Shimadzu Diagnostics Corporation

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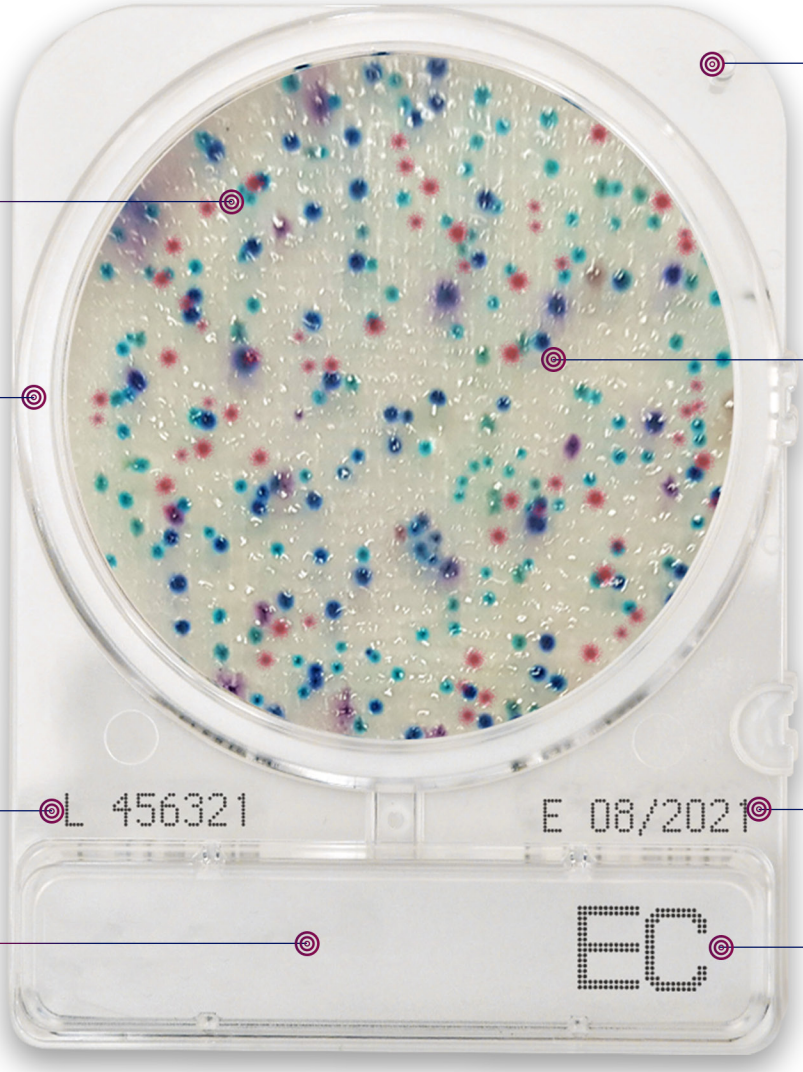
CompactDry™
There is always a better way.

The world's population is continuously growing, and so is the need for quality and safe products. People consume food for survival, take dietary supplement, and apply cosmetics for self-improvement. In general, food is available in the market as frozen, chilled, pre-cooked, and/or processed, while nutraceuticals and cosmetics are ready-to-consume and ready-to-apply, respectively. The common denominator for all of these products is the need to be properly processed as finished products.

Proper processing of food, dietary supplements, additives and cosmetics should be aligned with the standards set by the government or any recognized organization concerned with the said products. To be able to achieve this, manufacturers should start investing in different food safety testing methods that will ensure the quality and safety of their products. One of the most significant of which is subjecting the products to microbial analysis to verify if these fall within the set standards of particular product safety regulatory boards. The traditional way of doing the assay is through conventional plating method which can take about two (2) to seven (7) days depending on the microorganism of interest. Added to this are identification methods, especially for pathogens, which need further confirmation such as biochemical tests or even molecular identification tests. With these traditional methods, processed foods cannot be easily declared as "good to deliver" or "passed" by Quality Control. This leads to slower turnover, additional warehousing costs, and merchandise inventory with shorter shelf life.

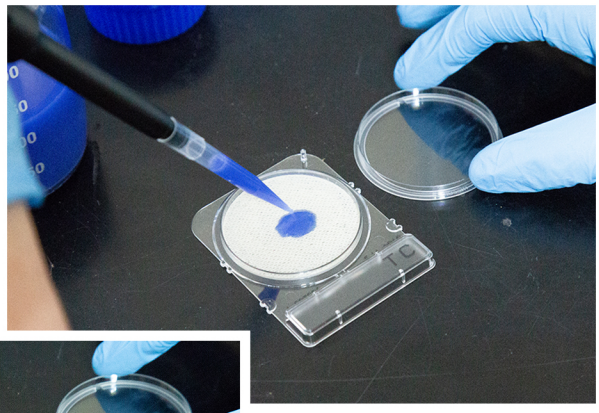
To address this problem, manufacturers turn to alternative tools that can do the same function but with faster results without sacrificing sensitivity and specificity. One such tool is CompactDry™, a product of Nissui Pharmaceuticals Co., Ltd. based in Japan, an innovation of the conventional plate method, and the future of microbial testing. CompactDry™ is a ready-to-use chromogenic media plate which can be used as direct substitute to conventional method. It eliminates the laborious work of media preparation and sterilization. In large manufacturing companies, a total of four (4) to six (6) hours daily can be saved in overall microbial testing, which can be used for other laboratory assignments. Moreover, for start-up micro, small, and medium companies with limited facilities, the use of rapid test kits is also an advantage because it only needs a small space due to minimum laboratory requirement.

CompactDry™ is very easy to use, and requires minimal training. This rapid test kit is available in 15 parameters which includes spoilage organisms, pathogens, and other special parameters. In comparison with other similar technologies, CompactDry™ is the only system with available tests for *Enterococcus*, *Vibrio parahaemolyticus*, *Pseudomonas aeruginosa*, and Total Count in Tea. It also offers reduced testing time for *E.coli*/coliform, and yeast and mold with only 24-72 hours of incubation time, respectively, another product development that is part of Nissui Corporation's commitment to continual innovation and provide the best solutions to various industries' microbial testing requirements.

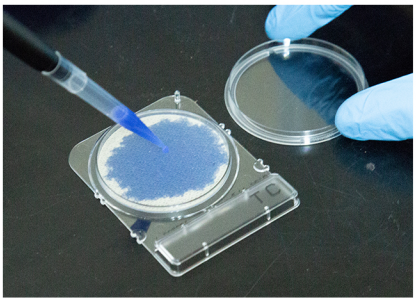

Compact plate
Chromogenic reaction
Rigid plate cover
Non-woven fabric
1 mL capacity
No spreader
Lot number
L 456321
Expiry date
E 08/2021
Label area
EC
Test parameter

CompactDry™
There is always a better way.

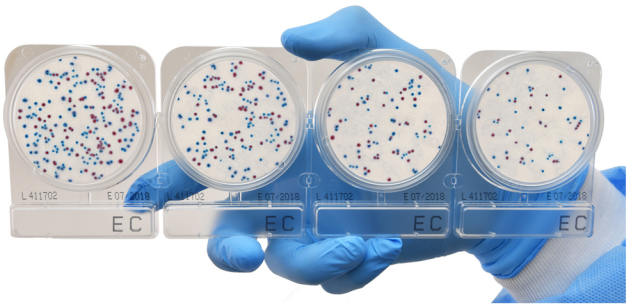
READY-TO-USE
- No need for sterilization
- Saves time, water, and energy
- Avoids series of contamination



SELF-DIFFUSING MEDIA
- Allows 10-second hands-on time
- Eliminates the use of other accessories such as spreader
- No "gelling time"; the medium infused in fabric pad readily solidifies upon inoculation
- No need for pressing, spreading, and tilting of plates



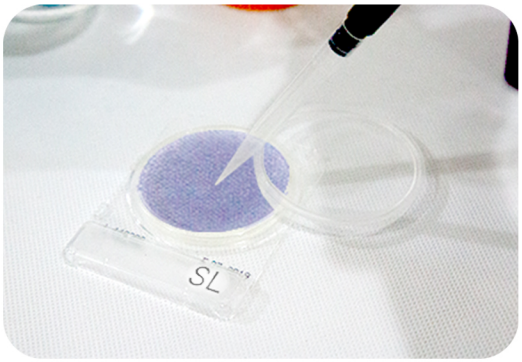
INNOVATIVE DESIGN
- Compact plate with robust plate cover to avoid contamination and spills
- With write-on area that is not easily erased due to matte finish
- Designed with easy to pick-up handle and air space for even distribution of oxygen
- Capable of unlimited stacking depending on incubator's capacity without compromising the growth of target microorganism



STRATEGIC PACKAGING
- Stable at room temperature for 18-36 months
- Clustered plates for grouping one sample type with multiple dilutions
- Plates can also be broken apart for single use only
- Packed by 4 plates per foil to avoid effect of secondary shelf life (30 days) upon opening of the foil

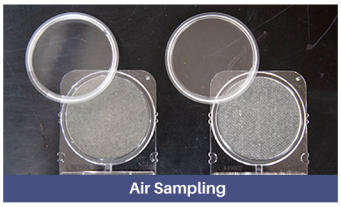
CompactDry™
There is always a better way.

CONVENIENT
- More streamlined workflow
- Uniform incubation temperature of 35°C for most parameters except for yeast & molds (25°C) and *Salmonella* (41°C)
- Microbial testing in 3 easy steps – inoculate, incubate, and interpret




Solid or Liquid Samples

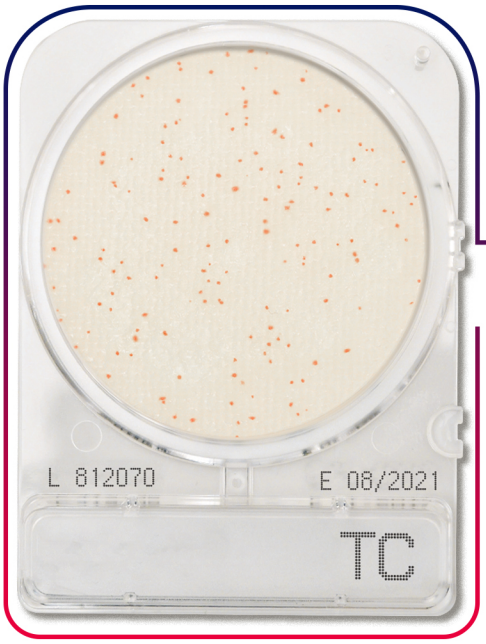

Membrane Filtration


Air Sampling


Environmental Samples

WIDEST AVAILABLE PARAMETERS & BROADEST APPLICATION
- Currently with a total of 15 parameters which includes pathogens, commonly tested and other special parameters
- Applicable for most solid and liquid samples, membrane filtration, air sampling, hand, and surface swabbing

FAST & ACCURATE RESULT
- Results in as fast as 24-48 hours for most parameters
- No need for additional confirmatory disks and procedure
- Distinct color contrast for better colony identification
- Easier differentiation between yeast and mold due to air space that allows 3D growth
- Easy to pick-up isolated colonies for further analysis
- International approvals – AOAC International, European Microval, Nordval, and HPFB Health Canada



Total Count

AOAC #010404 **NordVal** #033 **MicroVal** #20007LR01

Refers to all microorganisms present in a sample which can tolerate the presence of oxygen and does not have specific growth requirements typically not included in the formulation of general purpose media. In accordance to Food and Drug Administration's (FDA) regulation, Total Count is considered as one of the release parameters for food (frozen, chilled, precooked, and prepared food), pharmaceutical, and nutraceutical samples.

Result Interpretation

Red and otherwise colored colonies

PROCEDURE

Aseptically weigh 10.0 g or pipette 10.0 ml sample to appropriate sterile container (i.e. stomacher bag, dilution bottle, Whirl-Pak bag).

↓

Add 90.0 ml diluent to achieve 1:10 dilution and homogenize. Adjust pH if necessary. Suggested diluents for routine parameters are Butterfield's Phosphate Buffer, Maximum Recovery Diluent, and other appropriate diluents depending on BAM.

↓

If needed, dilute the sample further (refer to page no.38).

