User Guide
for microbial enumeration in liquid samples with nomad Testers

Introduction

The nomad Testers are simple, easy-to-use and ready-to-go devices for assessment of microbiological contamination levels when access to a laboratory, dedicated equipment or expert technique are limited.

Applications include microorganism enumeration in environmental waters, process waters, purified waters, equipment rinse waters, food and beverage products, manufacturing equipment including work surfaces for hygiene, environmental and/or process monitoring.

The devices unique all-in-one design allows for sampling and testing, thus eliminating the need for a sterile transport container and an aseptic liquid transfer step.

The nomad Testers are based on the established microbiological culture method and membrane filtration technique. The Testers combine a 0.45 μm membrane filter bonded to a culture media containing nutrient-pad, heat sealed into an easy-to-handle paddle.
The device configuration allows for the draw-through of 1 ml of sample to retain microorganisms on the filter surface, for subsequent culturing within its transparent plastic chamber. The filter is grid-marked to aid in counting the microbial colonies grown on its surface.

Each Tester assembly is packaged in a sealed plastic envelop.

Isolated tests may provide useful information when the result is comparable to an accepted standard. Since facilities, processes and procedures differ from one another, regular testing that results in the build-up of historical data can be beneficial for assessing then preventing contamination risks by:

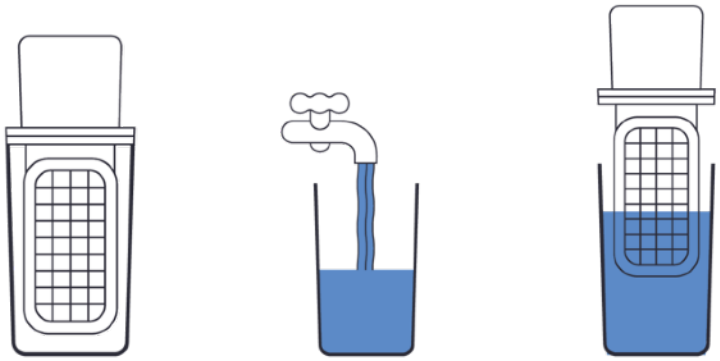
- Measuring the effectiveness of sanitary design, personnel practices, and operational methods.
- Providing information about indicator organisms, spoilage organisms, and/or pathogens of concern, so that appropriate corrective actions can be initiated to prevent potential microbial outbreaks.
- Acting as an early warning system for microbiological hazards in both the production and post-production environment
- Helping to identify harbourage niches and hot spots that may act as a source of contamination.
- Is a critical aspect of documenting the overall sanitary state of a facility.
- Validating the sanitation program and helps in determining the frequency required for cleaning and sanitation.

Consistency in the application of test protocols is important for building a solid data set.
If a microbial monitoring program is in place, existing practices should supersede the recommendations provided in this guide.

nomad
MICROBIAL TESTERS

Quick Start Guide - Liquids

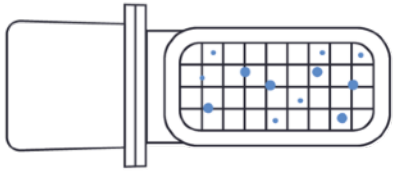
Sample & Test

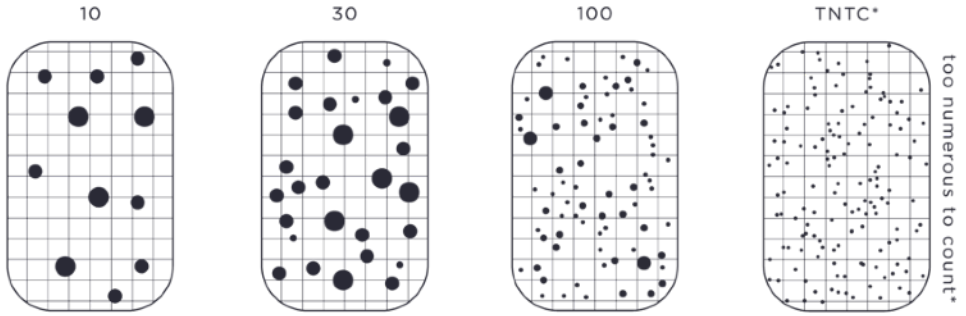


Incubate

Coliforms	35 °C / 95 °F	22-24 H
Total Count (HPC)	25-35 °C / 77-95 °F	48-72 H
Yeast & Mold	28-32 °C / 82-90 °F	48-72 H

