Workshop MRAMA: Lab Sessions

Wednesday, 22nd November · 9:15 a.m.-12 noon

- **Sample preparation and plating automated procedures**: dilutors Dilu*Flow*Pro, Smart Dilutor W, Diluwel and Dilumat, homogenizers BagMixer SW, Pulsifier, Masticator and Smasher, serial dilution systems Dilucup-Dilugent Shaker and Serial Diluter; spiral platers easySpiral Dilute and Eddy Jet 2W
- **Rapid viable cell count methods**: Vitroids and Lenticule discs (certified reference microorganisms); Petrifilm (plates and reader Advanced), DryPlates, Compact-Dry (plates and reader); Rapid YM agar, Quanti-P/A Clostricult; Colilert-18, Enterolert-DW, Pseudalert and Quanti-Tray; Milliflex Quantum; SimPlate; colony counters Scan 1200, SphereFlash and Quantica 500
- **Method for enumeration by miniaturized MPN**: TEMPO AC and EC

Thursday, 23rd November – Thanksgiving day · 9:15-11:55 a.m.

- **Environmental control procedures**: RODAC plates, Count-Tact and Lock&Block; contact slides; Quick Swab; muestreadores Spin Air, MicroBio, Airwel, AIR IDEAL 3P and Coriolis
- **Chromogenic culture media**: SALMA One Day, ALOA, ChromID Coli, ChromID EHEC, Baird-Parker RPF, *Brilliance* Salmonella (Oxoid Salmonella Precis method), *Brilliance* Listeria (Oxoid Listeria Precis method), *Brilliance* Staph 24 agar, *Brilliance* coliform agar, RAPID'*Salmonella,* RAPID'*E.coli* 2, RAPID'*L.mono*, IRIS *Salmonella* agar, COMPASS *Listeria* agar, Chromocult coliform agar, CondaChrome agar for *Salmonella*, CondaChrome agar for *E. coli*-coliforms
- **Diagnostic kits**: API (kit and reader), RapID ONE, Microbact, rhamnose test, EnteroPluri-*Test*, HACCP System *Plus*
- **Other environmental control procedures**:
  - **luminescence**: UltraSnap, AquaSnap, MicroSnap
- **Immunological detection methods**:
  - **ELFA**: VIDAS SPT
  - **immunoprecipitation**: 1-2 Test for *Salmonella*
  - **lateral immunomigration**: Singlepath, VIP Gold, Reveal 2.0 for *Salmonella*
- **Method for enumeration by miniaturized MPN**: reading of TEMPO AC and EC

Friday, 24th November · 9-11 a.m.

- **Diagnostic kits**: O·B·I·S·, membranas ID
- **Other environmental control procedures**:
  - **luminescence**: N-Light *Listeria monocytogenes*, luminometers Clean-Trace, AccuPoint Advanced, MVP ICON and EnSURE Touch
  - **colorimetry**: Contam Swab, FLASH, Clean Test, InSite, AllerSnap
  - **others**: BioFinder
- **Immunological detection methods**:
  - **confirmation by latex agglutination**: Oxoid latex test
  - **lateral immunomigration**: Reveal Q+ for aflatoxins and for DON (kits and reader Raptor), Reveal 3D for allergens (milk), AllerFlow gluten, DipSensor and Extenso for antibiotics
- **Molecular biology (methods other than PCR)**: Molecular Detection System (MDS)
- **Reading**: rapid viable cell count methods, environmental control procedures, chromogenic culture media, diagnostic kits, immunological detection methods

SAMPLE PREPARATION

25 g + 225 mL APT

25 g + 225 mL APT + o o

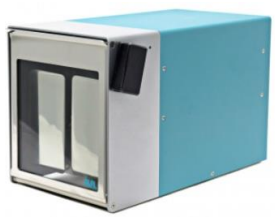
25 g + 200 mL + Reveal®

25 g + 225 mL Fraser 1/2

225 mL APT

200 mL H_2O + Reveal 2.0 *Salmonella*

225 mL ½ Fraser



25 g + 225 mL APT

25 g + 225 mL APT + IRIS/Rapid/ SALMA

25 g + 200 mL Reveal®

25 g + 225 mL Fraser 1/2

24 h incubation

2nd day

(continues on page 4)

