require an 18 hour overnight enrichment.

This may not sound long, but these in-process hold times have serious implications to the pharmaceutical industry. Throughout manufacturing, tests are conducted at various stages of processing and this time accumulates and lengthens the manufacturing timeline to finished product. Additionally, such long wait times for can quarantine materials from entering the next production stage. Pharmaceutical companies can lose a competitive edge due to extended production times, as efficient supply chain logistics resulting in lower cost-of-goods is the life blood of the industry.

Incomplete Solutions

Without an ability to make microorganisms grow any faster, pharmaceutical companies look towards more complex and sophisticated equipment to receive microbial tests results slightly faster than traditional culture methods.

Polymerase Chain Reaction (PCR) has proven to deliver results within minutes, but only after assays have undergone an 18 hour overnight enrichment process. Additionally, screening PCR may only detect the presence of DNA and cannot rapidly deliver viable results of bacteria growth. As such, PCR is typically followed up with PCR separation steps or a culture sample to verify if active-threat microorganisms are present.

In regard to Flow Cytometry, its main disadvantage is a low throughput rate that cannot handle large numbers of samples efficiently. This prolongs results and defeats the purpose of a rapid microbe detection system.

A Simple More Rapid Culture Method

NanoLogix advanced-culture technology fits well into the request from the FDA for sensitive, rapid methods. The new technology dramatically speeds up viable culture results by 4x to 24x, depending on the bacteria and product being used. This technology allows QA/QC technicians to view micro-colony growth and return detection and identification results significantly faster than traditional Petri, PCR or Flow Cytometry. With the NanoLogix method, pathogens like *Listeria* spp

and E.*coli* can be detected in just 5 hours and *Salmonella* in 4 hours, instead of the 18-24 hours or more required by PCR and traditional culturing respectively. Although NanoLogix technology is currently available in two standard size Petri dishes, the potential exists for expansion and adaptation of this technology to well plate arrays for use in high-throughput screening, thus meeting the FDA's three requests; sensitive, rapid and high-throughput.

How It Works

The advanced-culture method from NanoLogix uses a permeable, polymeric membrane sandwiched between two agar layers. The extremely thin, clear membrane allows tiny microorganisms to grow for a few hours, depending on the bacteria. Then after a fraction of conventional incubation time has passed, the membrane of this BioNanoPore (BNP) method is transported to a staining plate. Capillary action brings the staining agent through the membrane and into contact with the micro-colonies growing on the membrane. Ten to fifteen minutes on a staining agar makes previously invisible micro-colonies visible for detection. When testing a parenteral product, filtration of the sterile solution with the company's BioNanoFilter (BNF) technology can provide identification sensitivity for some microbes as low as one cell per liter, within 4 to 6 hours.

Available today for the QA/QC process of self-certifying organizations, such as pharmaceutical companies, the NanoLogix technology is very well suited for parallel development and validation, along with traditional Petri culture methods, during pharmaceutical R&D. Such rapid microbe detection can shorten the time needed for production by decreasing in-process hold times. The method's inexpensive, reliable, and rapid sample turnaround enables testing to be implemented at any stage of production. Issues of contamination can be better addressed, as faster results mean the more efficient use of manufacturing clean rooms, equipment, and personnel, which leads greater efficiency and a lower cost-of-goods.

Several researchers who have published a peer-reviewed paper [2] on the Nanologix technology regard it as the 'new gold standard' for the field of rapid diagnostics. With the millions of Petri dishes used every day, in a wide variety of applications, it is conceivable that the pharmaceutical industry may find the efficiency and accuracy of their operations completely transformed by a simple yet revolutionary application of this nanopore membrane.

[1] Pharma QBD. FDA Releases Strategic Plan for Advancement of Regulatory Science. August 18, 2011. http://www.pharmaqbd.com/fda_strategic_plan_regulatory_science/

[2] Jonathan Faro[1], Allan Katz[1], Karen Bishop[1], Gerald Riddle[1], Sebastian Faro[1]. "Rapid Diagnostic Test for Identifying *Group B Streptococcus.* American Journal of Perinatology". Thieme eJournals, August, 2011.