

**Analytical Laboratory**

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## Microbiology Test Description Summaries

Microbiological food safety is of increasing concern to consumers, producers and regulators. Food products are routinely tested to determine their acceptability based on microbial load. Often a company's HACCP plan, quality practices, supplier specifications, or regulatory mandates determine when and which micro tests are necessary before releasing a product.

The most commonly requested microbiological tests, for both environmental samples and finished products, are aerobic plate count, coliform, *E. coli*, *Staphylococcus aureus*, *Salmonella*, *Listeria*, yeast and mold. Turn around times vary depending upon the testing requested. While we test many types of foods, many of our analysts have extensive dairy backgrounds, which are especially advantageous for troubleshooting problems in dairy products and for using specialized tests or media.

See test descriptions below:

### **APC/SPC**

One of the most commonly requested tests - Aerobic Plate Count (APC) determines the overall microbial population in a sample. For dairy foods, Standard Plate Count (SPC) is used instead of APC. These two reference methods differ in their temperature of incubation, with APC incubated at 35°C and SPC incubated at 32°C.

The standard test method is an agar pour plate using Plate Count Agar for determination of the total aerobic microorganisms that will grow from a given sample. Variations in the general method allow for changes in incubation temperatures, time, aerobic and anaerobic conditions. The test takes at least two days after which results are given in CFU/g or ml (colony forming units per gram or milliliter). 3M Petrifilm™ can also be used to obtain aerobic or standard plate counts.

APC may also be referred to as Total Plate Count (TPC) or Total Viable Count (TVC).

### **Bacillus cereus**

*Bacillus cereus*, an aerobic spore forming bacteria widely distributed in nature and foods, is commonly found in soil, milk, cereals, starches, herbs, spices and other dried product and produces a toxin that can cause illness. Effective prevention and control depend on effective quality control or inhibiting spore germination and growth of vegetative cells in cooked, ready to eat foods. Spores can survive long periods of dryness and mild heat treatments. The presence of large numbers of *B. cereus* (greater than 10<sup>6</sup> organisms/g) in a food is indicative of active growth and proliferation of the organism and is consistent with a potential hazard to health. Enumerating *Bacillus* sp. is done using mannitol egg yolk polymyxin (MYP) agar. The MPN method can also be used when low levels of *B. cereus* are expected. Differentiation of *B. cereus* involves isolating suspect colonies and performing biochemical confirmation. By the plate method, incubation is generally two days if negative and up to six additional days for confirmation of suspect colonies.

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## **Coliforms**

Coliforms are assayed to determine the potential recontamination of a process system. Generally a selective medium incorporating bile salts is used to quantify this group of organisms.

Coliforms are very sensitive to heat processing. At temperatures above 120°F, coliforms will begin to die. They are all destroyed at pasteurization temperatures. Coliforms are gram negative, non-sporeforming bacteria, which are all heat sensitive and associated with most post processing product failures (spoilage). Testing can be done using pour plate method, Petrifilm™ or by MPN (most probable number which is a statistical estimate) and usually takes 24 hours using pour plate or Petrifilm™ and two days for MPN results if negative. An additional two days are needed to confirm coliform by the MPN method.

## **E. coli/ E. coli 0157:H7**

E. coli, a coliform notorious in recent years because a pathogenic strain (0157:H7) can cause severe food poisoning outbreaks. Food processors are at heightened awareness and concern that this organism not be present in their products or facilities. E. coli is tested by plating on VRB with MUG agar or Petrifilm™; or by MPN. VRB with MUG and Petrifilm™ are direct count methods, and MPN is a statistical estimate based on number of positive tubes. Commonly, the plate count method is used and results are final in one day. The advantage of MPN is that it has a lower (more sensitive) detection limit. Petrifilm™ takes 48 hours and MPN is 48 hours for negative results. An additional 2-3 days is necessary to confirm positive results. Specific testing is necessary to quantify E. coli 0157:H7. Typically an Immunoassay is used to determine presence or absence in food, with results in 1 day for a negative. Confirmation is available and takes up to an additional 5-7 days.