Serial Diluter

Dilucup/Dilushaker

Dilutions from -2 to -4

(continues on page 2)

SAMPLE PREPARATION

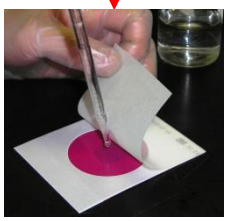
Decimal dilutions

Serial Diluter

Dilucup/Dilushaker

1/100 1/1000 1/10000

1.0 mL



Petrifilm

1.0 mL

DryPlates

Compact-Dry

1.0 mL+
9 mL Dil.

SimPlate

0.1 mL

Rapid YM

1.0 mL+
3 mL H_2O

TEMPO

Spiral
Log 100 µL

Eddy Jet 2W

easySpiral Dilute

H_2O CONTROL

100 mL H_2O

Milliflex Quantum

1 mL H_2O

Petrifilm AQHC

100 mL H_2O

Quanti-P/A Clostricult

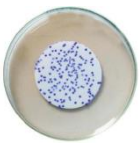
100 mL H_2O

Colilert
Pseudalert
Enteroalert

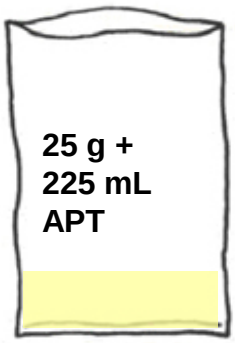
Quanti-Tray

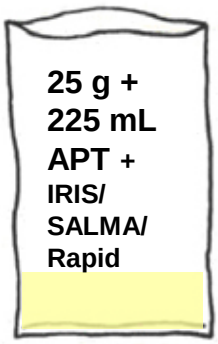
STANDARD CULTURES

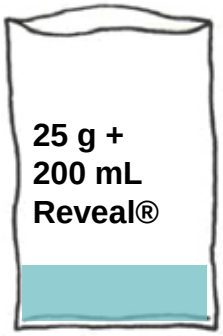
LENTICULE discs

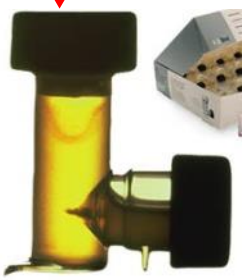


Vitroids





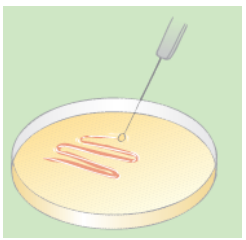




1-2 Test para *Salmonella*

VIDAS SPT

Chromogenic media plating



Reveal 2.0 *Salmonella*

Singlepath *L'mono*

VIP Gold *Listeria*

Molecular Detection System (MDS)

AUTOMATED COLONY COUNT

Scan 1200

Quantica 500

SPHERE FLASH

ENVIRONMENTAL CONTROL

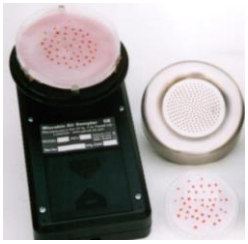
RODAC plates

Count-Tact plates

Laminocultives

Quick Swab

AIRWEL sample air



MicroBio sample air

AIR IDEAL 3P sample air

Coriolis COMPACT sample air

Coriolis sample air

Spin Air sample air

Sampling and counting (colonies)

Luminómetro Clean-Trace

EnSURE Touch

Luminómetro MVP ICON

Luminómetro AccuPoint Advanced

Bioluminiscence (ATP)

N-Light
Listeria monocytogenes

MicroSnap – EnSURE Touch

Bioluminiscence (enzymatic reaction)

ENVIRONMENTAL CONTROL

Colorimetry (proteins)

FLASH

Clean Test

AllerSnap

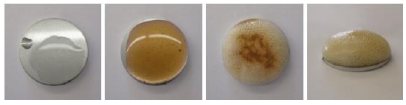
Colorimetry (bacteria)