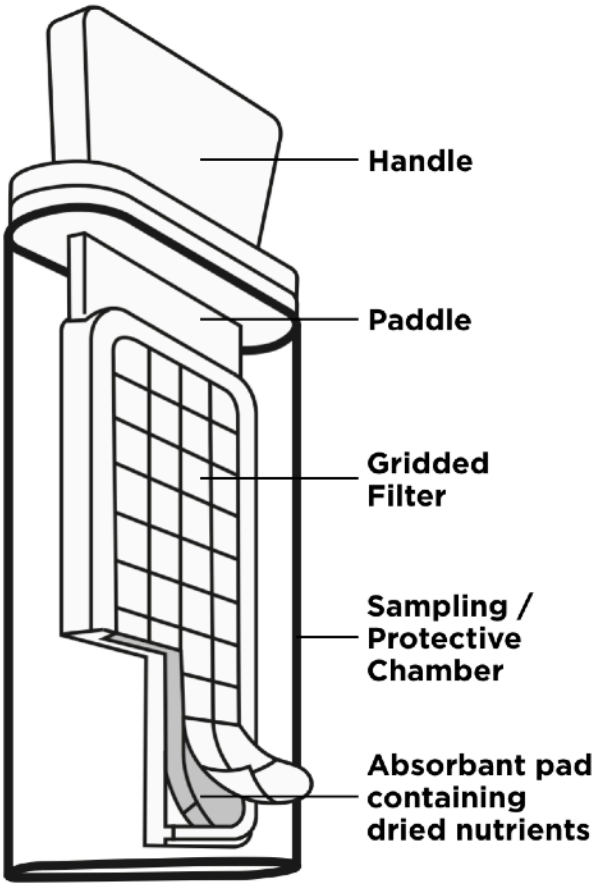
Count





Information Chapters

Tester components



A Tester consists of two parts:

•An outer plastic chamber used to collect liquid samples and as a protective cover for the paddle. Each chamber carries 2 graduation marks, one for the 18 ml sample collection, another 1,8 ml mark facilitating 10 x dilutions

•A plastic dip test handle (paddle) with a gridded retentive filter and an absorbent pad.

•The pad contains the dehydrated nutrient medium for recovery of organisms.

•The 0.45 μm membrane filter is bonded to the culture-media-containing nutrient-pad, heat sealed into an easy-to-handle paddle

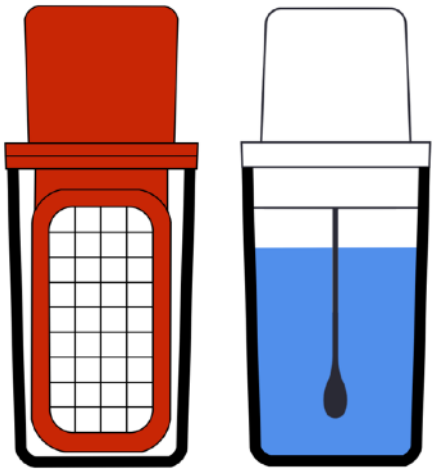
•Each Tester assembly is packaged in a sealed plastic envelop.

A Swab consists of two parts:

•A plastic cap connected to a polyester swab,

•An outer chamber, identical to the tester chamber, containing pre-measured 18 ml of a sterile dilution and neutralising buffer solution.

A Swab Test Kit combines a Tester with a Swab.



Instructions

Preamble

Notes:

A. It is recommended that samples containing residual chlorine should be neutralised with sodium thiosulfate (0.1 ml of a 10% thiosulfate solution for 120 ml of sample or 10 mg of thiosulfate for 100 ml of sample) prior to testing with the Testers.