↓

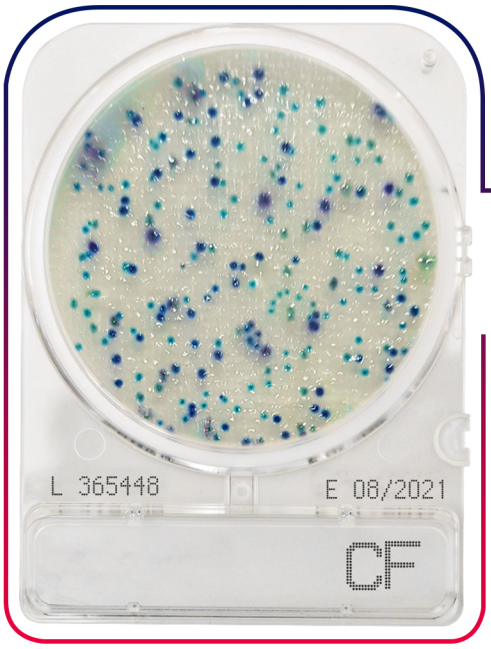
Open the cap. Dispense 1.0 ml of diluted sample in the middle of the CompactDry™ plate. Recap the plate.

↓

Invert and incubate the plates at specific incubation condition.
AOAC International: 35 ± 2°C for 48 ± 3 hours
MicroVal and NordVal: 30 ± 1°C for 48 ± 3 hours

↓

Read results.



Coliform

AOAC #110402 **NordVal** #035 **MicroVal** #MV0806-003 LR

Coliform are rod-shaped, Gram-negative, facultative anaerobe, non-spore forming bacteria commonly found in the gut of warm-blooded animals and soil. The term "coliform" is used to refer to enteric bacteria (both lactose and non-lactose fermenters) found in food samples like meat and shellfish, cosmetics, pharmaceutical raw materials, and most specially, water samples. Bacteria such as *Shigella, Salmonella, Yersinia, Citrobacter, Klebsiella,* and *Enterobacter* are considered as coliform but the most recognized coliform is *Escherichia coli*. Testing for the presence of coliform is important in the food industry especially in monitoring water quality.

Result Interpretation

Blue or blue green colonies

PROCEDURE

Aseptically weigh 10.0 g or pipette 10.0 ml sample to appropriate sterile container (i.e. stomacher bag, dilution bottle, Whirl-Pak bag).

↓

Add 90.0 ml diluent to achieve 1:10 dilution and homogenize. Adjust pH if necessary. Suggested diluents for routine parameters are Butterfield's Phosphate Buffer, Maximum Recovery Diluent, and other appropriate diluents depending on BAM.

↓

If needed, dilute the sample further (refer to page no. 38).

↓

Open the cap. Dispense 1.0 ml of diluted sample in the middle of the CompactDry™ plate. Recap the plate.

↓

Invert and incubate the plates at specific incubation condition.
AOAC International, MicroVal, and NordVal: 37 ± 1°C for 24 ± 2 hours

↓

Read results.

E. coli/Coliform

AOAC #110402 **NordVal** #036 **MicroVal** #MV0806-005 LR (*E. coli*) **MicroVal** #MV0806-004 LR (Coliform)

Escherichia coli is a coliform which is found in the gut of warm-blooded animals making it an excellent indicator for fecal contamination. Not all *E. coli* are pathogenic but there are strains which can be considered as opportunistic bacteria infecting an immunocompromised host. Testing for the presence of coliform and *E. coli* is important in food industry especially in monitoring water quality.

Result Interpretation

E. coli - Blue colonies
Total coliform - Red, blue, and purple colonies

PROCEDURE

Aseptically weigh 10.0 g or pipette 10.0 ml sample to appropriate sterile container (i.e. stomacher bag, dilution bottle, Whirl-Pak bag).

↓

Add 90.0 ml diluent to achieve 1:10 dilution and homogenize. Adjust pH if necessary. Suggested diluents for routine parameters are Butterfield's Phosphate Buffer, Maximum Recovery Diluent, and other appropriate diluents depending on BAM.

↓

If needed, dilute the sample further (refer to page no. 38).

↓

Open the cap. Dispense 1.0 ml of diluted sample in the middle of the CompactDry™ plate. Recap the plate.

↓

Invert and incubate the plates at specific incubation condition.
AOAC International: 35 ± 2°C for 24 ± 2 hours
MicroVal and NordVal: 37 ± 1°C for 24 ± 2 hours

↓

Read results.

Yeast and Mold

AOAC #100401 **NordVal** #043 **MicroVal** #RQA208LR10

Yeasts and molds (YM) are spore forming microorganisms. These spores are resistant to harsh conditions like extreme pH, drought, and extreme temperature. YM is the index of the cleanliness of environmental bioaerosol in different production plants. Failure to comply with the tolerable amount of YM in the production area will result to food spoilage. YM can easily spoil the food due to its wide tolerance to environmental conditions (e.g. water activity of less than 0.85, and as low as 0.45). Thus, based on Food and Drug Administration's regulation, it is part of the release parameters for food and beverages, and part of environmental monitoring system through air sampling.

Result Interpretation

Yeasts - Blue and white colonies
Molds - Cottony colonies with characteristic colors

PROCEDURE

Aseptically weigh 10.0 g or pipette 10.0 ml sample to appropriate sterile container (i.e. stomacher bag, dilution bottle, Whirl-Pak bag).

↓

Add 90.0 ml diluent to achieve 1:10 dilution and homogenize. Adjust pH if necessary. Suggested diluents for routine parameters are Butterfield's Phosphate Buffer, Maximum Recovery Diluent, and other appropriate diluents depending on BAM.

↓

If needed, dilute the sample further (refer to page no. 38).

↓

Open the cap. Dispense 1.0 ml of diluted sample in the middle of the CompactDry™ plate. Recap the plate.

↓

Invert and incubate the plates at specific incubation condition.
AOAC International, MicroVal, and NordVal: 25 ± 2°C for 3 to 7 days

↓

Read results.

Yeast and Mold Rapid

NordVal #050 **MicroVal** #2016LR61

Yeasts and molds (YM) are spore forming microorganisms. These spores are resistant to harsh conditions like extreme pH, drought, and extreme temperature. YM is the index of the cleanliness of environmental bioaerosol in different production plants. Failure to comply with the tolerable amount of YM in the production area will result to food spoilage. YM can easily spoil the food due to its wide tolerance to environmental conditions (e.g. water activity of less than 0.85, and as low as 0.45). Thus, based on Food and Drug Administration's regulation, it is part of the release parameters for food and beverages, and part of environmental monitoring system through air sampling.

Result Interpretation

Yeasts - Blue and white colonies
Molds - Cottony colonies with characteristic colors

PROCEDURE

Aseptically weigh 10.0 g or pipette 10.0 ml sample to appropriate sterile container (i.e. stomacher bag, dilution bottle, Whirl-Pak bag).

↓

Add 90.0 ml diluent to achieve 1:10 dilution and homogenize. Adjust pH if necessary. Suggested diluents for routine parameters are Butterfield's Phosphate Buffer, Maximum Recovery Diluent, and other appropriate diluents depending on BAM.

↓

If needed, dilute the sample further (refer to page no. 38).

↓

Open the cap. Dispense 1.0 ml of diluted sample in the middle of the CompactDry™ plate. Recap the plate.

↓

Invert and incubate the plates at specific incubation condition.
AOAC International: 25 ± 2°C for 48-72 hours
MicroVal, and NordVal: 25 ± 1°C for 3 days

↓

Read results.

Staphylococcus aureus

AOAC #081001 **NordVal** #042 **MicroVal** #2008LR14

Staphylococcus aureus is a common human microflora, it can naturally be found on the skin, nose, and hair. Due to this, it is commonly used as index of food handlers' personal hygiene. Thus, high staphylococcal population in food system indicates poor hygiene and sanitation. They are normally harmless but at high population, can begin forming endotoxins causing "*staph*" food poisoning. They can also tolerate low water activity and are opportunistic in high sugar food.

Result Interpretation

Blue or light blue colonies

PROCEDURE

Aseptically weigh 10.0 g or pipette 10.0 ml sample to appropriate sterile container (i.e. stomacher bag, dilution bottle, Whirl-Pak bag).

↓

Add 90.0 ml diluent to achieve 1:10 dilution and homogenize. Adjust pH if necessary. Suggested diluents for routine parameters are Butterfield's Phosphate Buffer, Maximum Recovery Diluent, and other appropriate diluents depending on BAM.

↓

If needed, dilute the sample further (refer to page no. 38).

↓

Open the cap. Dispense 1.0 ml of diluted sample in the middle of the CompactDry™ plate. Recap the plate.

↓

Invert and incubate the plates at specific incubation condition.
AOAC International: 35 ± 2°C for 24 ± 2 hours
MicroVal, and NordVal: 37 ± 1°C for 24 ± 2 hours

↓

Read results.

Salmonella

Salmonella is a genus of Gram-negative bacteria comprised of only two (2) species, *S. enterica* and *S. bongori*. Together, they have more than 2,500 serovars, most of which are pathogenic. Historical reservoirs are birds or poultry but has been isolated in pet, reptiles, and low water activity food such as spices and flour. Based on the standard set by the Food and Drug Administration, *Salmonella* should be totally absent in all food and environmental samples. No tolerable level was set because it has a low infective dose that can lead to fatal diseases. *Salmonella* infection is caused by cross-contamination during evisceration of animal, washing, and transportation of carcasses and can also occur during raw material preparation. Thus, environmental monitoring procedure should be present in industries like poultry because it is highly susceptible to the contamination.

Result Interpretation

Salmonella Positive
1. Black to green isolated or fused colonies
2. Medium around the colonies changes to yellow
3. Tailing due to motility

Salmonella Negative
1. No change in plate color
2. Red or reddish purple colonies

NOTE: Too high *Salmonella* count - Plate turns completely yellow without isolated colonies; spots of fused black and green colonies are present

PROCEDURE

Aseptically weigh 25.0 g or pipette 25.0 ml sample to appropriate sterile container (i.e. stomacher bag, dilution bottle, Whirl-Pak bag).

↓

Add 225.0 ml diluent and homogenize. Adjust pH if necessary. Suggested diluents for *Salmonella* parameter are Buffered Peptone Water and other appropriate diluents depending on BAM.

↓

Incubate at 36 ± 1°C for 20-24 hours.

↓

Obtain 0.1 ml of the pre-enriched sample and inoculate it at approximately 1 cm far from edge of CompactDry™ *Salmonella*. Then, add 1.0 ml of sterile distilled water on the opposite of the inoculum. Recap the plate.

↓

Invert and incubate the plates at specific incubation condition.
Manufacturer's recommendation: 42 ± 1°C for 22 ± 2 hours

↓

Read results.

Enterobacteriaceae

NordVal #034v **MicroVal** #MV0806-002 LR

Enterobacteriaceae is a family under the Gram-negative bacteria with known characteristic of being facultative anaerobe, non-spore former, and commonly motile but non-motile genera are also present. Enterobacteriaceae is commonly found in the environment as well as the gut of animals. Almost all foodborne pathogenic bacteria fall under this family including *Citrobacter, Enterobacter, Escherichia, Klebsiella, Proteus, Salmonella, Serratia, Shigella,* and *Yersinia*. Enterobacteriaceae is a release parameter in various food, beverages, pharmaceutical, nutraceutical, and cosmetic samples. As a recent development in food safety, *Enterobacter sakazakii* is being monitored in formula milk due to high risk of milk contamination, as well as its health impact when ingested by babies.

Result Interpretation

Red or purple colonies and otherwise colored colonies

PROCEDURE

Aseptically weigh 10.0 g or pipette 10.0 ml sample to appropriate sterile container (i.e. stomacher bag, dilution bottle, Whirl-Pak bag).

↓

Add 90.0 ml diluent to achieve 1:10 dilution and homogenize. Adjust pH if necessary. Suggested diluents for routine parameters are Butterfield's Phosphate Buffer, Maximum Recovery Diluent, and other appropriate diluents depending on BAM.

↓

If needed, dilute the sample further (refer to page no. 38).

↓

Open the cap. Dispense 1.0 ml of diluted sample in the middle of the CompactDry™ plate. Recap the plate.

↓

Invert and incubate the plates at specific incubation condition.
AOAC International: 35 ± 2°C for 24 ± 2 hours
MicroVal: 37°C for 24 hours, **NordVal**: 37 ± 1°C for 24 ± 2 hours

↓

Read results.

Enterococcus

MicroVal #2014LR48 **NordVal#** 047

Enterococci are abundantly found in gastrointestinal tract of humans and animals. Thus, this can be used as an index of good hygiene and sanitation. High count of *Enterococci* in the environment and food samples means that the company failed to comply with proper environmental monitoring. Most species under *Enterococcus* are regarded as "low-grade" pathogens but some are opportunistic which can produce various types of virulence factors with broad resistance against different antibiotics. Two of many species under *Enterococcus*, *Enterococcus faecalis* and *Enterococcus faecium*, have gained significant importance in clinical microbiology as they are known to cause various infection such as urinary tract infections, bacteraemia, meningitis, wound infections, and neonatal infections. In the food industry, *Enterococcus* is a release parameter of food products such as milk, meat, deli meat products, and fermented variant of milk and meat.