Contam Swab

InSite

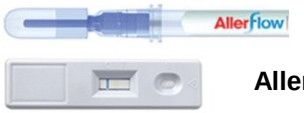
Others

BioFinder



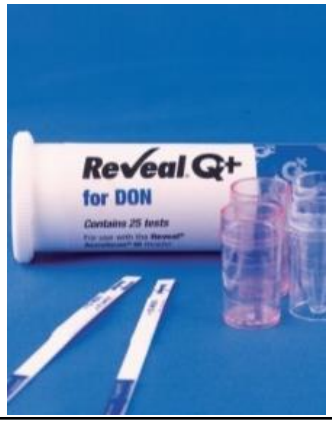
Inmunology (proteins)

Reveal 3-D leche



AllerFlow gluten

MYCOTOXIN DETECTION



Reveal Q+ y lector Raptor

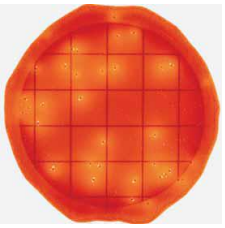
ANTIBIOTICS DETECTION

DipSensor

Extenso

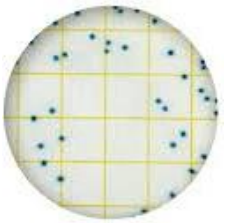
CHROMOGENIC CULTURE MEDIA

Coliformes / *Escherichia coli*

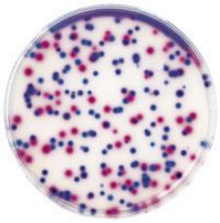


Petrifilm recuento d coliformes

Petrifilm recuento E. coli / coliformes

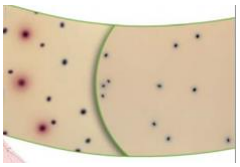


Petrifilm Select recuento de *E. coli*

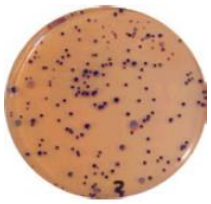


Chromocult coliformes

RAPID'*E.coli* 2

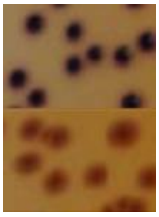


chromID Coli



chromID EHEC

CondaChrome *E. coli*-coliformes

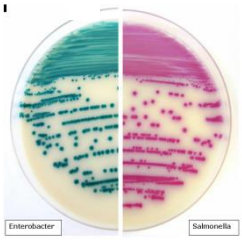


Brilliance cromogénico coliformes

Salmonella spp.