## **Lactic Acid Bacteria**

The manufacture of cheese often involves enumerating and isolating the main groups of bacteria present in milk, cheese curd and cheese ripening, as well as that in other fermented dairy products. Various selective media are used dependent upon the purpose of the testing. Ellikers is a selective media used for total lactic count including Lactobacillus and Lactococcus. Rogosa is used to determine the amount of environmental or non-starter Lactobacillus. It is a selective medium with a low pH (5.4) and high concentration of acetate ions which suppresses the growth of all organisms except non-starter Lactobacilli. MRS media supports the growth of dairy Lactobacilli as well as Pediococci and Leuconostoc. M17 agar is selective for the enumeration of lactic acid Streptococci (Lactococcus). These tests take two days. MRS + Fructose media is used to test for Lactic Acid Heterofermentative bacteria (lactic spoilage organisms). This is an MPN method (most probable number which is a statistical estimate) and takes four days to complete.

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### **Listeria**

Many food manufacturers test products, drains, vents, filters, and equipment for Listeria. Listeria is especially troubling because it can grow at refrigerator temperatures, and Listeria monocytogenes can cause serious illness. The DuPont Qualicon BAX® system is used in our laboratory to rapidly screen product and environmental samples for the presence of Listeria species.

The automated BAX system screens samples for Listeria using polymerase chain reaction (PCR). PCR allows detection at the genetic level, which gives improved accuracy over traditional screening methods and minimizes false positives. The test takes two-three days for a suspect positive or negative result and up to an additional 5 days for confirmation of a suspect result. Confirmation includes identification of isolates to Listeria species identification (for example; genus = Listeria, species = monocytogenes).

### **Microorganism Identification**

Microorganism identification is available to determine the identity of select organisms of interest or to estimate the prevalence of microorganisms in a sample. Identification of an unknown microbial isolate can be achieved through biochemical characterization and/or genetic characterization methodology.

bioMerieux VITEK 2®: The VITEK 2® automated biochemical characterization system offers isolate identification to species for most organisms. Microbial identity is obtained based on a series of biochemical reactions that create a biochemical pattern. For uncommon bacteria, our experienced technical staff can use this pattern, along with morphological information from selective agars, ancillary tests and direct microscopic observation to narrow the field to a select few possibilities.

DuPont Qualicon RiboPrinter®: For genetic characterization and tracking of strains, our lab utilizes the RiboPrinter® microbial characterization system. Using DNA fingerprinting analysis, the RiboPrinter® can identify environmental isolates, spoilage microorganisms and pathogenic isolates. The bacterial isolate's genetic pattern is compared to a database containing over 6900 RiboPrint® patterns, representing almost 220 bacterial genera and over 1440 species and serotypes.

### **Proteolytics**

Starter Lactococci produce peptides and amino acids by their proteolytic action on casein, which contributes to flavor in these cheese and yogurt products. The skim milk agar method is used to help determine the amount of proteolysis that has occurred in a product. The test takes three days.

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### **Salmonella**

Salmonella is an enteric pathogen which can cause serious illness. Foods may become contaminated through poor sanitation and manufacturing practices. The DuPont Qualicon BAX® system is used in our laboratory to rapidly screen product and environmental samples for the presence of Salmonella species.

The automated BAX system screens samples for Salmonella using polymerase chain reaction (PCR). PCR allows detection at the genetic level, which gives improved accuracy over traditional screening methods and minimizes false positives. The BAX® method takes two days to a suspect positive or negative result. If confirmation is requested, it is another 5-6 days for complete confirmation, using the FDA-BAM confirmation procedure.

### **Staphylococcus aureus**

Staphylococcus aureus is a common cause of food poisoning. Once these organisms grow to a certain number, they produce a heat stable enterotoxin which causes vomiting and diarrhea in those who eat the contaminated food. Because the toxin is heat stable, once it is produced in a food, no amount of pasteurization can eliminate its presence. Staph is assayed for by a direct plate method using Baird Parker agar, or by MPN which is a statistical estimate of the number of colonies in the sample. Plating takes 48 hours if negative with an additional day if suspect colonies are present, to screen for coagulase activity (coagulase is an enzyme usually produced by Staphylococcus aureus). MPN takes two to four days for a negative result plus an additional day if confirmation is necessary. Staph enterotoxin can be tested for by an ELISA method which usually takes one day.

### **Yeast and Mold**

Yeasts and molds are determined quantitatively by using standard FDA – BAM plating methodology. Plate count agar with the addition of an antibiotic is used. This method will detect the majority of yeast and molds commonly found in foods, some may require specialized conditions. Yeast and mold determination requires five days of incubation.

Osmophilic yeast organisms can cause spoilage in high sugar and/or low water activity products and are determined quantitatively by using a reduced water activity agar and diluent. This assay requires 7 days of incubation.