B. Where exact counts are not required, samples containing an estimated microorganism level of up to 300 microorganisms growing into colonies (a.k.a. cfu) per ml may be tested without dilution.
For exact counts, any sample containing more than 100 cfu/ml should be diluted.
If a dilution level of 1:10 is required, simply fill the Tester chamber with the liquid up to the lower graduated line (1.8 ml) add sterile water (or buffer) to the upper line (18 ml), insert Tester paddle and proceed with test.

C. For most water samples, the Tester paddle should be immersed in the sample for 20 seconds.
For some application, like viscous samples, the paddle may require immersion for up to 2 minutes to allow the 1 mL aliquot to wet thoroughly.
If the entire membrane is properly wetted, the filter will appear dark grey with the Total Count Tester and the Yeast & Mould tester and a very light grey with the Coliform Tester.

D. Do not immerse paddle longer than is required, otherwise a loss of medium through the membrane and into the sample may occur.

E. Sample dilutions should be made with a sterile phosphate buffer (pH 7.2).
If this is unavailable, sterile, chlorine-free tap water may be used.

F. Paste samples can be tested by the following method:
- Weigh a given amount of the paste in a sterile bag or container, for example 10 gr or 10 ml.
- Pour a known volume and at least 20 ml of sterile peptone water or phosphate buffer into the bag or container, for example 90 ml.
  - (a) Seal the bag and knead with the fingers for a known period of time and at least 2 minutes, or
  - (b) In the container, mix with a sterile stem, for example the sanitized end piece of a mini mixer for a known period of time and until the sample is homogeneous.
  - Allow the large suspended solids to settle (if any)
- Pour 18 ml of the upper liquid layer into the Tester chamber case (fill to the upper mark).
- Test in the usual manner.
- Report results with correction for dilution factor (10 x in this example)

G. Solid samples can be tested by the following method:
- Weigh a given amount of the paste in a sterile container, for example 10 gr or 10 ml.
- Pour a known volume and at least 20 ml of sterile peptone water or phosphate buffer into the bag or container, for example 90 ml.
- Mix with a sterile stem, for example the sanitized end piece of a mini mixer for a known period of time and until the sample is homogeneous.
- Allow the large suspended solids to settle (if any)
- Pour 18 ml of the upper liquid layer into the Tester chamber case (fill to the upper mark).
- Test in the usual manner.
- Report results with correction for dilution factor (10 x in this example)

Sampling

Collection of food, water and environmental samples may occur in a variety of locations.
Each location and reason for sampling will be associated with its own risks. It is important to make an assessment of these risks and put appropriate control measures in place before any sampling is carried out.

Examples of hazards include:

- Wet floors that present a slip hazard
- Working at heights when ladders/steps are required to reach sampling points
- Working in confined spaces when sampling from difficult-to-reach parts
- Exposure to aerosols when sampling from cooling towers and showers
- Exposure to irritant, corrosive or toxic chemicals
- Exposure to moving mechanical parts
- Lone working in isolated areas such as plant rooms

A. Sampling from a container

1. Catch the paddle handle between the index and middle fingers, with the membrane carrying part on the backside of your hand. Hold the chamber between thumb and index finger. The other hand is free for handling the sample container and other sample collection actions.

2. If the sample level is above the 18 ml mark, tilt the chamber to an approximate 45° angle. The excess sample will spill.

3. Proceed with testing as suggested below

B. Sampling from a sampling port or a point of use e.g. a tap

1. Catch the paddle handle between the index and middle fingers, with the membrane carrying part on the backside of your hand. Hold the chamber between thumb and index finger. The other hand is free for opening and closing the port and other sample collection actions.

2. Clean or sanitise the sampling port for example with a clean disposable cloth (and detergent if necessary).
   Alternatively, immerse the end of the tap is a suitable sanitant solution for 2 to 3 minutes, then rinse flush the sampling port thoroughly e.g. for 1 minute or 1 litre.
   Alternatively, use a wash bottle to spray a suitable sanitant onto the outside and inside of the tap spout. Leave for 2-3 minutes before rinsing.

3. If the sample level is above the 18 ml mark, tilt the chamber to an approximate 45° angle. The excess sample will spill.