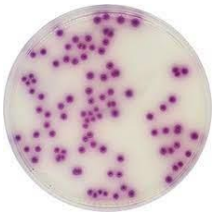
RAPID'*Salmonella*

Brilliance Salmonella



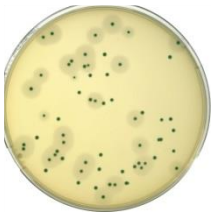
IRIS *Salmonella*

SALMA One Day

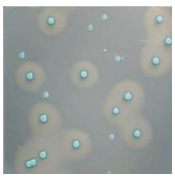


CondaChrome *Salmonella*

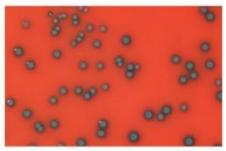
Listeria monocytogenes



COMPASS *Listeria*



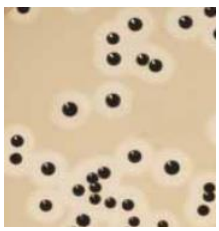
Brilliance Listeria



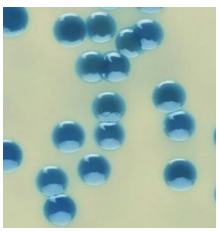
RAPID'*L.mono*

ALOA

Staphylococcus aureus



Baird-Parker RPF

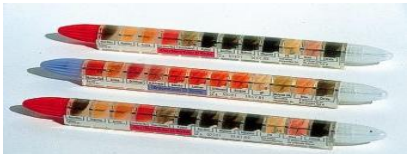


Brilliance Staph 24

DIAGNOSTIC TESTS AND KITS

Enterobacterias

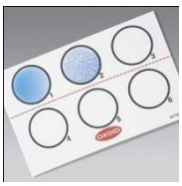
API



EnteroPluri-*Test*

RapID ONE

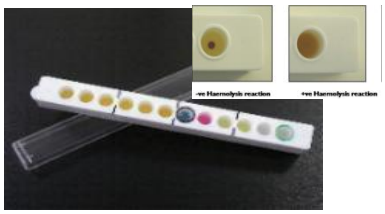
Salmonella spp.



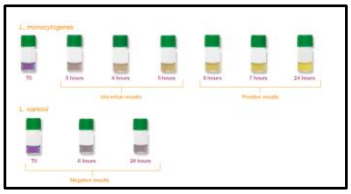
Agglutination tests

Oxoid latex test

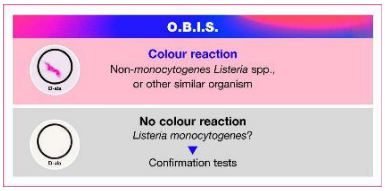
Listeria monocytogenes



Microbact Listeria 12L



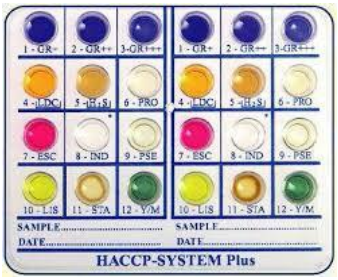
Test de la ramnosa



O·B·I·S·

API Listeria

Different microorganisms



HACCP System *Plus*

Membranas ID

Overview of the methods employed

1. Wednesday

1.1. Sample preparation and plating automated procedures

- Homogenizers: Stomacher vs Pulsifier:

In the case of Stomacher, the sample and diluent are put into a sterile plastic bag which is vigorously pounded on its outer surfaces by paddles when placed inside the machine. The resulting compression and shearing forces effectively remove even deep-seated bacteria. In contrast, Pulsifier is used for dislodging microorganisms from foods without excessively breaking the food structure. It has an oval metal ring that can house a plastic bag with sample and diluents. When the instrument is activated, the ring will vibrate vigorously for a predetermined time (around 30-60s). During this time, microorganisms on the food surface or in the food will be dislodged into the diluents with the minimum destruction of the food.

Fung and colleagues in 1988 evaluated the Pulsifier against the Stomacher with 96 different food items (included beef, pork, fish, shrimp, a variety of vegetables, cereal, etc) and found that the systems gave essentially the same viable cell count in the food but the "Pulsified" samples were much clearer than the "Stomached" samples and they had less debris.

- BagMixer SW:

The BagMixer 400 SW is a new generation lab blender. It is silent, very sturdy and user friendly. The window door enables a quick check-up of the blending, in process. It is adapted to all types of applications and compatible with all blender bags.
The peristaltic movement of the paddles allows optimal bacterial extraction during blending, without risk of cross-contamination. In 30 seconds, the sample is ready for analysis.

- Pulsifier:

Pulsifier employs a revolutionary technology for the processing of food samples for microbiological examination. Unlike paddle-type instruments the Pulsifier II® incorporates an oscillating ring that beats the outside of the plastic bag containing the food sample at high frequency producing a combination of shock waves and intense stirring which drives the microbes into suspension. Pulsification has been shown in most food types to be less destructive to the sample producing lower concentrations of debris.

A programmable digital display is used for cycle times, program setting and pause feature. There is also a self-contained area at the bottom of the instrument capable of collecting a complete sample volume in the event of spills or leaks. The lid has a unique locking device so the bag is sealed tightly to prevent aerosol release. This hinged lid can also be released for easy clean-up.

