# ADS Pathogen Detection Protocols

**Introduction**

One pathogenic bacterium Colony Forming Unit (CFU) is neither detectable, other than through microscopy, nor a danger to human health. One million pathogenic CFUs are both detectable by a number of means and a danger to human health. The problem for most sanitarians is how to prevent the one from becoming the one million, where people start getting sick or dying.
The problem with sickness or death due to bacterial infection is that there is no margin of acceptability, not that customers will accept. We expect problems that are addressable (solvable at least in theory) be addressed. So what to do?

We clean. We disinfect. We test. But the sanitarian is always faced with the same issue. How much to clean? How much to disinfect? How much to test? The new bacterial testing product we offer sanitarians provide an opportunity to test more often and more locations without greater cost. The ideas presented below are intended to assist sanitarians in devising plans that extend the efficiency, capability and effectiveness of their pathogen protection programs.

**What we offer**

A better way to detect pathogens when speed, ease of use and cost are factors to be considered: The how and why of fluorometric pathogen detection, and how to do better microbial surveillance.

All facilities subject to the dangers of pathogenic bacteria require a protection plan. In the food industry it might be based on HACCP. In medical institutions an infection control officer may be charged with the planning and implementation tasks. Pharmaceutical companies also have their own strategies and implementation programs.

The discussion below is not meant, and should not be interpreted, as a substitute for industry standards and practices. It is only meant to be suggestive of the ways that fluorometric detection can be incorporated to enhance microbial surveillance.

A fluorometer measures in Relative Fluorescent Units (RFU). These units correspond to the reactions which occur between the bacterial enzymes present in the sample (if bacteria are present) and the fluorescent reagent. Different bacteria produce different amounts of enzymes. (Fluorescence is a process of absorption of light energy at one wavelength and emitting light energy at longer wavelength.)

Fluorescence is easily observed when a substance is illuminated with invisible ultraviolet light and emits light that is visible to the human eye. This phenomenon is frequently used to detect presence of a substance by "tagging" it with a fluorescing material. The RFU value is a simple Pass or Fail which is utilized in several applications such as ATP. For example, Pass < 500 RFU Fail > 501 RFU.

For some bacteria a RFU reading may indicate …
A RFU measurement of 500 or less* indicates no CFUs.
A RFU measurement of 501 to 10,000* indicates 1 to 1,000 CFUs.
A RFU measurement of 10,001 to 25,000* indicates 1,001 to 10,000 CFUs
A RFU measurement of 25.001 or greater* indicates greater than 10,000 CFUs.
* Within a ten-minute time frame.

The measurement of CFUs is a moving target because bacteria colonies are growing all the time, sometimes slower with refrigeration, sometime faster in a warm environment. The danger to human health is not low numbers of pathogenic bacteria. The body can defend itself quite admirably against low levels of most pathogens. However, the body's defenses are overwhelmed at high levels, say 100,000 or more of CFUs.

The great advantages of fluorometric detection are low cost, ease of use and speed while still allowing specificity as to target pathogens, and the fact that more tests can be performed, thus providing for confirmation. Yes, if direct counting of CFUs at extremely low levels could be achieved without a 24 or 48- hour culture, fluorometric techniques might be less useful. But the 24 to 48-hour wait to see results is not suitable in many situations.

You will note that we recommend two test measurements per site. The first set of tests indicates whether there are high enough levels of bacteria to threaten human health, sooner or later. If the results are positive in this regard, all activity comes to a halt and action is taken (cleaning and/or disinfection). The second set of tests indicates whether any low levels of bacteria are likely to grow over time into a threat to human health. If the results are positive in this regard, all activity comes to a halt and action is taken (cleaning and/or disinfection).

**Three Industry Examples (sanitation)**

*Kits are also available for liquids and products (food/cosmetic). The testing protocols are similar but adjusted to the requirements (where, when and how) to the specific targets to be tested.*
*Hospitals and Medical Facilities*
a) Identify those items in rooms that are likely to come in contact with patients and/or medical staff (minimum 5).

b) Swab all 5 contact items immediately after cleaning and disinfection (two swabs per test site).

c) Test 5 swabs immediately (no incubation). If the tests fail, re-disinfect.

d) Incubate 5 swabs for five hours (per instruction) and test. If the tests fail, re-disinfect.

e) Repeat procedures b) through d) when necessary.

f) Repeat this procedure whenever there is a change of patients or personnel shift change or every 48 hours.

**Fresh Vegetables (Packing Houses and Fresh Cut Facilities)**

a) Identify those places or items in the production facility that are most likely to come in contact with products (minimum 15).

b) Identify those difficult locations where there is no contact with products (10 minimum).

c) Swab the 25 location (two swabs per test site).

d) Test 25 swabs immediately (no incubation). If the tests fail, re-disinfect.

e) Incubate 25 swabs for five hours (per instructions) and test. If the tests fail, re-disinfect.

f) Repeat procedures c) through e) when necessary.

g) Repeat this procedure every production or work shift.

**Processed Food and Beverages**

a) Identify those places in the production facility that are most likely to come in contact with products (minimum 15).

b) Identify those difficult locations where there is no contact with products (10 minimum).

c) Swab 25 of the locations (two swabs per test site).

d) Test 25 swabs immediately (no incubation). If the tests fail, re-disinfect.

e) Incubate 25 swabs for five hours (per instructions) and test. If the tests fail, re-disinfect.

f) Repeat procedures c) through e) when necessary.

g) Repeat this procedure every production or work shift.

**Conclusion**

After a facility (one room or many) has gone through a cleaning and disinfection cycle there is an expectation, a belief, that the room and the objects in the room are safe from pathogenic threats to human health. But testing provides the certainty (knowledge) that this is so at that moment in time. A small green grocer for example can't use (afford) the sophisticated equipment that would provide this certainty. Counting CFUs after 24 or 48 hours is inexpensive, but too late. The products have already been sold. Fluorometric detection does what no other technology can do. It provides users with a level of certainty that they can afford while meeting the requirement of timeliness (information when you need it).