Considerations for Choosing a Rapid Microbiological Method: Aligning Your Needs with Available Technology

Running title: Considerations for Choosing an RMM

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Microbiology labs are under pressure to find ways to accelerate results. These pressures come from corporate quality initiatives, the need to save money, and regulatory agencies. According to an article in American Pharmaceutical Review, microbiologically-related recalls are on the rise (Sutton and Jimenez 2012). This type of data places more emphasis on the importance of good microbial quality control testing in the lab. Many labs are looking to modernize their facilities and processes, but choosing an appropriate rapid method can be daunting. There are many rapid methods available, and none are "one-size-fits-all". Experts such as Michael Miller have identified the various technologies available and some of their benefits and shortcomings (Miller and Schwedock 2011). Because a system that works well in one situation may have serious shortcomings in another, the QC microbiologist must educate him or herself on the advantages and disadvantages of each method in order to make a wise choice based specifically on his or her laboratory's needs.. The goal of this chapter is to help the user frame the appropriate questions to guide the choice of a rapid method for his or her product and/or process.

Drivers for Rapid Methods

In the last few years, rapid microbial detection methods have become more prevalent as manufacturers look for way to streamline production and bring high quality products to market faster. New initiatives within the quality control lab have driven management to look at the QC lab from a different perspective. One popular initiative is Lean in the lab (Jones and Severns 2011). Lean processes have been helping manufacturers create more efficient, less wasteful production environments for decades. Lean represents one kind of corporate initiative that is driving QC labs to look into rapid methods as a tool to reduce waste and increase productivity.

Performing microbial quality control testing may appear straightforward and inexpensive. Samples come in, are prepared, incubated and any contamination is given time to grow until sufficient time has passed or growth has become visible. However a deeper inspection into this process looking through the lens of Lean concepts reveals an inefficient and wasteful process. Protocols are repetitive and include manual counting and data entry steps. The incubation process itself takes valuable time that requires product to wait or processes to be at risk.

Will one method cover all testing needs?

Manufacturers and products are as diverse as the types of rapid micro methods available in the market. It is unlikely that a single rapid method will cover all of a company's microbiological testing needs. Which and how many to choose depends on the diversity of testing performed, the size of the company, and the desired throughput. In many cases it may be wise to choose a single rapid method that can be used in the

widest variety of situations in order to get the best return-on-investment. However, if your company has diverse high-throughput needs, or a high value test that has very specific requirements, it may be cost-effective to adopt more than one rapid method to deal with the various situations.

What is the minimum time savings needed to create value?

Are same day results useful/necessary?

Obviously a rapid method should have a shorter time-to-result than the traditional method, but the fastest method is not always necessary nor always the best choice, and other considerations may become more important. In fact, in some circumstances a moderate time savings may be all that is needed to create the maximum value. Consider the 14-day Sterility Test (Chapter <71> in USP 2011). If same-day results mean that product can be shipped immediately, then using a very quick method such as one that uses viability staining to detect single live cells (Gressett, Vanhaecke et al. 2008) can save up to 13 days of hold time. However, often other tests will become rate limiting, such as those from the environmental monitoring program, or the chemical analysis. In those cases, getting sterility results in 5-7 days may be sufficient to render the sterility test no longer rate-limiting.

On the other hand, immediate results may be useful in certain manufacturing situations. If manufacturing occurs "at risk" before microbiological results are back or if a manufacturing process stops while waiting for results to return, then it may be that the rapidity of the method is the most important issue. In fact, rapid testing could even provide the impetus to change an "at-risk" manufacturing process into one where results are known before the process is continued. Less dramatically, but still of value, it could mean that the manufacturing process continues operating "at risk" for a shorter time. Being able to stop a process sooner if a contamination is found can lead to savings. One "save" every few years could more than pay for the expense of the alternate method.

One should also consider both the "time-to-positive" and "time-to-negative" results. Some assays, particularly those that are growth-based with continuous monitoring (e.g. BacT/Alert , BACTEC, GrowthDirect), can often give very fast results in the case of certain common types of contamination, even though they might take longer to confirm a negative or final result. This should be contrasted with methods that require waiting to the specified end time to find out anything.

Is your sample compatible with the rapid method?

Clearly it is crucial for the sample type to be compatible with the rapid method. In the simplest case, if the method is based on filtration, the product must be filterable, or convertible into something filterable. But there are many other considerations. Products with many particulates may interfere with or cause false positives with some RMMs, such as those based on cytometry or that otherwise measure fluorescence. Biologics or similar products may contain ATP, which could interfere with the detection system of certain luminescence-based methods. There are products containing growth inhibitors that may not be compatible with direct-inoculation growth-based methods. Another point to consider is the maximum sample volume accommodated by the RMM. Those based on direct inoculation of a liquid growth medium, or flow cytometry, may have a maximum volume, whereas those based on filtration may be much more flexible.