4. Proceed with testing as suggested below

C. Sampling from a body of liquid

Note that this procedure implies that the device chamber is dipped into the sampled body, potentially contaminating the sampled body with microorganisms that have contaminated the external part of the device during handling or with sanitant residues if the chamber outer surfaces have been decontaminated. If the body of liquid is intended for processing, ensure the sampling procedure does not negatively affect the process.

Note that dipping the paddle filter section directly into the body of liquid might result in an uneven dispersion of the microorganisms on the filter surface and affect the result.

1. Choose a location at the water body, which is characteristic for the whole water.
   An appropriate site for taking a single sample may be where the water velocity is likely to be at its

lowest and away from inlets or outlets. It may be advisable to take samples from potential “dead spots” in the water/liquid circulation.

2. Open the device. Catch the paddle handle between the index and middle fingers with the membrane carrying part on the backside of your hand. With the opposite hand, hold the chamber between thumb and index finger. The other hand is free for other sample collection actions.

3. Immerse the chamber with its opening pointing downwards into the water with continuous motion and directing away from the body but parallel with the edge of the water body, until completely immersed, (for large bodies of water, about 200-400mm (8-16”) below the surface), tilt the chamber to allow it to fill then remove the chamber from the water body.

4. Tilt the chamber to an approximate 45° angle. The excess sample will spill.

5. Proceed with testing as suggested below

Instructions for testing Liquid Samples

1. Open the Tester package, lift out the device.

2. Write on the Tester case with indelible marker the date, type and location of sampling.

3. Carefully remove the "paddle" from its case.
   To make it easier to remove the paddle, hold the Tester chamber with the membrane facing you and twist the handle towards you.
   If both hands are requires for sampling, catch the paddle handle between the index and middle fingers, with the membrane carrying part on the backside of your hand
   Avoid touching the gridded filter surface at all times.

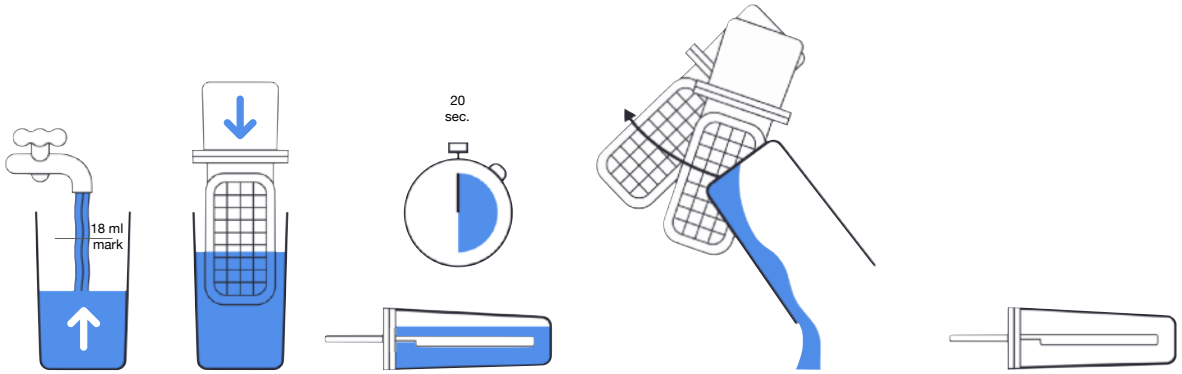
4. Pour the liquid sample into the chamber, filling to the upper 18 ml mark.

5. Position the paddle over the chamber and drop the paddle into the liquid.
   Quickly insert the paddle firmly into the chamber and lay the unit with membrane facing down onto a flat surface.
   Make sure the membrane is uniformly wetted, and while in this position, the unit should not be agitated.
   Allow 20 seconds for sample to be drawn through the filter and ensure there are no more bubbles coming out of the vent prior to removing the paddle from the sample.
   The filter should appear a uniform grey.
   If the sample is viscous, the paddle should remain in the case for additional time (up to 2 minutes).

6. Remove the paddle and, with a firm snap of the wrist, shake off the excess liquid. Empty the chamber and reinsert the paddle. To prevent the paddle from drying out during incubation, it should be seated firmly in the case to form an air-tight seal.