Result Interpretation

Blue or blue green colonies

PROCEDURE

Aseptically weigh 10.0 g or pipette 10.0 ml sample to appropriate sterile container (i.e. stomacher bag, dilution bottle, Whirl-Pak bag).

↓

Add 90.0 ml diluent to achieve 1:10 dilution and homogenize. Adjust pH if necessary. Suggested diluents for routine parameters are Butterfield's Phosphate Buffer, Maximum Recovery Diluent, and other appropriate diluents depending on BAM.

↓

If needed, dilute the sample further (refer to page no. 38).

↓

Open the cap. Dispense 1.0 ml of diluted sample in the middle of the CompactDry™ plate. Recap the plate.

↓

Invert and incubate the plates at specific incubation condition.
MicroVal: 37 ± 1°C for 20-24 hours
NordVal: 36 ± 1°C for 24 ± 2 hours

↓

Read results.

Bacillus cereus

MicroVal #2011LR41 **NordVal** #045

Bacillus cereus is a Gram-positive, aerobic, spore-forming bacterium which usually thrive in soil, vegetable, and various raw and processed food products. *Bacillus* spores can survive adverse condition and germinate if conditions improve, a trait which makes completely eliminating them difficult. Studies showed that when the bacterial population of *B. cereus* reached more than 10^6 cells/g toxins are produced, it can either be emetic or diarrhetic enterotoxin which is attributed with acute attack of nausea and vomiting, or with abdominal pain and non-bloody diarrhea, respectively. With this, *B. cereus* is considered as a release parameter for cooked meat and vegetables, boiled and fried rice, vanilla sauce, custards, soups, and raw vegetable sprout.

Result Interpretation

Round blue or light blue colonies

PROCEDURE

Aseptically weigh 10.0 g or pipette 10.0 ml sample to appropriate sterile container (i.e. stomacher bag, dilution bottle, Whirl-Pak bag).

↓

Add 90.0 ml diluent to achieve 1:10 dilution and homogenize. Adjust pH if necessary. Suggested diluents for routine parameters are Butterfield's Phosphate Buffer, Maximum Recovery Diluent, and other appropriate diluents depending on BAM.

↓

If needed, dilute the sample further (refer to page no. 38).

↓

Open the cap. Dispense 1.0 ml of diluted sample in the middle of the CompactDry™ plate. Recap the plate.

↓

Invert and incubate the plates at specific incubation condition.
Manufacturer's recommendation: 35 ± 2°C for 24 ± 2 hours
MicroVal: 30°C for 48 hours, **NordVal:** 30 ± 1°C for 48 ± 2 hours

↓

Read results.

Listeria

Listeria are Gram-positive, motile (at mesophilic condition), psychrotrophic bacteria with six (6) known species - *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. ivanovii*, and *L. grayi*. They are associated with clinical importance due to their virulence activity. But among the six species of *Listeria*, the most prevalent is *L. monocytogenes* causing listeriosis including sepsis, meningitis, encephalitis, corneal ulcer, pneumonia, cervical infection, and abortion, where elderly, immunocompromised, pregnant women, and infants are the most susceptible. Due to the increasing *Listeria* outbreak, certain high-risk foods became necessary to test for *Listeria* contamination. These high-risk foods are as follows: ready-to-eat seafoods, pre-packed fruits and vegetables, drinks made from fresh fruits, deli meats without further cooking process, unpasteurized milk, soft-serve ice cream, soft cheeses, salad dressings, and raw vegetable garnishes.

Result Interpretation

Blue or light blue colonies of about 1-2 mm in diameter

PROCEDURE

Aseptically weigh 25.0 g or pipette 25.0 ml sample to appropriate sterile container (i.e. stomacher bag, dilution bottle, Whirl-Pak bag).

↓

Add 225.0 ml diluent and homogenize. Adjust pH if necessary. Suggested diluents for *Listeria* parameter are Buffered Peptone Water and other appropriate diluents depending on BAM.

↓

Pre-enrich at 20°C for 1 hour.

↓

Open the cap. Dispense 1.0 ml of the pre-enriched sample in the middle of the CompactDry™ *Listeria* plate. Recap the plate.

↓

Invert and incubate the plates at specific incubation condition.
Manufacturer's recommendation: 35 ±1°C for 24 ± 2 hours

↓

Read results.

Vibrio parahaemolyticus

Vibrio is a Gram-negative, rod-shaped bacteria which is characterized by a rigid curve, it is usually motile due to the single polar flagellum. *V. cholerae, V. parahaemolyticus,* and *V. vulnificus* are the three known pathogenic species of *Vibrio* with documented cases. *Vibrio* are commonly found in seawater as they can tolerate high salt concentration. Foods which are contaminated with *Vibrio* can lead to negative health implication starting with diarrhea, and worst, with death. Thus, it is considered as a release parameter for seafoods and seafood-based products.

Result Interpretation

Blue or blue green colonies

PROCEDURE

Aseptically weigh 10.0 g or pipette 10.0 ml sample to appropriate sterile container (i.e. stomacher bag, dilution bottle, Whirl-Pak bag).

↓

Add 90.0 ml diluent to achieve 1:10 dilution and homogenize. Adjust pH if necessary. Suggested diluents for routine parameters are Butterfield's Phosphate Buffer, Maximum Recovery Diluent, and other appropriate diluents depending on BAM.

↓

If needed, dilute the sample further (refer to page no. 38).

↓

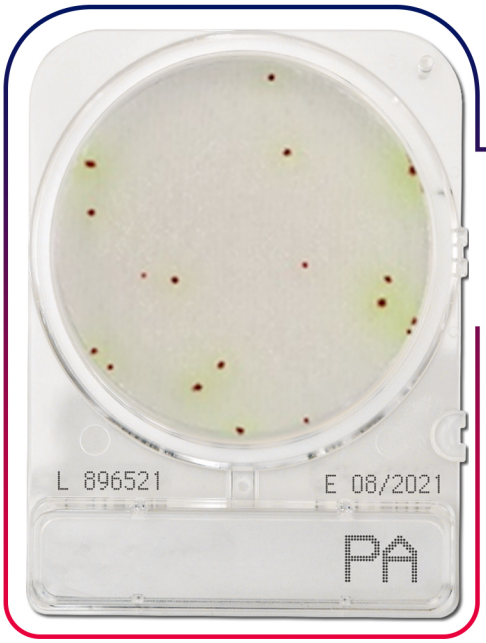
Open the cap. Dispense 1.0 ml of diluted sample in the middle of the CompactDry™ plate. Recap the plate.

↓

Invert and incubate the plates at specific incubation condition.
Manufacturer's recommendation: 35-37°C for 20-24 hours

↓

Read results.



Pseudomonas aeruginosa

Pseudomonas is a psychrotropic-bacteria which can survive and thrive at low temperature; due to this, it is a known causative agent of spoilage of refrigerated products such as red meat, poultry, fish, and milk and milk products. They are ubiquitous in nature and they can thrive in soil, fresh water, and marine environment. Their survival ability is based on the wide range of environmental requirements such as water activity, salt concentration, and temperature. Thus, aside from food products, they are also known to contaminate clinical environment as well as cosmetic and pharmaceutical products. Increase in the number of such bacteria tends to lower the quality and safety of the food, and in return, the risk of getting diseases. Some species are known with pathogenic activity while some are opportunistic bacteria for immunocompromised individuals. Furthermore, *Pseudomonas aeruginosa* is the common target in food and pharmaceutical industries due to its versatile metabolic capacity, persistence, and multiple virulence factors; one of which is prolific biofilm formation.

Result Interpretation

Red colonies with greenish-yellow ring or halo

PROCEDURE

Aseptically weigh 10.0 g or pipette 10.0 ml sample to appropriate sterile container (i.e. stomacher bag, dilution bottle, Whirl-Pak bag).

↓

Add 90.0 ml diluent to achieve 1:10 dilution and homogenize. Adjust pH if necessary. Suggested diluents for routine parameters are Butterfield's Phosphate Buffer, Maximum Recovery Diluent, and other appropriate diluents depending on BAM.

↓

If needed, dilute the sample further (refer to page no. 38).

↓

Open the cap. Dispense 1.0 ml of diluted sample in the middle of the CompactDry™ plate. Recap the plate.

↓

Invert and incubate the plates at specific incubation condition.
Manufacturer's recommendation: 35 ±1°C for 48 ± 3 hours

↓

Read results.

Heterotrophic Bacteria in Water

In accordance to the awareness set by World Health Organization (WHO), drinking water, and process water should be free from any contaminating microorganisms. Similar to food samples, total microbial count of water should be determined which is called Heterotrophic Plate Count (HPC). HPC includes all microorganisms including bacteria and yeast and mold that require high carbon source. Thus, the medium for HPC is different from Aerobic Plate Count (APC). In terms of incubation condition, conventional method of enumerating HPC requires elevated temperature of up to 40°C for five (5) to seven (7) days depending on the method used. HPC is required to test for piped-water samples, especially at the stagnant part of the pipeline, due to the accumulation of different microorganism in the form of biofilm. Furthermore, HPC testing is part of the national standard for drinking water aside from obtaining the total thermotolerant coliform and total *E. coli* count.

Result Interpretation

Red and otherwise colored colonies are counted

PROCEDURE

Aseptically weigh 100 mL sample to appropriate sterile sampling bottle.

↓

Open the cap. Dispense 1.0 ml of pure sample in the middle of the CompactDry™ plate. Recap the plate.

↓

If needed, dilute the sample further (e.g. environmental or surface water), refer to page no. 38.

↓

Invert and incubate the plates at specific incubation condition.
Manufacturer's recommendation: 36 ± 2°C for 44 ± 4 hours
ISO 6222: 22 ± 2°C for 68 ± 4 hours
Filter / SMEWW: 35 ± 1°C for 48 ± 2 hours

↓

Read results.

Total Count in Tea

Total microbial count is the index of food stability, and is an important release parameter in food industry. Consequently, general purpose medium used for Total Count cannot be used to determine the microbial population in tea samples due to the presence of antiseptic substance, catechin. This substance will only inhibit the growth of bacteria which will lead to underestimation. Thus, to properly count the microbial population in tea, the medium should have catechin neutralizer to counterbalance the effect of antiseptic substance of tea.

Result Interpretation

Red and otherwise colored colonies are counted

PROCEDURE

Aseptically weigh 10.0 g or pipette 10.0 ml sample to appropriate sterile container (i.e. stomacher bag, dilution bottle, Whirl-Pak bag).

↓

Add 90.0 ml diluent to achieve 1:10 dilution and homogenize. Adjust pH if necessary. Suggested diluents for routine parameters are Butterfield's Phosphate Buffer, Maximum Recovery Diluent, and other appropriate diluents depending on BAM.

↓

If needed, dilute the sample further (refer to page no. 38).

↓

Open the cap. Dispense 1.0 ml of diluted sample in the middle of the CompactDry™ plate. Recap the plate.

↓

Invert and incubate the plates at specific incubation condition.
Manufacturer's recommendation: 35 ± 2°C for 48 ± 3 hours

↓

Read results.

ENVIRONMENTAL SAMPLING

A. Surface Sampling

Swab: Commonly tested parameters
Sponge: Pathogens *(Salmonella, Listeria)*

Obtain the sterile swab or pre-moistened sponge.

↓

Swab or wipe a target surface area of 4" x 4" or 10" x 10" respectively using cross-hatch technique.

↓

Return the swab in the tube or place the sponge in a sterile Whirl-Pak.

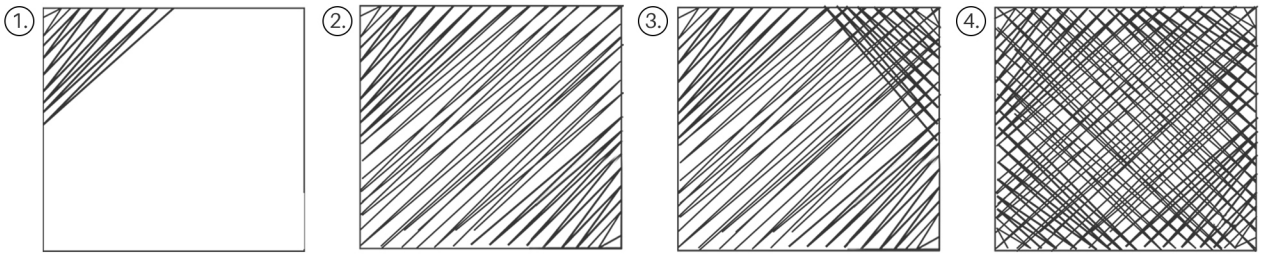
↓

Add recommended diluent to obtain 1:10 dilution.