Does the RMM add labor or reduce labor?
Some rapid methods may save time in time-to-results, but dramatically increase the labor necessary to prepare and handle the sample. One must consider the tradeoff of potential increased labor with the time savings that the method will afford. Some methods may have additional upfront work, while saving downstream labor. Other RMMs, such as those that have an automated component, may ultimately save labor, not only by eliminating tasks such as counting and scoring, but by reducing manual paperwork tasks. One must also consider how the RMM will change existing operations. What are the changes to the sample preparation, and how much retraining of the staff will be required?

Combing paperwork, sample preparation, incubation and monitoring, the number of steps a lab technician must complete for each sample can be significant. In some worst case scenarios, where there is an interim read of the plate, a traditional 7-day water test can require 14 different steps, if not more. Even if each step takes only seconds, the time quickly increases as the number of samples increases. Furthermore, the activities are repetitive, manual and prone to error. Ideally, any RMM system that reduces overall assay time should also reduce steps. If the 7 day water test results are available in 1 day, but require 3 additional steps, the overall assay time has been reduced, but at the cost of additional labor and work. Is that a viable trade-off?

Will automation help me by reducing human error?
Automation has been a standard in manufacturing since Henry Ford. The benefits of automating a process are clear. Personnel that would have been involved in manual tasks are freed to perform more valuable tasks for the organization. When aligned with automation, standard operating procedures become "automated" standard operating procedures and are always consistent.

Automated rapid methods can result in improved standardization by eliminating human variation and giving more reproducible results regardless of the technician. In addition to improving reproducibility, RMMs with automation can reduce the places where human errors can occur, particularly those that occur during the counting of plates or data entry. In contrast, RMMs that lack automation, especially those that add manual steps, may increase the possibility for human error.

Does the RMM provide a count or a presence/absence (+/-, or qualitative) result?
For some assays, an accurate enumeration result is required. Obviously, methods that only provide a yes or no answer would not satisfy. For the remaining RMMs, the accuracy and the dynamic range of the enumeration should be evaluated. Some RMMs do not accurately enumerate at low bioburdens whereas others can only provide accurate counting when the bioburden is low. For example, a method that can enumerate accurately from 30 CFU (colony forming units) to 1,000CFU may be ideal in situations where there is always a bioburden. In other cases, a method that can accurately count live microorganisms from zero to 300 might be necessary. There are even methods that can give accurate enumeration information over a large dynamic range (0->1,000 CFU) and such methods may be useful in eliminating dilution steps. Be cautious when evaluating a method's dynamic range, for in addition to the inherent limits of the

RMM, the dynamic range and accuracy of a method can be affected by the sample or product being tested. This must be considered when assessing the usable dynamic range.

If my counts are usually zero, can I get away with using a qualitative RMM as a screen?
An interesting case can be made for using a rapid method that provides a +/- result as a screen before taking the time to do a traditional enumeration assay. This model works only when the enumeration result is usually zero. In this scenario, if the result comes back negative, the sample passes. If the result comes back positive, it is followed-up by traditional testing. This scenario can be quite successful in saving a company time and money, so long as the results of the screen are typically negative. It has been implemented successfully with RMMs that use technologies such as ATP bioluminescence to look for bulk growth (Jimenez 2004).

One might also consider the accuracy needs of an enumeration assay. In some cases an order of magnitude result will satisfy. Therefore, even if the RMM cannot give accurate results, particularly at low counts, it may still provide timely information if the assay need only distinguish between <100 CFU and 1000 CFU. This approach has been implemented for the testing of batches of egg fluids in vaccine production (Bhusari and Steger 2008).

How easy or difficult is it to validate the RMM?

Both the United States and European Pharmacopoeia's offer guidance regarding validation of microbiological methods. A recent USP presentation highlights some of the chapters that deal with validation of rapid methods (Tirumalai 2009).

- US Pharmacopoeia Chapter <1223>, "Validation of Alternative Microbiological Methods" (USP 2011)
- The European Pharmacopoeia Chapter 5.1.6 section 3.1 (European Directorate for the Quality of Medicines 2009) states "For example, a sterility test by membrane filtration may be performed according to the pharmacopoeial procedure up to the point of combining the processed filter with the recovery media, and after that the presence of viable cells might then be demonstrated by use of some of the available (RMM) methods. Validation of this application would, therefore, require validation of the recovery system employed rather than the entire test."
- PDA Technical Report 33, "Evaluation, Validation, and Implementation of New Microbiological Testing Methods" (PDA 2000). This report highlights details around validation of new test methods, stating that, "The two critical components of any definition of validation are appropriateness of a specific product or process (it does what it purports to do) and reproducibility (it continues to perform)."

If the methods used in the QC/QA lab at the production facility are explicitly defined in the product license, then a formal application to change the license will be required. Based on the risk of the change to the product quality, the route of license change may be as simple as an annual report. If the risk is higher, the more complex CBE 30 (Changes Be Effected 30 days,)

PDA 2000) may be required, or for a very critical change a prior approve supplement (PAS) may be required. The FDA has an initiative called the comparability protocol that facilitates faster license changes (FDA 2003). The use of this route effectively allows a CBE 0 to be used, helping to streamline the process.