7. Incubate the Tester, filter side down, using the time and temperature specified in the table shown in the "Culture-Incubation Guide".

8. For examination and counting please refer to the « Colony Counting Guide" section.



Culture-Incubation Guide

When using the blue Tester i.e. performing coliform analysis, the time and temperature shown below must be followed.
The other Testers may be incubated at any temperature shown below as long as the temperature is consistently followed.
The mid-points of these ranges are preferable.
Time of incubation (except Coliforms) should be at least 48 hrs.
Incubation periods exceeding this will be dictated by practical time limitations.

The incubation period should be consistent.

	Incubation	
	Temperature (°C / °F)	**Duration (Hours)**
Blue (Coliforms)	35 / 95	22-24
Red (Total Count)	25 / 77	72
or	35 / 95	48
Starved, heat or chemically stressed microorganisms	Room temperature	3-7 days
Yellow (Yeast and Mould)	28 / 82	72
or	32 / 90	48

Colony Counting Guide

After incubation is complete, remove paddle from its chamber and examine the filter surface, preferably with an illuminated magnifier (5X to 10 X).

A Smartphone may also be used as a magnifier, in one of 2 ways:

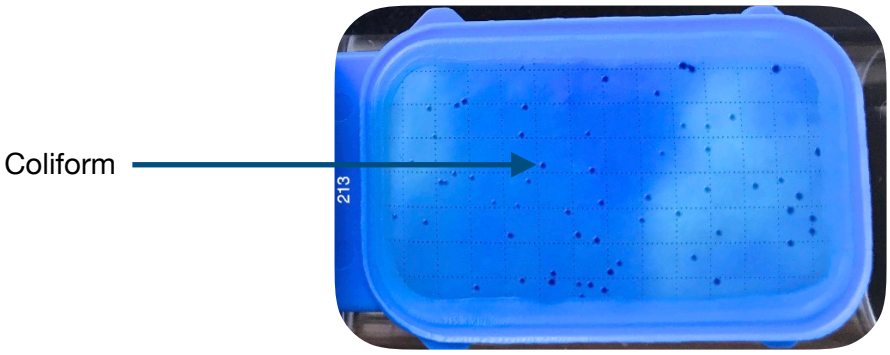
- Using the camera function with maximum zoom
- Taking a picture at a distance of 4 inches or 10 cm, then zooming in on the picture.
  This approach also allows the counting to be performed later or by another person.
  Make sure the date, type and location marked on the device are visible on the picture.

The appearance of the microbial colonies will vary, depending upon the type of device used and the organisms recovered.

Generally they will appear as follows:

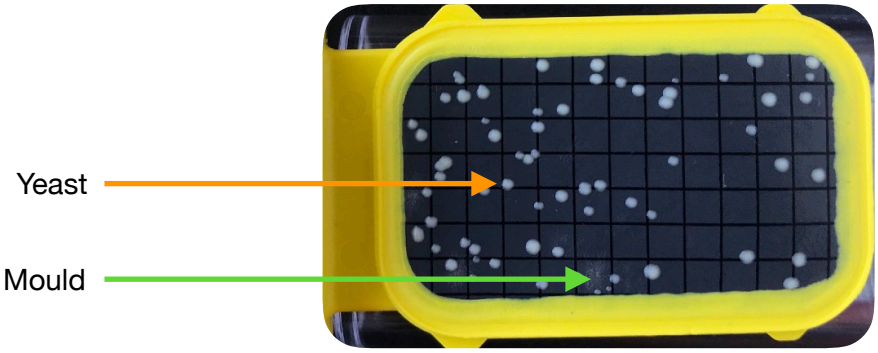
Blue nomad Testers:

- Coliform colonies are blue or blue/green.
- Non-coliform colonies may be green, grey, or cream.