↓

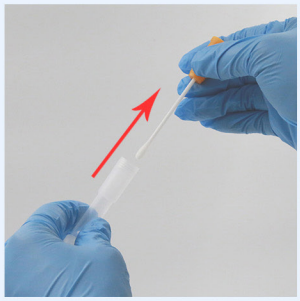
Agitate and proceed to analysis using CompactDry™ of target parameter.

Cross-Hatch Technique

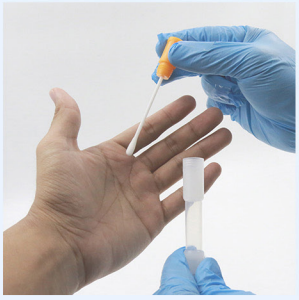


ENVIRONMENTAL SAMPLING

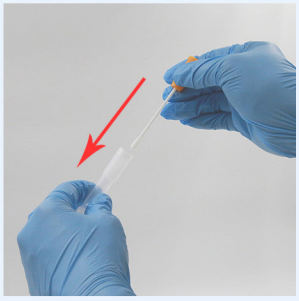
B. Hand Sampling



1. Remove swab from the tube.



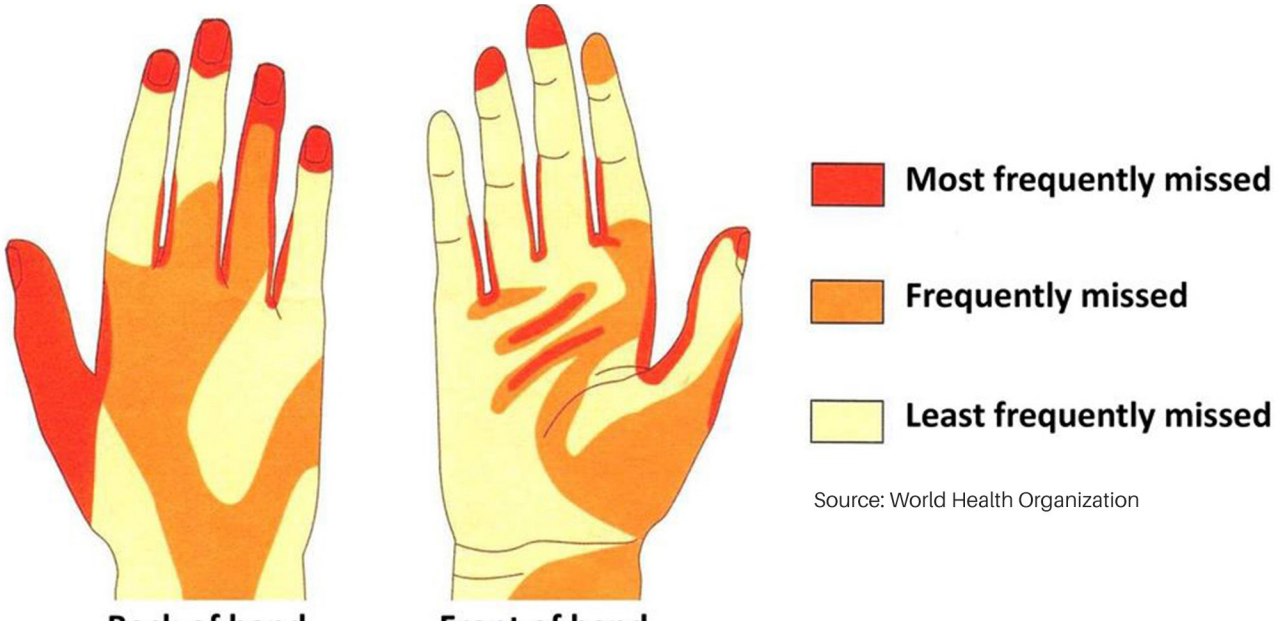
2. Swab the whole surface of the hand especially the **frequently missed areas.**



3. Return swab to tube.

4. Shake to mix. Remove cap of the tip. Proceed to analysis using CompactDry™ of target parameter.

Areas frequently missed during handwashing

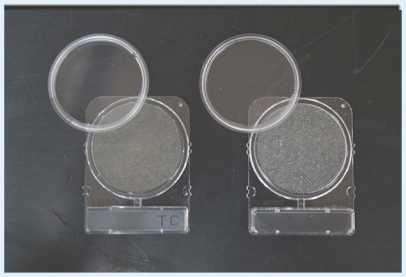


Source: World Health Organization

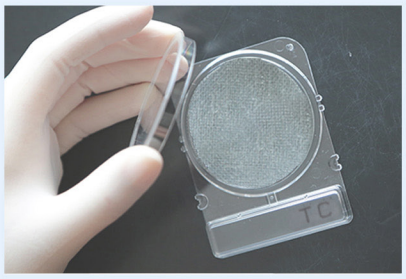
ENVIRONMENTAL SAMPLING

C. Air Sampling

1. Rehydrate CompactDry™ plate with 1mL sterile diluent.



2. Expose plate to air for 15 minutes.



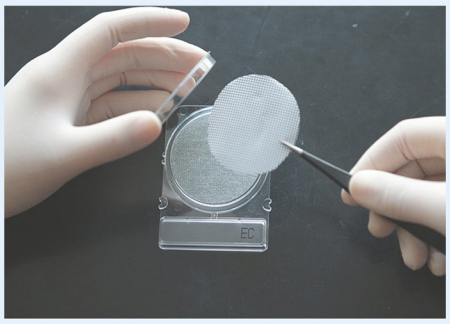
3. Return the cap of the plate. Invert and incubate the plates at specific incubation condition (refer to page no. 37).

MEMBRANE FILTRATION

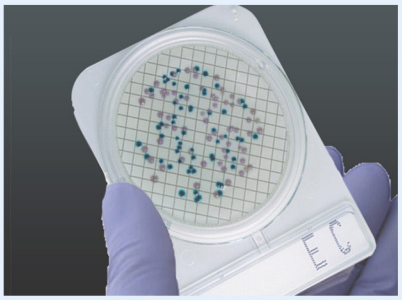
1. Dispense 1mL sterile distilled water onto CompactDry™ plate.

2. Filter sample by filling the sterile chamber with the required amount of liquid sample. Open the vacuum to start filtration.

3. After filtration, wash the inner surface with 20-30 mL sterile distilled water. Repeat 20-30 times.



4. Transfer membrane filter onto CompactDry™ plates, trapped side up. Ensure that there are no bubbles between the plate and the filter by performing rolling motion. Incubate the plates at specific incubation condition (refer to page no. 37).



5. Read results.

Bakery Industry

COMMONLY TESTED PARAMETERS

Total Count (TC), *E. coli* and Coliform (EC), Yeast & Mold (YM), *Staphylococcus aureus* (XSA), *Bacillus cereus* (XBC), *Salmonella* (SL), and *Listeria* (LS)

SAMPLE PREPARATION

For indicator organisms (TC, EC, YM, XSA, and XBC) - Cut out standardized sample size (e.g. 10 g or 25 g) using sterile knife or scissors. Place in a sterile container, then add the appropriate volume of sterile diluent (e.g. 90 mL or 225 mL of Buffered Peptone Water or BPW, Maximum Recovery Diluent or MRD, or 0.1% Peptone Water). Homogenize and dilute accordingly.

For pathogens (SL and LS) - Weigh 25 g and dilute with 225 mL sterile BPW.

REGULATORY LIMITS

Refer to Codex Alimentarius, Food & Drug Administration (FDA), Food Standards Agency (FSA), or other local regulatory bodies.

PROCESSING NOTES

- Use 25 g sample size for samples that absorb a large volume of diluent.
- Use filter bags to minimize inclusion of sample particles during inoculation.

Effect of Sample Color to Result Interpretation

Color of the sample may interfere with the interpretation of the results (Figure 1). Plate the highest dilution possible without affecting the needed sensitivity of the test, e.g. if allowable level for total count is 800 CFU/g, plate 1 mL of the 10^{-2} dilution (Figure 2) but not 10^{-3} since it cannot detect the required level.

Sample: Chocolate Muffin

Dilution: 10^{-1}
Remark: No visible colonies

Figure 1

Dilution: 10^{-2}
Remark: Visible colonies

Figure 2

Similar to other tests that rely on β-Glucuronidase activity to detect *E. coli*, CompactDry™ EC cannot detect *E. coli* O157:H7 as an *E. coli*.

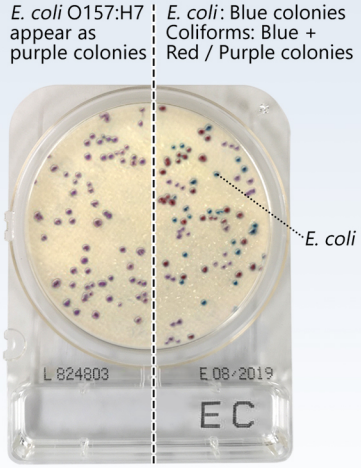


Figure 3

Beverage Industry

COMMONLY TESTED PARAMETERS

Total Count (TC), *E. coli* and Coliform (EC), Yeast & Mold (YM), *Staphylococcus aureus* (XSA), *Salmonella* (SL), and *Listeria* (LS)

SAMPLE PREPARATION

For indicator organisms (TC, EC, YM, and XSA):

- For filterable liquid samples - Filter 100 mL of liquid sample using a 47 mm membrane filter with 0.45 µm pore size.
- For non-filterable liquid and powder samples - Weigh standardized sample size (e.g. 10 g or 25 g). Place in a sterile container, then add the appropriate volume of sterile diluent (e.g. 90 mL or 225 mL of Buffered Peptone Water or BPW, Maximum Recovery Diluent or MRD, or 0.1% Peptone Water). Homogenize and dilute accordingly.

For pathogens (SL and LS): Weigh 25 g and dilute with 225 mL sterile BPW.

REGULATORY LIMITS

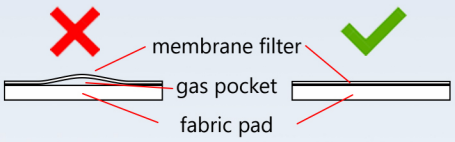
Refer to Codex Alimentarius, Food & Drug Administration (FDA), Food Standards Agency (FSA), or other local regulatory bodies.

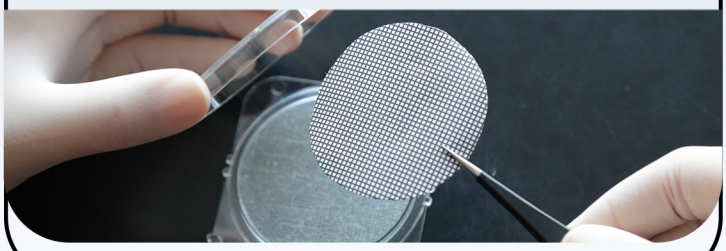
PROCESSING NOTES

Check sample pH after the 10^{-1} dilution. Ensure that sample pH is between 6 and 8, otherwise neutralize using sterile 0.1 N Sodium Hydroxide (NaOH) if lower than 6, or 0.1 N Hydrochloric Acid (HCl) if higher than 8.

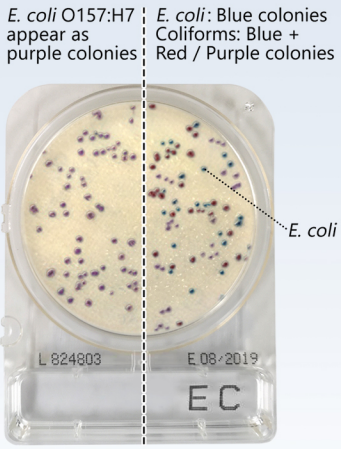
For filtered samples, rehydrate CompactDry™ plates with 1 mL sterile diluent before transferring membrane filter using a rolling motion to avoid gas pockets between the filter and the fabric.

Filtered Samples





Like other tests that rely on β-Glucuronidase activity to detect *E. coli*, CompactDry™ EC cannot detect *E. coli* O157:H7 as an *E. coli*.