For pharmaceutical companies that are navigating the validation process, the first step is the determination of the guidelines from which to base the validation. Typically, companies look to the RMM supplier to perform the IQ and OQ steps of the process. For RMMs that involve instruments, the OQ step may be validated according to the requirements of USP <1058> Analytical Instrument Qualification (USP 2011), which is simply a validation that the instrument operates as expected. Though required, this on its own does not validate the RMM.

If the method can be considered an automated compendial method as may be the case for systems that simply provide an automated way to count growing colonies, then the easier USP <16> "Automated Methods Analysis" may be applied for method validation (Thomas 2011). If the RMM is an alternate method, then USP <1223> must be applied.

One should also consider if the RMM has been designed specifically for the pharmaceutical industry. Many RMMs were designed with other industries in mind, and are only begrudgingly adapted to the regulatory needs of the pharmaceutical industry. This can become particularly apparent if the software is not 21 CFR Part 11 (FDA 2003) compliant. Also enquire if the vendor helps with the validation process. Vendor involvement can be invaluable for help with determining the approach and overcoming technical obstacles.

Is the count in real CFU, or estimated from another parameter, such as relative fluorescent units?
Another aspect to consider is whether or not the RMM gives results in CFU. Some methods that claim to "enumerate" actually take a measurement of some other output that then must be converted to CFU. Methods such as these may require more experimental work to prove equivalence and set action and alert limits.

Do the microorganisms survive the RMM, such that they are available for identification?
Good manufacturing practices dictate that contamination control includes the identification of contamination found during quality control testing (Sandle 2011, Sutton 2010). Detection of the organism is just one part of the process. RMM's are designed with detection in mind, but not all allow for ease of identification. If compliance with GMP is a priority, then RMM's that allow for ease of identification will be on your shortlist.

Will the RMM easily integrate with the LIMS (laboratory information system) or other data management platform?
Many organizations already have LIMS in place. Hence the ease with which a rapid method can integrate with it may be a consideration. It is important to determine if the instrumentation associated with the method can accept information from or exchange information with the LIMS. An RMM system that can integrate to LIMs eliminates the chance of user-errors that could result from the tedious step of typing in the results of sample analysis.

In addition, a method that can integrate with the LIMS can help with the popular paperless lab initiative. In this environment, typical documents such as work orders and count sheets are replaced with electronic forms, reducing waste associated with large filing cabinets of paper. The LIMS is generally at the heart of paperless labs and a rapid method that operates seamlessly within the LIMS can be an asset.

Does the RMM provide reporting? How much reporting do I need?
A popular report in the area of environmental monitoring is the trend report. The data is used to determine if there are continual or increasing contamination issues in certain manufacturing areas. This report is critical to manufacturing. As RMMs are considered, an area that may be important is the ability of the RMM to provide trend and other types of reports. The need for reporting becomes more important when the facility does not have a LIMS system already in place.

What is the RMM's record with false positives? What is the impact to me when I get a false positive?
When assessing the usefulness of a method, one must consider the impact of a false positive. For example, if the assay will be used for trending of bioburden, counting 23 instead of 22 may be no big deal. However, in other circumstances, counting 1 instead of 0 can lead to unnecessary and time-consuming investigations. Therefore it is important to match the false positive rate with the needs of the testing regime.

In addition, one must consider whether the user can distinguish false positives from real positives during validation. Some RMMs, particularly those that are not growth-based, are more sensitive than the compendial methods. This can make it difficult to determine if higher numbers of positives are due to false positives or increased sensitivity. If there is a third objective criteria that can make the distinction, the uncertainty can be resolved but unfortunately often there is not.

As with many other aspects of a RMM, the false positive rate may be affected by the sample type. In some cases, as discussed above, the false positive rate can affect the dynamic range of an assay by eliminating accuracy at low counts.

What is the RMM's record with false negatives? What is the LOD (limit of detection)?
False negatives are clearly problematic, but again their impact will depend on the circumstances. As with false positives, a bioburden count that is off by 1 or 2 will not likely cause a manufacturing problem. Yet in other circumstances, finding 0 when there really should have been 1 or 2 can have devastating consequences, such as product recalls or harm to patients. When accurate counts are needed at low numbers, the false negative rate and the LOD of the assay become important considerations. Again, the false negative rate must be assessed in the context of the sample or product being tested, as product interference can have a dramatic affect on the rate of false negatives.

Conclusion:

Several distinct technologies exist under the umbrella of rapid microbial methods. A one-size-fits all approach will not deliver the expected results. Microbiologists need to understand the company, their laboratory environment, and the unique requirements of both their product and manufacturing. With that information, the technology that best fits the company's specific need can be chosen.

Disclaimer: This chapter is not meant to be a thorough examination of all rapid microbiological methods available. Rather it is meant to help the reader tailor the choosing of a rapid method to the needs of a particular site or company.

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