Yellow nomad Testers:

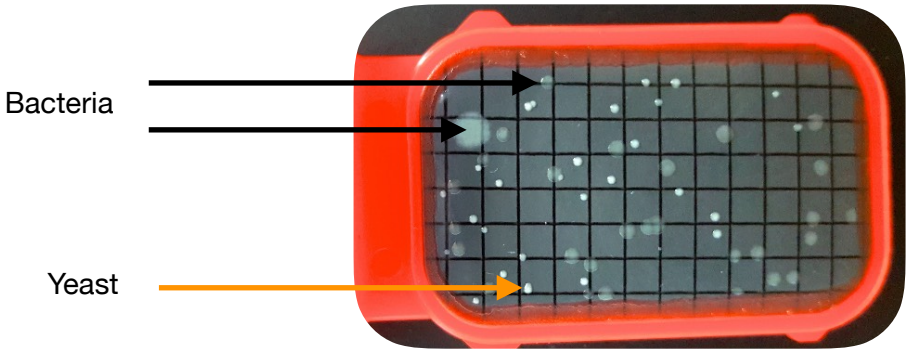
- Mould colonies appear white, green or brown/black and filamentous.
- Bacterial colonies may appear but are usually smaller and more glistening and transparent than the yeast colonies.



Red nomad Testers:

- Bacteria colonies appear glistening and translucent or transparent, circular or irregular in shape, with colours varying from colourless to white, cream yellow or occasionally pigmented.

- Mould colonies often appear filamentous whitish grey, with fuzzy edges. They usually turn into a different colour, from the center outwards
- Yeast colonies appear satiny, opaque, white coloured or may turn green over time.



Counting Colonies

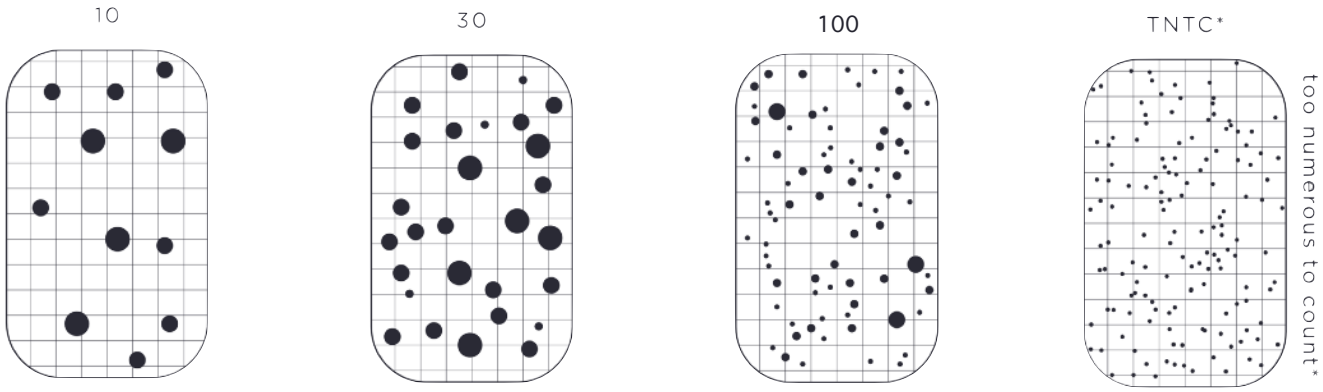
Colonies growing on the filter surface are counted as individual organisms.

In recording your count with the blue Tester for coliforms, count only the blue colonies.
Coliform and faecal coliforms are always reported as the number per 100 mL sample.
Therefore in undiluted samples, count the number of blue colonies obtained and multiply this result by 100. If the sample is diluted, multiply the 100 ml count by the appropriate dilution factor.

For all other samples, the count per ml is the generally accepted system for recording your results.
Therefore, for non-diluted samples, the number of colonies observed on the filter will be the number recorded (as sample count/ml).
For diluted samples, the count obtained must be multiplied by the dilution factor.