- Masticator:

Masticator paddle blenders enable thorough homogenization of samples while isolating them from any possible contamination. The Masticator's high-quality durable motors ensure safe and efficient homogenization. This solution is for food, cosmetic, pharmaceutical and clinical laboratories that seek reliable homogenization that is cross-contamination-free.

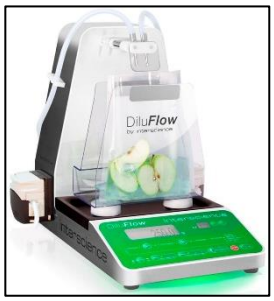
The Masticator blenders disperse samples inside sterile bags with paddles that drive a masticating action while moving the sample from side to side. This key step in sample preparation ensures homogenous distribution of microorganisms throughout the diluent. Several models encompass a wide array of possibilities.

- Smasher:

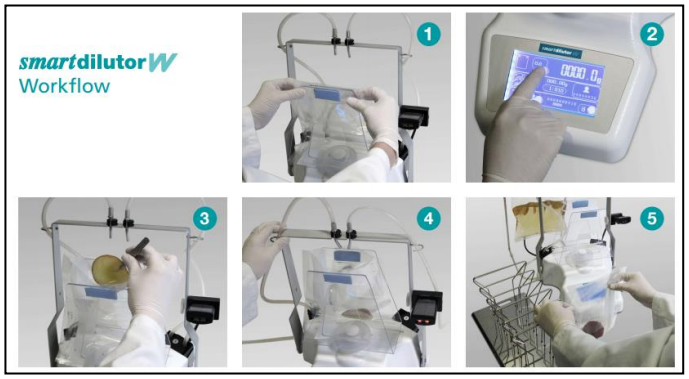
Smasher is a high performance bag blender, that ensures sample homogeneity before microbial detection or identification tests. It crushes the sample in record time and then automatically adjusts its mixing speed for a perfectly homogenised enrichment broth.
A study conducted by an independent laboratory showed that the Smasher tested at 15 and 30 second blending times made it possible to obtain results similar to those of the compared blender tested at 60 second blending time.

- Dilu*Flow* Pro:

The DiluFlow Pro automatically dilutes a solid sample in seconds, with the appropriate weight of diluent, up to a total weight of 5 kg. Its robotic arm enables easy work under a laminar flow cabinet.



- Smart Dilutor W:



Its precise weigh cell and powerful peristaltic pumps approach optimized gravimetric dilutions/ liquid dispensing to food, pharmaceutical and cosmetic quality control labs.

- Diluwel:

Diluwel is a rapid, accurate gravimetric diluter for combining solid samples with the correct amount of diluent, automating the laborious task of making and standardising initial sample dilution.

- Dilumat:

Dilumat diluter is fully automated and demonstrate high performance in food sample preparation. With automated solutions for your sample preparation, your will standardize your sample preparation process and increase your lab productivity. You can dilute food samples from 3 g to 375 g. It meets BAM and ISO 7218 requirements.

- Dilucup-DiluShaker:

The Dilucup is delivered in blisters of rows of either 3 or 6 containers, each filled with 9 ml diluting media. It is easy to separate the number of Dilucups needed. The Dilucups are placed on the tray of the Dilushaker. The Dilucups are opened by tearing off the film covering the holes in the lids. When the Dilushaker is turned on, the liquid in all Dilucups rotates. A sample of 1 ml is added to the first cup and is instantly mixed with the media. After changing the pipette tip, 1 ml of sample is withdrawn from the first cup and added to the second. With the Dilushaker operating the process is repeated until the required dilution factor is achieved.