Dairy Industry

COMMONLY TESTED PARAMETERS

Total Count (TC), *E. coli* and Coliform (EC), Enterobacteriaceae (ETB), Yeast & Mold (YM), *Staphylococcus aureus* (XSA), *Bacillus cereus* (XBC), *Salmonella* (SL), and *Listeria* (LS)

SAMPLE PREPARATION

For indicator organisms (TC, EC, ETB, YM, XSA, and XBC*):

- **Solid and Liquid Samples -** Cut or weigh out standardized sample size (e.g. 10 g or 25 g) using a sterile knife or scissors. Place in a sterile container, then add the appropriate volume of sterile diluent (e.g. 90 mL or 225 mL of Buffered Peptone Water or BPW, Maximum Recovery Diluent or MRD, or 0.1% Peptone Water). Homogenize and dilute accordingly.
- **Butter -** Based on ISO 6887-5:2010, weigh 10 g or 25 g of sample into sterile flask and warm until melted on a 45°C water bath. Add sterile 90 mL or 225 mL diluent pre-warmed to 45°C and mix thoroughly. The use of stomacher is highly recommended.

**Bacillus cereus* (XBC) is required to be tested for powdered products only.

REGULATORY LIMITS

Refer to Codex Alimentarius, Food & Drug Administration (FDA), Food Standards Agency (FSA), or other local regulatory bodies.

PROCESSING NOTES

For CompactDry™ EC, interference may be observed for fermented dairy products (e.g. yogurt and soft cheese) due to their natural β-Galactosidase content. Dilute accordingly.

Sample: Yogurt

Dilution: 10^{-1}
Remark: looks like TNTC

Figure 1

Dilution: 10^{-2}
Remark: <100 CFU/g

Figure 2

Like other tests that rely on β-Glucuronidase activity to detect *E. coli*, CompactDry™ EC cannot detect *E. coli* O157:H7 as an *E. coli.*

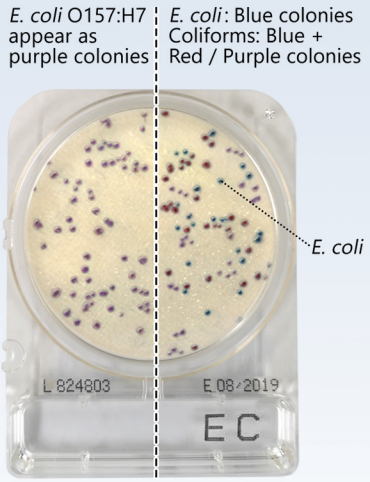


Figure 3

Meat Industry

COMMONLY TESTED PARAMETERS

Total Count (TC), *E. coli* and Coliform (EC), Enterobacteriaceae (ETB), Yeast & Mold (YM), *Staphylococcus aureus* (XSA), *Salmonella* (SL), and *Listeria* (LS)

SAMPLE PREPARATION

- **Destructive or Excision Method -** Cut out standardized sample size (e.g. 10 g or 25 g) from the meat carcass using sterile knife or scissors. Place in a sterile container, then add the appropriate volume of sterile diluent (e.g. 90 mL or 225 mL of Buffered Peptone Water or BPW, Maximum Recovery Diluent or MRD, or 0.1% Peptone Water). Homogenize and dilute accordingly.
- **Carcass Swab -** Using a sterile swab or sponge, swab a standardized sampling area (e.g. 10x10 cm) on the surface of the carcass. Resuspend swab or sponge in sterile diluent. Dilute accordingly.

For pathogens (SL and LS) - Weigh 25 g of meat sample and dilute with 225 mL sterile BPW. Some countries require the absence of *Salmonella* in 10 g sample instead of 25 g for minced meat (non-poultry origin) and mechanically separated meat (MSM).

REGULATORY LIMITS

Refer to Codex Alimentarius, Food & Drug Administration (FDA), Food Standards Agency (FSA), or other local regulatory bodies.

PROCESSING NOTES

Raw meat generally has high microbial load; ground or minced meat even more so. For TC, plate using 10^{-3} (Figure 2) or 10^{-4} (Figure 3) dilution while using 10^{-1} for other indicator organisms. For TC of swab or sponge samples, plate using 10^{-2} or 10^{-3} as these tend to have lower microbial load.

Sample: Raw Meat

Dilution: 10^{-2}
Remarks: TNTC, no visible colonies

Figure 1

Dilution: 10^{-3}
Remark: Countable

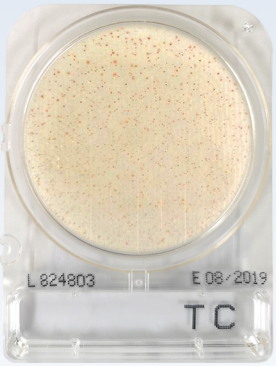


Figure 2

Dilution: 10^{-4}
Remark: Easily countable

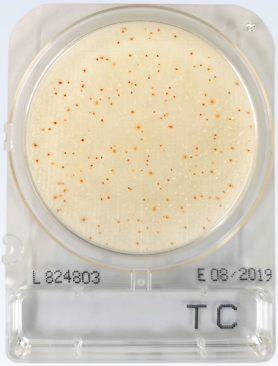


Figure 3

Similar to other tests that rely on β-Glucuronidase activity to detect *E. coli*, CompactDry™ EC cannot detect *E. coli* O157:H7 as an *E. coli*.

E. coli O157:H7 appear as purple colonies

E. coli: Blue colonies
Coliforms: Blue + Red / Purple colonies

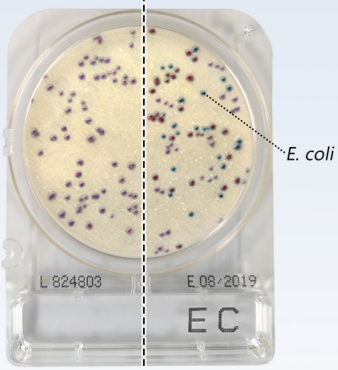


Figure 4

Snacks Industry

COMMONLY TESTED PARAMETERS

Total Count (TC), *E. coli* and Coliform (EC), Enterobacteriaceae (ETB), Yeast & Mold (YM), *Staphylococcus aureus* (XSA), *Bacillus cereus* (XBC), and *Salmonella* (SL)

SAMPLE PREPARATION

For indicator organisms (TC, EC, ETB, YM, XSA, and XBC) - Cut out standardized sample size (e.g. 10 g or 25 g) using sterile knife or scissors. Place in a sterile container, then add the appropriate volume of sterile diluent (e.g. 90 mL of Buffered Peptone Water or BPW, Maximum Recovery Diluent or MRD, or 0.1% Peptone Water). Homogenize and dilute accordingly.

For pathogen (SL) - Weigh 25 g and dilute with 225 mL sterile BPW.

REGULATORY LIMITS

Refer to Codex Alimentarius, Food & Drug Administration (FDA), Food Standards Agency (FSA), or other local regulatory bodies.

PROCESSING NOTES

- Use 25 g sample size for samples that absorb a large volume of diluent (e.g. wafer and biscuits).
- Use filter bags to minimize inclusion of sample particles during inoculation.
- Check sample pH after the 10^{-1} dilution especially for acidic samples. Ensure that sample pH is between 6 and 8, otherwise neutralize using sterile 0.1 N Sodium Hydroxide (NaOH) if lower than 6, or 0.1 N Hydrochloric Acid (HCl) if higher than 8.

Effect of Sample Color to Result Interpretation

Color of the sample may interfere with the interpretation of the results. Plate the highest dilution possible without affecting the needed sensitivity of the test. For example, if allowable level for aerobic count is 800 CFU/g, plate 1 mL of the 10^{-2} dilution but not 10^{-3} since it cannot detect the required level.

Sample: Chocolate

Dilution: 10^{-1}
Remark: No visible colonies

Figure 1

Dilution: 10^{-2}
Remark: Visible colonies

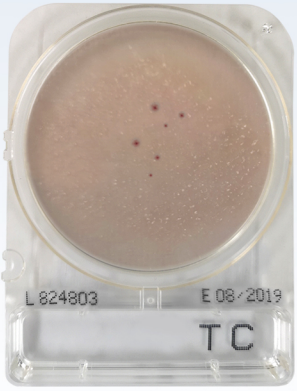


Figure 2

Similar to other tests that rely on β-Glucuronidase activity to detect *E. coli*, CompactDry™ EC cannot detect *E. coli* O157:H7 as an *E. coli*.

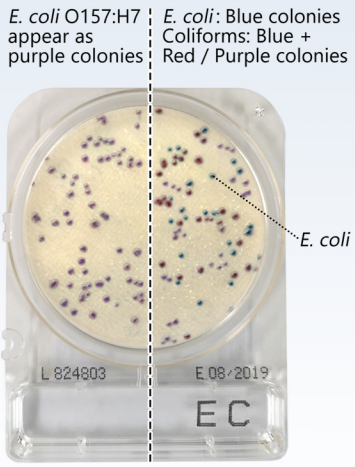


Figure 3

Fresh Produce Industry

COMMONLY TESTED PARAMETERS

Total Count (TC), *E. coli* and Coliform (EC), Enterobacteriaceae (ETB), Yeast & Mold (YM), *Staphylococcus aureus* (XSA), *Salmonella* (SL), and *Listeria* (LS)

SAMPLE PREPARATION

For indicator organisms (TC, EC, ETB, YM, and XSA) - Cut out standardized sample size (e.g. 10 g or 25 g) from the fruit or vegetable using sterile knife or scissors, then place in a sterile container. Add the appropriate volume of sterile diluent (e.g. 90 mL or 225 mL of Buffered Peptone Water or BPW, Maximum Recovery Diluent or MRD, or 0.1% Peptone Water), then homogenize and dilute accordingly.

For pathogens (SL and LS) - For cut up samples, weigh 25 g and dilute with 225 mL sterile BPW. For whole fruits or vegetables, test samples as is, including any visible dirt. Add sufficient sterile BPW to float the sample.

REGULATORY LIMITS

Refer to Codex Alimentarius, Food & Drug Administration (FDA), Food Standards Agency (FSA), or other local regulatory bodies.

PROCESSING NOTES

Check sample pH after the 10^{-1} dilution especially for acidic fruits. Ensure that sample pH is between 6 and 8, otherwise neutralize using sterile 0.1 N Sodium Hydroxide (NaOH) if lower than 6, or 0.1 N Hydrochloric Acid (HCl) if higher than 8.

For CompactDry™ EC, interference may be observed as most fruits (e.g. apples and tomatoes) naturally have β-Galactosidase. Dilute accordingly.

Sample: Apple

Dilution: 10^{-1}
Remark: looks like TNTC

Figure 1

Dilution: 10^{-2}
Remark: <100 CFU/g

Figure 2

Like other tests that rely on β-Glucuronidase activity to detect *E. coli*, CompactDry™ EC cannot detect *E. coli* O157:H7 as an *E. coli*.

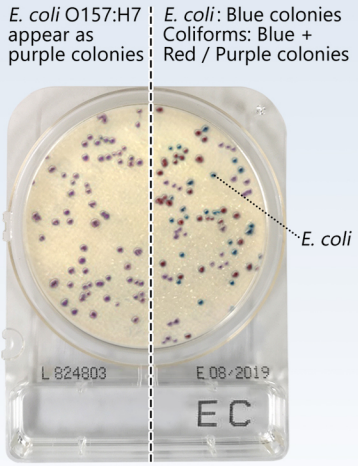


Figure 3

Seafood Industry

COMMONLY TESTED PARAMETERS

Total Count (TC), *E. coli* and Coliform (EC), Enterobacteriaceae (ETB), Yeast & Mold (YM), *Staphylococcus aureus* (XSA), *Salmonella* (SL), *Listeria* (LS), and *Vibrio parahaemolyticus* (VP)

SAMPLE PREPARATION

For indicator organisms (TC, EC, ETB, YM, and XSA) - Cut out or weigh standardized sample size (e.g. 10 g or 25 g) using sterile knife or scissors. Place in a sterile container, then add the appropriate volume of sterile diluent (e.g. 90 mL or 225 mL of Buffered Peptone Water or BPW, Maximum Recovery Diluent or MRD, or 0.1% Peptone Water). Homogenize and dilute accordingly.

For pathogens (SL and LS) - Weigh 25 g of sample and dilute with 225 mL sterile BPW, then homogenize.

For pathogen (VP) - Weigh 50 g of sample and add 450 mL of sterile Butterfield's Phosphate Buffer (BPB), then homogenize. Use the surface tissues, gills, and gut for fish while for shellfish, use the meat and juice.

REGULATORY LIMITS

Refer to Codex Alimentarius, Food & Drug Administration (FDA), Food Standards

PROCESSING NOTES

Raw seafood generally has high microbial load. For TC, plate using 10^{-3} (Figure 2) or 10^{-4} (Figure 3) dilution while using10^{-1} for other indicator organisms. Cooked seafood tends to have lower microbial load. For TC, plate using 10^{-1} or 10^{-2}.

