Why Fluorometric Detection is Cost Efficient and Necessary

The New and Old Method of Identifying Pathogenic Microorganisms – Fluorometric Detection

Where We Are Now

If you are in the food business, you know that sanitation can be as important as productivity making the product, and taste pleasing the customer. The economic and reputational effect of product recalls, and needless to say, sickness and/or the loss of life, can be catastrophic to any company's bottom line. There is a growing list of both food manufacturers and fast-food providers who have injured, killed, and put into doubt the cleanliness of their products and establishments.

For some time now there have been two primary testing methods for pathogenic microorganisms. The tried and true method has been to swab, incubate 24 to 48 hours and count bacterial colonies. Most of the time the results are negative no bacterial growth, but not always. That's when panic sets in (recalls, holds, immediate destruction or returns). The most advanced method for pathogen testing has been Nucleic Acid Amplification/Polymerase Chain Reaction (NAAT/PCR) - very effective, fairly rapid but requiring expensive equipment and properly trained personnel. We have discovered through our research and experience with test and measurement in plants, that there may be a better way; fluorometric detection of microorganisms through identification of the enzyme particular to a specific organism.

Some History

Since the early 1950's microbiologists have been studying and utilizing enzymes as a method of detecting bacteria. Why? They discovered the specificity of enzymes to microorganisms. Still, most companies stopped using enzyme methods (if they ever started) and/or never undertook the expense in equipment and highly trained personnel required to use NAAT/PCR technologies (or in fact antigen/antibody tests which were primarily used in testing for infectious diseases). As a result, most companies continued to rely on traditional culture methods.

Consider the context of the decision to continue using the traditional culture methods. As long as most food production utilized high heat as in cooking and canning, or pasteurization, or low pH (as in soft drinks) HAACP plans found it convenient to rely on ATP technology to guarantee the sanitation of the production line and surrounding areas. Adenosine Triphosphate detection identifies any organic contamination, which in and of itself requires re-cleaning and/or re-disinfection. Still, there was (and is) a downside – false positives. Yes, the contamination was there in terms of organic material, but the companies never found out whether this contamination was pathogenic. And it is the pathogens that make people sick.

Moreover, tiny colonies of bacteria, when undetected, can over time become large and dangerous colonies. In a manner of speaking traditional cultures became a backup to ATP, a secondary method that, hopefully, guaranteed that pathogens were not a threat to the consumer.

The Return of the Enzyme

Proprietary substrates which, when hydrolyzed by a specific enzyme (during peptide hydrolysis), produce a fluorescence detectable by a fluorometric device. This is the basis of an inexpensive and rapid method of identifying pathogens in any setting, food, medical and pharmaceutical. The foundation for success is that the technology is based on the detection of specific enzymes produced by specific microorganisms or bacteria. Continued enzymatic research discovered specific bacterial enzymes for a host of microorganisms. Over the years, proprietary substrates identifying and linking to the specific enzymes have been developed. These substrates have been optimized into various platforms focusing on the early detection of bacteria.

Other diagnostic systems, including NAAT/PCR, must find the bacteria cell itself, and then may require more than a day to obtain results from the time of sample collection to test results. More importantly, bacteria producing thousands of molecules of an enzyme increase the odds and shortens the time for detection utilizing the fluorometer.
As an aside fluorometric assays can also detect the presence of biofilm. Consider, while the bacteria can remain hidden within the biofilm, the enzymes escape the barrier and can be detected by such assays.

More on the Fluorometric Approach to Identifying Pathogenic Microorganisms.
Because enzymes are unique to the microorganic life of which they are a part (whether bacteria, viruses, fungi, etc.), they are the best identifier of a specific microorganism (like the ones that are pathogenic) provided we can identify the specific enzyme itself.

Why is this so?

Enzymes can be thought of as organic catalysts. That is, they make combinations of organic material possible without themselves ending up as part of the combination. We need not understand how this is achieved (that is a subject for different paper), but recognize that this process gives us a definitive reason why a specific enzyme can be related to a specific organism.

A small chain of 140 amino acids (the building blocks of proteins) combine and recombine into new configurations (actually three dimensional shapes) to become specific enzymes. This happens well over a trillion (yes a trillion) times per second. (Please keep in mind that the physics of the nano world is different than that of our world. For example it is difficult to even imagine the microbiologic storm that surrounds us. Think of a blinding snow storm except we can't see the flakes with our non-nano eyes.) One (or many) of these combinations – configurations unlocks (so to speak - catalyzes) the possibility that certain organic materials can combine to become a specific organism, e.g. a pathogen. There it is. Identify the enzyme and you have identified the microorganism.

We believe the future of pathogen testing where low cost, rapid results, ease of use and expansive application are valuable, will be well received and have universal appeal.

How is a Typical Test Accomplished
1. Add a buffer to a swab and then swab a four-by-four-inch area.
2. Fill a vial with buffer and insert the swab.
3. Remove the swab and add two drops of reagent.
4. Place the vial in the fluorometer, wait one minute and push "measure" then "blank."
5. Press "measure" and wait ten minutes.

This fluorometric detection method is also available for both liquid and product samples.

The Advantages of Fluorometric Analysis of Microorganisms

- Easy to use with little training required
- Greater number of test with the same or less employees and time spent
- Potential for broadening the scope of testing both in locations and time between tests
- Low cost of instrument and kits
- Elimination of the false positives of other methods (like ATP)

● Immediate test results in most cases, plus a five-hour incubation procedure for any low to no CFU requirement