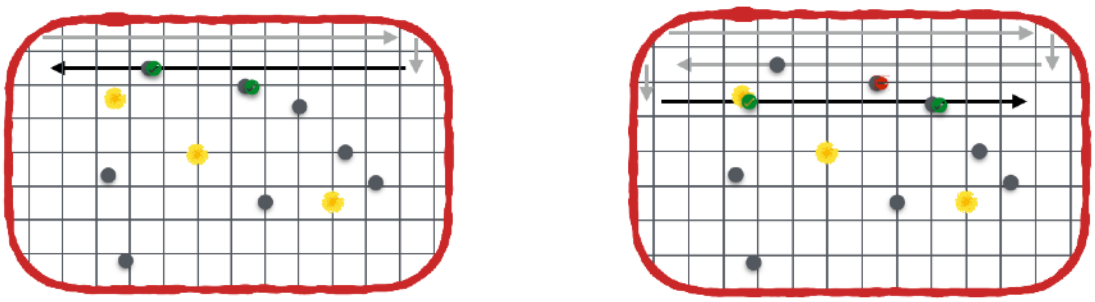
For example:
- Number of colonies on filter = 60
- Sample dilution = 1:10 (dilution factor is 10)
- Sample count/mL = 60 x 10 = 600 cfu/ml (colony forming units)

A rapid count estimate can be made by comparing the filter to the examples shown below.



Precise counting technique

Starting from a corner of the grid-marked filter, follow the long grids and count.
When a colony has grown over a grid, to avoid double counts only count the colony the first time i.e. on the first pass.



Alternatively, starting from a corner of the grid-marked filter, follow the long grids and using a multi-surface pen, mark each colony position by a dot on the chamber. Once all the filter surface has been observed, count the dots.

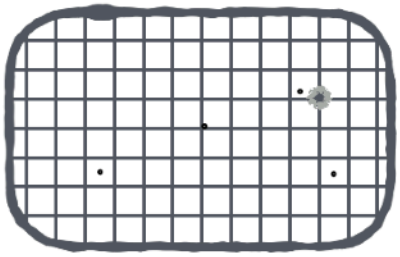
Multiple readings

Some populations of microorganisms grow faster than others.

To improve the quality of the result, it is desirable to perform additional readings at incubation periods corresponding to 2/3 and 4/3 of the recommended incubation time.

These additional readings allow:

- to improve the counting of slow-growing populations whose colonies are difficult to see and count after a normal incubation period
- to reduce equivocal counts resulting from the fact that some fast-growing colonies overlap, join or come to confluence with others.In this illustration, the observations are done on a Total Count Tester incubated at room temperature.



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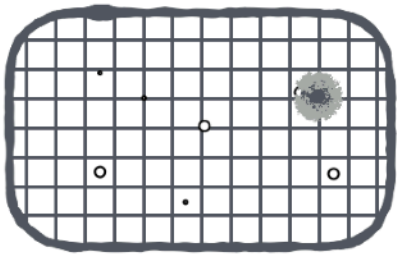

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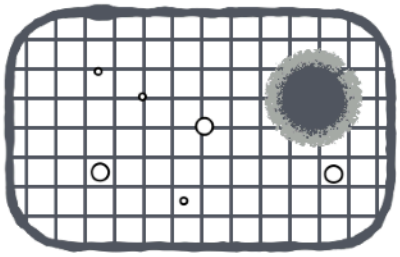

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After the recommended incubation period, a mould has grown quickly and potentially overgrown a nearby bacteria colony.

In addition a small colony has appeared, located closely to a grid mark, which could be overlooked.

The final count is 8, while 8 individual colonies are not easy to observe on any of the individual readings.

In this case, the use of a multi-surface marker for marking each colony with a dot is recommended.

Ordering Information

nomad Testers for testing of liquids

Description	Color	Qty/Pk	Catalog #
HPC Total Count Tester	Red	25	NTRD TTC 25
Coliform Tester	Blue	25	NTRD COL 25
Yeast & Mould Tester	Yellow	25	NTRD YM0 25

nomad Swab Kits for testing of surfaces

Description	Color	Qty/Pk	Catalog #
HPC Total Count Tester with Swab in Neutralising Buffer	Red	25	NTRK TTC 25
Coliform Tester with Swab in Neutralising Buffer	Blue	25	NTRK COL 25
Yeast & Mould Tester with Swab in Neutralising Buffer	Yellow	25	NTRK YM0 25
Swab in Neutralising Buffer	Red	25	NTRD SWB 25