Sample: Raw Seafood

Dilution: 10^{-2}
Remarks: TNTC; no visible colonies

Figure 1

Dilution: 10^{-3}
Remark: Countable

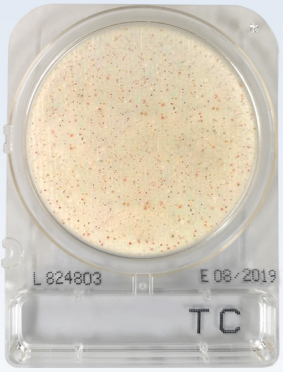


Figure 2

Dilution: 10^{-4}
Remark: Easily countable

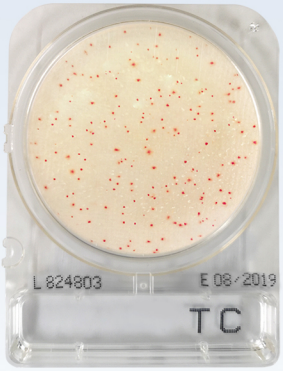


Figure 3

Similar to other tests that rely on β-Glucuronidase activity to detect *E. coli*, CompactDry™ EC cannot detect *E. coli* O157:H7 as an *E. coli*.

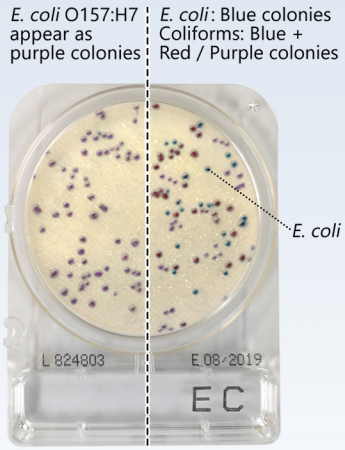


Figure 4

Water Industry

COMMONLY TESTED PARAMETERS

Heterotrophic Bacteria in Water (AQ), *E. coli* and Coliform (EC), *Enterococcus* (ETC), *Pseudomonas aeruginosa* (PA), and *Salmonella* (SL)

SAMPLE PREPARATION

For indicator organisms (AQ, EC, ETC, and PA):

- **For samples with very low microbial load (e.g. potable water) -** Filter 100 mL of liquid sample using a 47 mm membrane filter with 0.45 µm pore size.
- **For samples with low to high microbial load (e.g. surface water and waste water)** Directly inoculate 1 mL of sample or if suspected to be high, dilute to 10^{-1} using sterile diluent.

For pathogen (SL):

- **Direct -** Measure 100 mL water sample and dilute with 100 mL sterile double strength Buffered Peptone Water (BPW).
- **Filtration -** Filter 100 mL water sample and transfer filter to 100 mL sterile BPW.

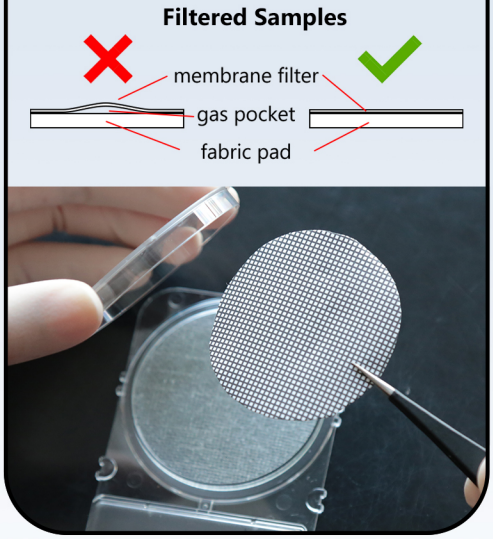
REGULATORY LIMITS

Refer to World Health Organization (WHO), Environmental Protection Agency (EPA), or other local regulatory bodies.

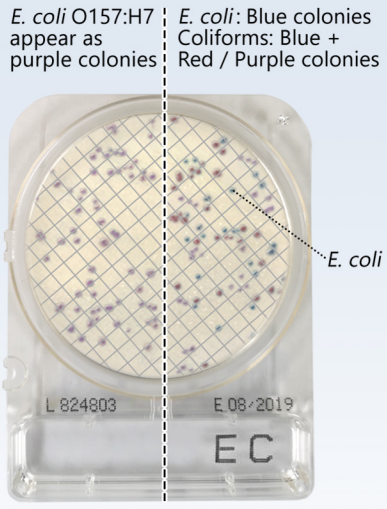
PROCESSING NOTES

For chlorinated samples, make sure that the sterile container has enough sterile sodium thiosulfate ($Na_2S_2O_3$) to neutralize chlorine in the water. For 100 mL samples, add 100 µL of 3% $Na_2S_2O_3$ for potable water and 100 µL of 10% $Na_2S_2O_3$ for chlorinated waste or effluent water. Commercially prepared sampling containers usually have $Na_2S_2O_3$ tablets.

For filtered samples, add 1 mL sterile diluent to rehydrate CompactDry™ plates. Transfer membrane filter using a rolling motion to avoid gas pockets between the filter and the fabric.



Similar to other tests that rely on β-Glucuronidase activity to detect *E. coli*, CompactDry™ EC cannot detect *E. coli* O157:H7 as an *E. coli*.



Sugar Industry

COMMONLY TESTED PARAMETERS

Total Count (TC), *E. coli* and Coliform (EC), Yeast & Mold (YM), *Staphylococcus aureus* (XSA), and *Salmonella (SL)*

SAMPLE PREPARATION

For indicator organisms (TC, EC, YM, and XSA):

- **For samples with low microbial load -** Weigh standardized sample size (e.g. 10 g or 25 g). Place in a sterile container, then add the appropriate volume of sterile diluent (e.g. 90 mL or 225 mL of Maximum Recovery Diluent or MRD or 0.1% Peptone Water). Agitate to dissolve. Filter 100 mL of liquid sample using a 47 mm membrane filter with 0.45 µm pore size.
- **For samples with high microbial load or darkly colored -** Weigh standardized sample size (e.g. 10 g or 25 g). Place in a sterile container, then add the appropriate volume of sterile diluent (e.g. 90 mL or 225 mL of MRD or 0.1% Peptone Water). Homogenize and dilute accordingly.

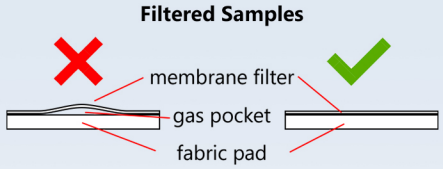
For pathogen (SL) - Weigh 25 g and dilute with 225 mL sterile Buffered Peptone Water (BPW).

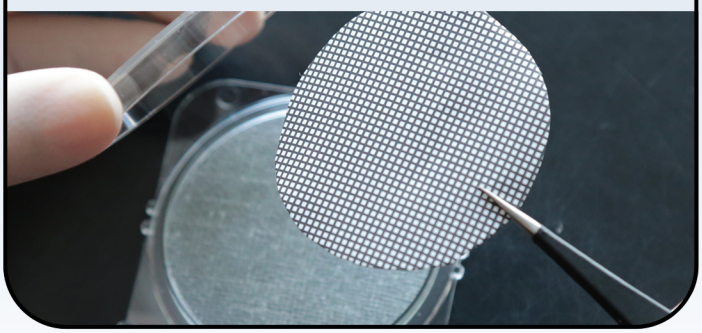
REGULATORY LIMITS

Refer to the International Commission for Uniform Methods of Sugar Analysis (ICUMSA), Codex Alimentarius, Food & Drug Administration (FDA), Food Standards Agency (FSA), or other local regulatory bodies.

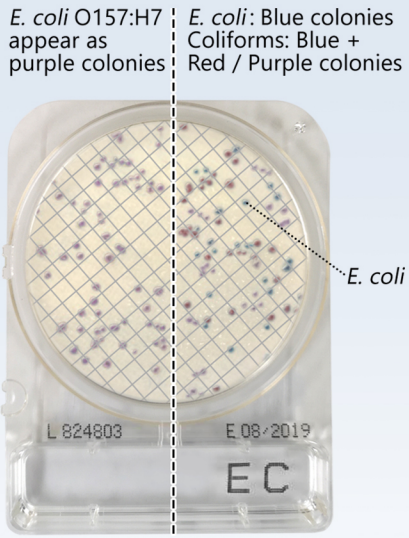
PROCESSING NOTES

For filtered samples, add 1 mL sterile diluent to rehydrate CompactDry™ plates. Transfer membrane filter using a rolling motion to avoid gas pockets between the filter and the fabric.





Like other tests that rely on β-Glucuronidase activity to detect *E. coli*, CompactDry™ EC cannot detect *E. coli* O157:H7 as an *E. coli*.



Feed Industry

COMMONLY TESTED PARAMETERS

Total Count (TC), *E. coli* and Coliform (EC), Enterobacteriaceae (ETB), Yeast & Mold (YM), *Staphylococcus aureus* (XSA), *Bacillus cereus* (XBC), *Salmonella* (SL), and *Listeria* (LS)

SAMPLE PREPARATION

For indicator organisms (TC, EC, ETB, YM, XSA, and XBC) - Weigh standardized sample size (e.g. 10 g or 25 g), place in a sterile container, and add the appropriate volume of sterile diluent (e.g. 90 mL or 225 mL of Buffered Peptone Water or BPW, Maximum Recovery Diluent or MRD, or 0.1% Peptone Water). Homogenize and dilute accordingly.

For pathogens (SL and LS) - Weigh 25 g of sample and dilute with 225 mL sterile BPW, then homogenize.

REGULATORY LIMITS

Refer to Codex Alimentarius and local regulatory bodies.

PROCESSING NOTES

- Use 25 g sample size for samples that absorb a large volume of diluent.
- Use filter bags to minimize inclusion of sample particles during inoculation.

Animal feeds generally has high microbial load. For TC, plate using 10^{-3} (Figure 2) or 10^{-4} (Figure 3) dilution while using 10^{-1} or 10^{-2} for other indicator organisms.

Sample: Animal Feeds

Dilution: 10^{-2}
Remark: TNTC

Figure 1

Dilution: 10^{-3}
Remark: Countable

Figure 2

Dilution: 10^{-4}
Remark: Easily countable

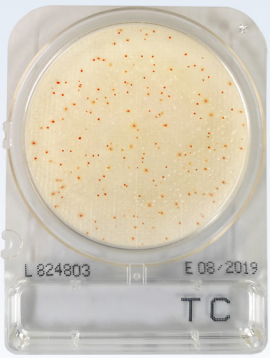


Figure 3

Similar to other tests that rely on β-Glucuronidase activity to detect *E. coli*, CompactDry™ EC cannot detect *E. coli* O157:H7 as an *E. coli*.

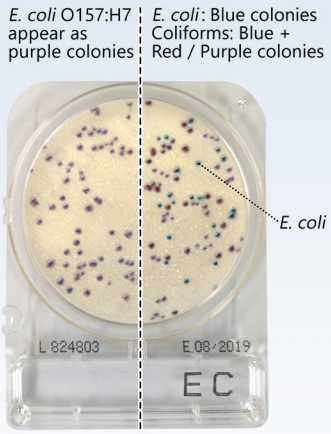


Figure 4

Nutraceutical Industry

COMMONLY TESTED PARAMETERS

Total Count (TC), *E. coli* and Coliform (EC), Enterobacteriaceae (ETB), Yeast & Mold (YM), *Staphylococcus aureus* (XSA), *Pseudomonas aeruginosa* (PA), *Salmonella* (SL), and *Listeria* (LS)

SAMPLE PREPARATION

For indicator organisms (TC, EC, ETB, YM, XSA, and PA) - Weigh 10 g and dilute with buffered diluent supplemented with Tween (Polysorbate) 80 or 20 (e.g. Butterfield's Phosphate Buffer or BPB, Fluid Soybean Casein Digest Medium, or Tryptic Soy Broth or TSB). Dilute as needed.

For pathogens (SL and LS) - Weigh 25 g and dilute with 225 mL sterile TSB.

REGULATORY LIMITS

Refer to the United States Pharmacopeia (USP), Food & Drug Administration (FDA), or other local regulatory bodies.

PROCESSING NOTES

The addition of Tween or Polysorbate (4% v/v) not only aids in dispersion of non-water soluble samples, but also acts as a neutralizer to the antimicrobial agents found naturally in most dietary supplements of plant origin.