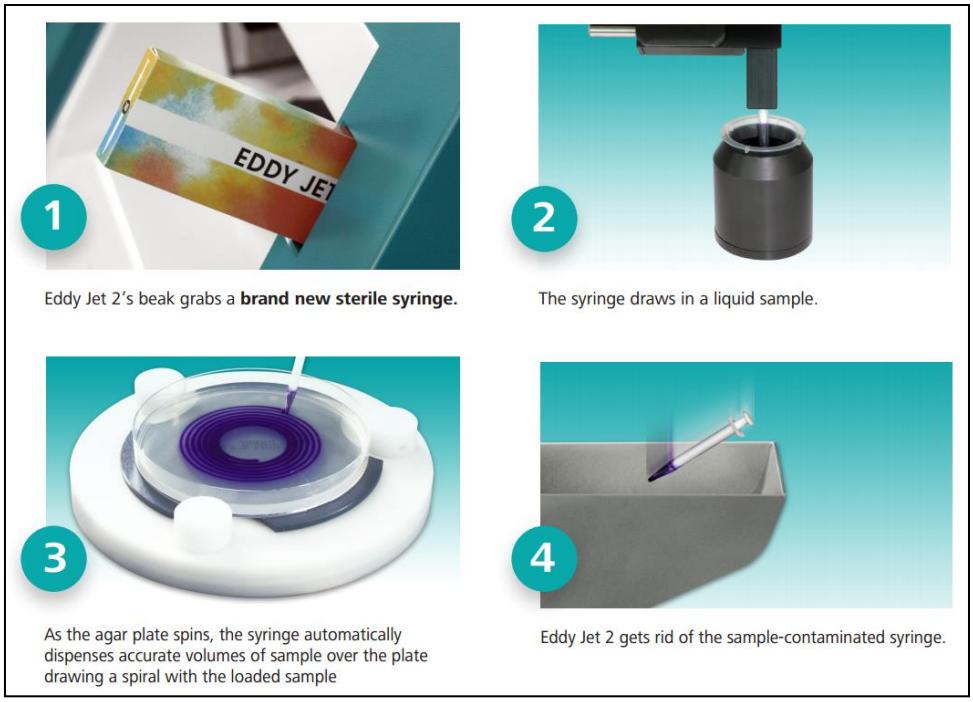
- Serial Diluter:

The Serial Diluter is an innovative device to enable simple, fast and accurate execution of serial dilutions for bacterial counts in food safety testing. It saves you time by eliminating the washing, filling, sealing and autoclaving of test tubes; the need for volume control after autoclaving; the need for the storage of the prepared dilution tubes; the manual handling of test tubes and caps during pipetting; and finally, the manual mixing.

- easySpiral Dilute:

It is a 2-in-1 equipment: automatic diluter and plater. It allows to do 5 x serial 1/10 dilutions and automatic plating on 1 single Petri dish, with a countable range of 30 to 10^{12} CFU/ml. Controlled by microprocessor it is possible to use Petri dishes with diameter: 55, 90 and 150 mm. With sample mixing before dilution/plating and monthly cleaning program. Successive plating capacity with the same sample: 20 Petri dishes (50 µl).

- Spiral plater Eddy Jet 2:



This cutting-edge spiral plater automates, standardizes and streamlines plate inoculations with its patented cross-contamination free technology. Microsyringes confer the system unique, unmatched advantages that make it a bestseller. The user interface is very intuitive and there are no setup times.

1.2. Rapid viable cell count methodologies

- Vitroids:

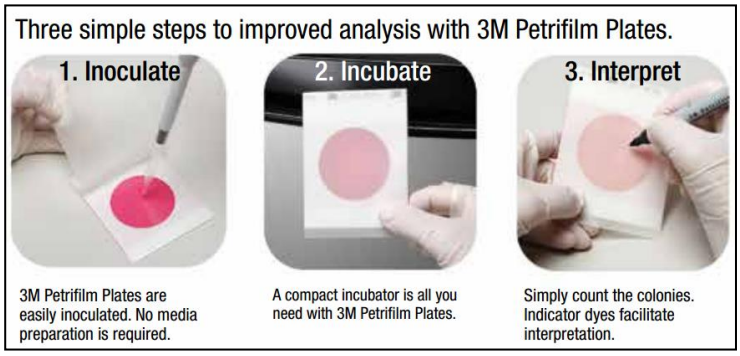
Vitroids are discs that contain viable microorganisms in a certified quantity. Consisting of pure cultures of bacteria or fungi in a solid water soluble matrix, they are stable for at least one year and are in a viable state with a shelf life of 1-3 years.