Effect of Sample Color to Result Interpretation

Color of the sample may interfere with the interpretation of the results. Plate the highest dilution possible without affecting the needed sensitivity of the test. For example, if allowable level for total count is 200 CFU/g, plate 1mL of the 10^{-2} dilution but not 10^{-3} since it cannot detect the required level.

Sample: Herbal Capsule

Dilution: 10^{-1}
Remark: No visible colonies

Figure 1

Dilution: 10^{-2}
Remark: Visible colony

Figure 2

Similar to other tests that rely on β-Glucuronidase activity to detect *E. coli*, CompactDry™ EC cannot detect *E. coli* O157:H7 as an *E. coli*.

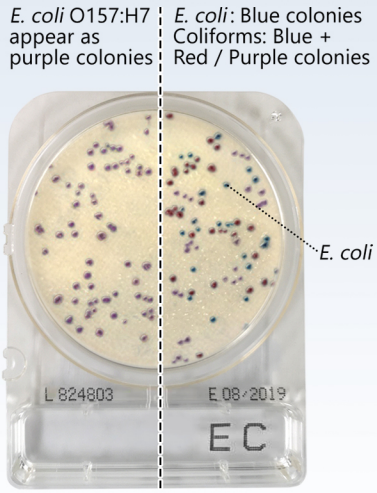


Figure 3

Cosmetic Industry

COMMONLY TESTED PARAMETERS

Total Count (TC), Enterobacteriaceae (ETB), Yeast & Mold (YM), and *Staphylococcus aureus* (XSA)

SAMPLE PREPARATION

For indicator organisms (TC, ETB, YM, and XSA):

- Water Soluble Samples - Weigh 10 g of sample and dilute with 90 mL Modified Letheen Broth (MLB).
- Oil-based Samples and Powders - Weigh 10 g of sample and dilute with 80 mL sterile MLB supplemented with 10 mL Tween (Polysorbate) 80 or 20, then homogenize.

REGULATORY LIMITS

Refer to the United States Pharmacopeia (USP) or other local regulatory bodies.

PROCESSING NOTES

The addition of Tween (Polysorbate) not only aids in dispersion of non-water soluble samples, but also acts as a neutralizer to the antimicrobial agents found in cosmetics.

Effect of Neutralizer to Result Interpretation

MLB is necessary to neutralize antimicrobial compounds in the formulation. Using other diluent without neutralizer, e.g. Butterfield's Phosphate Buffer (BPB) in Figure 2, will result to poor recovery of the target organism.

Sample: Facial Toner

Diluent: MLB
Dilution: 10^{-1}
Colonies: 40
Remark: Better recovery

Figure 1

Diluent: BPB
Dilution: 10^{-1}
Colonies: 20
Remark: Poor recovery

Figure 2

Effect of Sample Color to Result Interpretation

Color of the sample may interfere with the interpretation of the results (Figure 3). Plate the highest dilution possible without affecting the needed sensitivity of the test, e.g. if allowable level for total count is 500 CFU/g, plate 1mL of the 10^{-2} dilution (Figure 4) but not 10^{-3} since it cannot detect the required level.

Sample: Lipstick

Dilution: 10^{-1}
Remark: No visible colonies

Figure 3

Dilution: 10^{-2}
Remark: Visible colonies

Figure 4

INCUBATION TIME AND TEMPERATURE

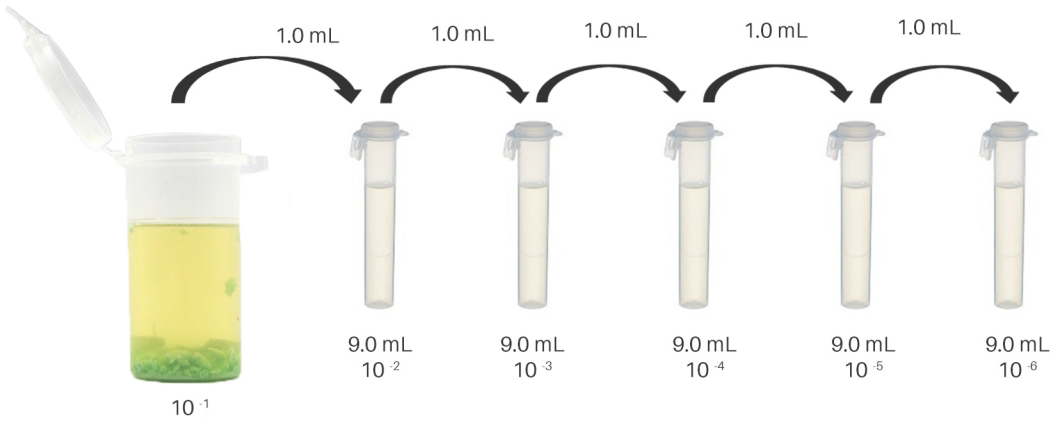
CompactDry™
There is always a better way.

TEST PARAMETER	TIME AND TEMPERATURE	APPROVAL
Total Count	35 ± 2°C for 48 ± 3 hours[B] 30 ± 1°C for 48 ± 3 hours[C, D]	AOAC #010404 NordVal #033 MicroVal #20007LR01
Coliform	37 ± 1°C for 24 ± 2 hours[B, C, D]	AOAC #110402 NordVal #035 MicroVal #MV0806-003 LR
E. coli / Coliform	35 ± 2°C for 24 ± 2 hours[B] 37 ± 1°C for 24 ± 2 hours[C, D]	AOAC #110402 NordVal #036 MicroVal #MV0806-005 LR(*E. coli*) #MV0806-004 LR (Coliform)
Yeast and Mold	25 ± 2°C for 3 - 7 days[B, C, D]	AOAC #100401 NordVal #043 MicroVal #RQA208LR10
Yeast and Mold Rapid	25 ± 2°C for 48 - 72 hours[B] 25 ± 1°C for 3 days[C, D]	NordVal #050 MicroVal #2016LR61
Staphylococcus aureus	35 ± 2°C for 24 ± 2 hours[B] 37 ± 1°C for 24 ± 2 hours[C, D]	AOAC #081001 NordVal #042 MicroVal #2008LR14
Salmonella	pre-enrichment: 36 ± 1°C for 22 ± 2 hours[A] plate: 42 ± 1°C for 22 ± 2 hours[A]	On-going
Enterobacteriaceae	35 ± 2°C for 24 ± 2 hours[B] 37°C for 24 hours[C] 37 ± 1°C for 24 ± 2 hours[D]	MicroVal #MV0806-002 LR NordVal #034
Enterococcus	37 ± 1°C for 20 - 24 hours[C] 36 ± 1°C for 24 ± 2 hours[D]	MicroVal #2014LR48 NordVal# 047
Bacillus cereus	35 ± 2°C for 24 ± 2 hours[A] 30°C for 48 hours[C] 30°C for 48 ± 2 hours[D]	MicroVal #2011LR41
Listeria spp.	pre-enrichment: 20°C for 1 hour[A] plate: 35 ± 1°C for 24 ± 2 hours[A]	On-going
Vibrio parahaemolyticus	35 - 37°C for 22 ± 2 hours[A]	On-going
Pseudomonas aeruginosa	35 ± 1°C for 48 ± 3 hours[A]	On-going
Heterotrophic bacteria in water	36 ± 2°C for 44 ± 4 hours[A] 22 ± 2°C for 68 ± 4 hours [E] 35 ± 1°C for 48 ± 2 hours[F]	On-going
Total Count in Tea (Tea products)	35 ± 2°C for 48 ± 3 hours[A]	On-going

[A] Manufacturer's Recommendation
[B] AOAC International
[C] MicroVal
[D] NordVal
[E] ISO 6222
[F] Filter / SMEWW Method

DILUTION SCHEME

Ready-to-use diluent



NOTE:
- Use the same diluent for the initial dilution
- For most parameters: Obtain 1.0 ml inoculum from the desired dilution

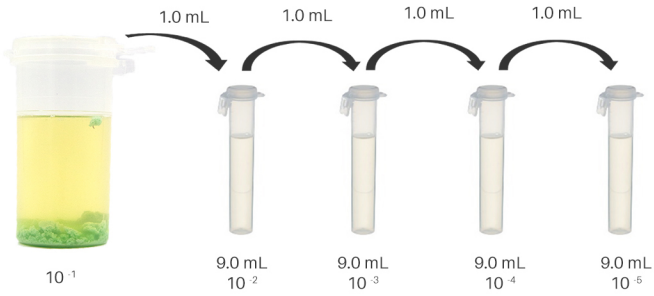
General Formula:

$$Dilution = \frac{Volume\ transferred\ \ (ml)}{Total\ volume\ (ml)} \text{ x } Previous\ dilution$$

Where:

Total volume = sum of the volume transferred and volume of the diluent used for serial dilution
Previous dilution = reference dilution of the computation

Example: Determine the dilution of the "tube x" given that the volume transferred is 1.0 ml, and this sample is from tube with 10^{-3} dilution (volume of the diluent = 9.0 ml).



$$Dilution = \frac{1.0\ ml}{10.0\ ml} \text{ x } 10^{-3}$$

$$Dilution = 0.01 \text{ x } 0.001$$

$$Dilution = 0.0001\ or\ 10^{-4}$$

General Formula:

$$\frac{cfu}{g} = \frac{A}{V} \times Df$$

Where:

A = Average count of colonies within the same dilution and sample
V = Volume plated (in terms of CompactDry™, 1.0 ml)
Df = Dilution factor; reciprocal of dilution (example: 10^{-3} is $1/10^{-3} = 1000$)

Reminder: In conventional method, acceptable count for bacteria and YM should be within 30-300 and 15-150, respectively. This limit is based on the statistical analysis where the size of the plate is considered versus the minimum space of different colonies that can be grown on the plate without overlapping. Thus, avoiding underestimation.

1. **One sample using single dilution (single plating)**

Dilution	10^{-1}
Count	47

Count:

$$cfu/_g = \frac{47}{1.0\ ml} \times 10$$

$$cfu/_g = 470\ or\ 4.7 \times 10^2$$

2. **One sample using single dilution (duplicate plating)**

Dilution	10^{-2}	
Count	47	52

Count:

$$cfu/_g = \frac{\frac{(47+52)}{2}}{1.0\ ml} \times 100$$

$$cfu/_g = 4950\ or\ 4.95 \times 10^3$$

3. One sample using serial dilutions (duplicate plating)

Dilution	10^{-1}		10^{-2}		10^{-3}	
Count	500	470	52	47	4	3

Count:

$$cfu/_g = \frac{\frac{(47+52)}{2}}{1.0\ ml} \text{ x } 100$$

$$cfu/_g = \ 4950\ or\ 4.95 \text{ x } 10^3$$

Formula two succeeding dilutions:

$$cfu/_g = \frac{\Sigma C}{[(1 \text{ x } n1) + (0.1 \text{ x } n2)] \text{ x } D}$$

Where:

ΣC = Summation of colonies (within the range)
n_1 = Number of plates with counts within the range on first dilution
n_2 = Number of plates with counts within the range on second dilution
D = Lower dilution with counts within the range

4. One sample with two succeeding dilution (duplicate plating)

Dilution	10^{-1}		10^{-2}		10^{-3}	
Count	325	275	34	35	4	3

Count:

$$cfu/_g = \frac{275 + 34 + 35}{[(1 \text{ x } 1) + (0.1\ x\ 2)] \text{ x } 0.1}$$

$$cfu/_g = \frac{344}{0.12}$$

$$cfu/_g = \ 2,866.67 \text{ or } 2.87 \text{ x } 10^3$$

Formula for Compact Dry Counting using Gridlines:

$$cfu/_g = \frac{A_G \text{ x } m}{V} \text{ x } Df$$

Where:

A_G = Average count of colonies (in at least 3 grids) within the same dilution and sample
V = Volume plated (in terms of Compact Dry, 1.0 ml)
m = multiplier (for big grids = 20; for small grids = 80)
Df = Dilution factor; reciprocal of dilution (example: 10^{-3} is $1/10^{-3} = 1000$)

Reminder: The client can only use the grids if there is an even distribution of the growth on the plate. Also, it is the client decision if they will count the colonies using either small grids or big grids.

5. Counting using Big Grids

Dilution	10^{-2}		
Count	4	5	7

Count:

$$cfu/_g = \frac{\left(\frac{4+5+7}{3}\right) \text{x } 20}{1.0\ ml} \text{ x } 100$$

$$cfu/_g = \frac{106.6}{1.0\ ml} \quad 100$$

$$cfu/_g = \ 10{,}660 \ or \ 1.07\text{x}10^4$$

6. Counting using Small Grids

Dilution	10^{-2}		
Count	1	2	1

Count:

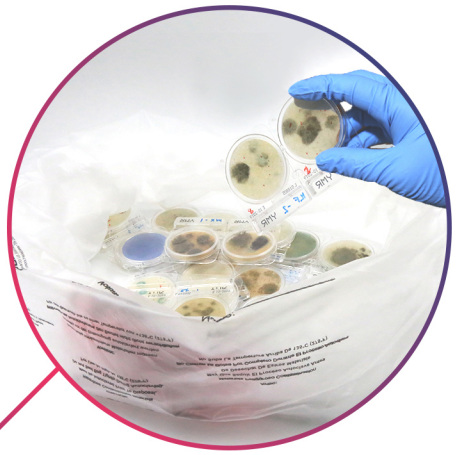
$$cfu/_g = \frac{\left(\frac{1+2+1}{3}\right) \text{x } 80}{1.0\ ml} \text{ x } 100$$

$$cfu/_g = \frac{106.7}{1.0\ ml} \text{ x } 100$$

$$cfu/_g = \ 10{,}666 \ or \ 1.07\text{x}10^4$$

Any reagent and material used with the CompactDry™ must be decontaminated by autoclaving after use

Place the used CompactDry™ plates in a disposable autoclave bag.
Note: To avoid re-opening of the plastic after decontamination, separate CompactDry™ plates from other materials which are disposable.



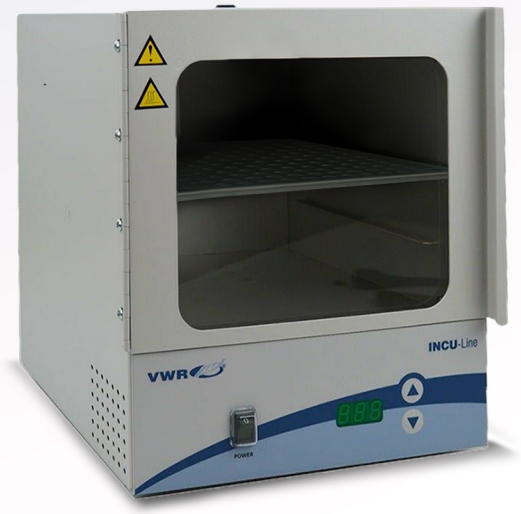
Place the bag in the autoclave or industrial pressure canner and decontaminate. (Decontamination condition: 121°C at 15 psi for 20 min or more depending on the load)

After decontamination, dispose the plates as industrial waste in accordance to official state regulations.

As mentioned in different parts of the CompactDry™ End-user manual, establishing a laboratory using rapid test kit would only need a minimum number of equipment and consumable. The recommended pieces of equipment that can be incorporated in the laboratory are as follows:

INCUBATOR

The suggested number of incubators needed by a facility is equal to the number of different incubation temperatures. If the laboratory is aiming to test their sample for commonly tested parameters such as total count, *E. coli*, and yeast and mold, they would need two (2) incubators. However, if they are planning to have an additional test for *Salmonella*, they need to have an additional one (1) incubator. It is highly recommended not to frequently change the temperature of an incubator as this may affect the sensor of the unit resulting to a deviation between the internal temperature and the set temperature. Thus, the need to be callibrated again



Incubator temperature:
Commonly tested parameters: 35 ± 2°C
Yeast and Mold : 25 ± 2°C
Salmonella : 42 ± 1°C

AUTOCLAVE OR PRESSURE CANNER

As mentioned in the "Waste Disposal" protocol, all materials used in the microbial analysis should be subjected to decontamination before disposal. The validated procedure to kill the microorganisms is to heat the materials to 121°C at 15 psi for 20 minutes or more. For starters, industrial pressure canner is enough to decontaminate the products. This can also be used in sterilization of different materials intended as part of analysis.

PIPETTOR

Transferring of exact volume of inoculum for serial dilution and actual assay is an important factor to obtain a reliable result at the end of the test. Both inoculated and transferred volume (dilution factor) are considered in the computation of the result of microbial population. Thus, a well-calibrated tool like pipettor should be used. In microbial analysis using CompactDry™, two volumes are needed, thus, two types of pipettor should be available in the laboratory – for 0.1 ml (*Salmonella* analysis) and 1.0 ml (other parameters). There are also pipettors with 5.0 and 10.0 ml volumes which are useful in aseptic transferring of sterile medium in smaller containers.

Note: Pipettors intended for microbiology laboratory should only be used for microbial analysis to avoid contamination.

PIPETTE TIPS WITH RACK

For a pipettor to be useful, it should be in-tandem with sterile pipette tips (placed in a rack). Tips are made from plastic, and is considered autoclavable but it is not recommended to be re-used. In Microbiology, the tips should be used only once. Reheating of the tips at too high temperature and pressure (similar to sterilization or decontamination) can cause deformity of the tips making the withdrawn liquid sample less accurate compared when using the unused tips.

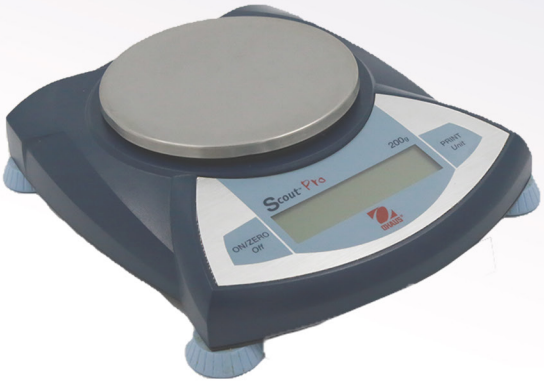
UV STERILIZER

Total absence of contaminating microorganism or asepsis in testing area should be observed. With this, rooms can be cleaned using disinfectants and other solutions that can eliminate such contaminations. Aside from using different cleaning solutions, laboratory can be subjected to complete sterilization using UV sterilizer with germicidal activity specifically with wavelength ranging from 260-270 nm. The efficacy of sterilization is dependent on the height or placement of the UV sterilizer from the target surface (8 feet). Rooms should be subjected for UV sterilization for 30 minutes. After the sterilization period, let the UV particulates dissolve for 10-15 minutes. No personnel should enter the premise within the dissolution period to avoid health risk.

Note: Particulates of UV light and UV sterilizer itself has mutagenic property.

MICROBIOLOGICAL SAFETY CABINET

Microbiological Safety Cabinet, or more popularly called as Biosafety Cabinet (BSC), is an equipment used to contain microorganisms especially pathogens which require higher biosafety level during microbial assay. There are different types of BSC depending on the biocontainment requirement. In connection with its biocontainment function, each BSC should be equipped with HEPA filter to sterilize the air coming from the area of assay. Laminar flow hood is sometimes used in other companies but this should not be recommended due to lack of filtration.



WEIGHING SCALE

Weighing scale is an important equipment inside a laboratory. It has two major functions – 1.) to weigh dehydrated culture media for media preparation, and 2.) to weigh samples for microbial analysis. There are several kinds of weighing scale that can be used in a laboratory, and these are based on the quantity that a scale can weigh. The most common weighing scales are top loading balance for heavy samples (10-500 grams) and analytical balance for lighter samples and medium supplements (0.001 to 5.000 grams).

CHILLER

Some media need to be stored at refrigerated temperature, and some can be stored at room temperature. Proper storage should be observed when storing these media to maintain its effectiveness. Chiller is also used to store reference strains as positive control for different tests as well as validation protocols. In large scale laboratories, it is recommended to acquire an industrial type refrigerator or a deep freezer which can be set at -86°C for storing of enzymes and other supplements.

1. **What happens if more than 300 colonies were observed in a CompactDry™ plate? What should be done?**

   A. *With observed colonies*
   If there are visible colonies, it is not advisable to count a plate with more than 300 colonies, as this may result to underestimation due to possible overlapping colony growth.

   B. *With no observed colonies*
   If a sample contains too high bacterial population (≥ 300), then there may be no isolated colonies observed, thus reported as "Too Numerous to Count (TNTC)". TNTC growth in CompactDry™ plates shows changed color of the fabric pad similar to the positive result of the respective test result (e.g red for CompactDry™ TC, blue for CompactDry™ YM and YMR).

   If either of these happens, the sample can be further diluted until they get a specific dilution where there is an observable count (within 1-300 colonies).

2. **What if 1.0 mL of sample does not diffuse completely and evenly onto the plate due to viscosity?**

   A. *High microbial load*
   Highly viscous samples should be appropriately diluted. This will result to ≥ 10^{-2} dilution which is less viscous and will diffuse easily onto the fabric pad of CompactDry™ plate.

   B. *Low microbial load*
   Obtain 1.0 mL inoculum in 10^{-1} dilution, and inoculate small volume unto at least 5 different points in the CompactDry™ plate.

3. **How to deal with highly pigmented samples without compromising the standard dilution?**

   A. *High microbial load*
   Similar to highly viscous samples, highly pigmented samples can also be diluted until the strong pigmentation fades.

   B. *Low microbial load*
   For highly pigmented samples with low microbial load, the dilution can be increased (e.g. 10^{-2}) but inoculum volume must be adjusted as well. This can be done by filtering 10 mL of the 10^{-2} dilution using a membrane filter to retain the needed sensitivity.

4. **Can CompactDry™ plate be stored at refrigerated temperature?**

   Yes, it can be stored at refrigerated temperature. However, it should be tempered to room temperature before use.

5. **What is the limit of detection of CompactDry™ plate?**

   The advised limit is 1-300 colonies, otherwise, adjust dilution.

6. **Will the pH of the sample affect interpretation?**

   No, the result interpretation is strictly chromogenic and relies on enzymatic activity of target organism. However, pH can affect the recovery of pH sensitive microorganisms. To avoid this, adjust the pH of the sample by adding 1N NaOH or 1N HCl (based on BAM protocol) if the sample is too acidic or too basic, respectively.

7. **The result from CompactDry™ *Salmonella* is considered as presumptive positive, what should be done to confirm the presence of *Salmonella*?**

   To confirm the presumptive positive *Salmonella*, pick an isolated colony from CompactDry™ *Salmonella* plate. Perform stab and streak method to TSIA (Triple Sugar Iron Agar) slant, and without flaming, inoculate LIA (Lysine Iron Agar) slant by stabbing the butt twice and then streaking the slant. Incubate TSIA and LIA slants at 35°C for 24 hours. *Salmonella* in culture typically produces alkaline (red) slant and acid (yellow) butt, with or without Hydrogen Sulfide (H_2S) production (blackening of agar) in TSIA. In LIA, *Salmonella* produces alkaline (purple) reaction in butt of tube. Most *Salmonella* cultures produce H_2S in LIA. Furthermore, positive results from TSIA or LIA shall be subjected to latex agglutination test.

   An isolated presumptive colony of *Salmonella* will be picked from the positive TSIA/LIA slant, and will be transferred to a latex agglutination kit reaction card with 1 drop of saline where the saline will emulsify the picked colony. At the first two minutes of reaction, it will be observed for auto-agglutination. After the first reaction, 1 drop of test latex will be added, and will be observed for further agglutination. The presence of agglutination confirms the presence of *Salmonella*. The agglutination reaction is due to the latex particles coated with polyvalent antisera where it is raised against various flagellar antigens of *Salmonella*. In addition to this, non-motile *Salmonella* are detected not by flagellar proteins but due to protein basal bodies similar to the protein found in the attachment point of flagella.

8. **What is the difference between CompactDry™ Total Count (TC) and CompactDry™ Heterotrophic Plate Count (AQ)?**

   These two CompactDry™ plates are both used for measuring the total microbial count but designed for different sample types. CompactDry™ TC is used for determining the microbial population in most solid, liquid, environmental, and air samples. On the other hand, CompactDry™ AQ is designed for heterotrophic microorganisms or all microorganisms that require organic nutrient for growth, and these are universally present in water, food, soil, vegetation, air, among others (Gensberger et al., 2015). With its use to detect and measure total microbial count in drinking water, CompactDry™ AQ is a significant tool to comply with national standards, for instance, the Philippine Department of Health Administrative Order 2017-0010, which mandates that heterotrophic plate count should be measured in all drinking water.

9. **What is the difference between CompactDry™ Total Count (TC) and CompactDry™ Total Count for Tea (TCC)?**

   CompactDry™ Total Count (TC) and CompactDry™ Total Count for Tea (TCC) are both used for measuring the total microbial count. Tea products naturally contain catechin that inhibits growth of microorganisms. CompactDry™ TCC contains catechin neutralizer to counter the effect of antiseptic substances of tea. Hence, to check for total microbial growth for tea products, it is recommended to use CompactDry™ TCC over CompactDry™ TC.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