The preparation can be performed in most solid and liquid medium or rehydration buffer can also be used. Discs can be rehydrated in as little as 100 µL buffer, or in larger volumes, e.g. 100 mL medium. It is also possible to add the disc to a cooled molten medium used for pour plate techniques. The rehydration process takes approximately 10 minutes. On solid media, the disc forms a droplet that can be spread with a sterile loop. In liquid media, the disc dissolves very quickly.

- **Lenticule discs:**

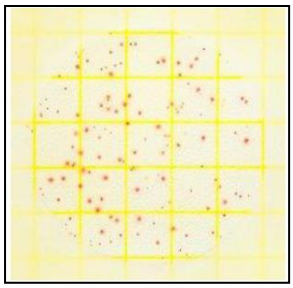
Lenticule discs contain viable microorganisms in a certified and narrow defined quantity (ISO/IEC 17025), produced under reproducible conditions (ISO Guide 34). The bacterial and fungal strains are prepared directly from strains selected from PHE's National Collection of Type Cultures (NCTC) and National Collection of Pathogenic Fungi (NCPF). The discs consist of bacteria or fungi in a solid water soluble matrix. Microorganisms in this form are stable for at least one year and are in a viable stage (no lag phase or recovery time). Each batch is provided with a comprehensive certificate of analysis that specifies the mean number of colony forming units (CFU), an expanded uncertainty about the mean value, details about the method used to determine the product data and the number of passages (subcultures) from the original strain.

- Petrifilm and Petrifilm Aqua (plates and reader):

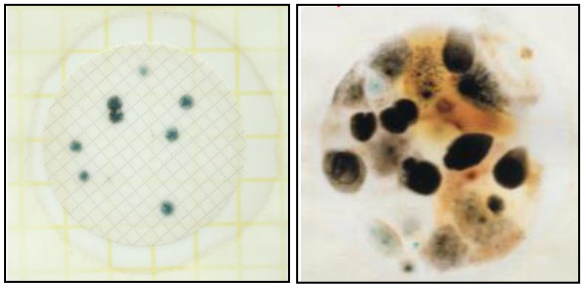


It is an all-in-one plating system. They are heavily used in many microbiology-related industries and fields to culture various microorganisms and are meant to be a more efficient method for detection and enumeration compared to conventional plating techniques.

3M Petrifilm™ Aerobic plate count

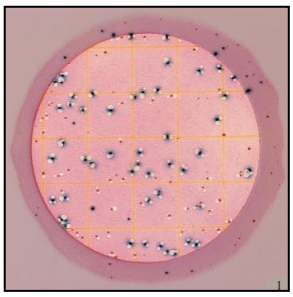


3M Petrifilm™ Aqua yeasts and moulds



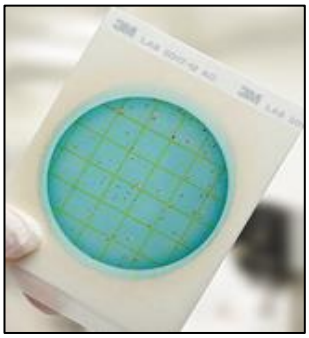
The 3M Petrifilm Rapid Yeast and Moulds Plate is a sample-ready culture medium system which contains nutrients supplemented with antibiotics, a cold-water-soluble gelling agent, and an indicator that facilitates yeast and mold enumeration.

3M Petrifilm™ EC/Coliform plate counts



Petrifilm *E. coli*/Coliform Count (EC) plates contain Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, an indicator of glucuronidase activity, and an indicator that facilitates colony enumeration. Most *E. coli* (about 97%) produce beta-glucuronidase which produces a blue precipitate associated with the colony. The top film traps gas produced by the lactose fermenting coliforms and *E. coli*. About 95% of *E. coli* produce gas, indicated by blue to red-blue colonies associated with entrapped gas on the Petrifilm EC plate (within approximately one colony diameter).

Petrifilm LAB

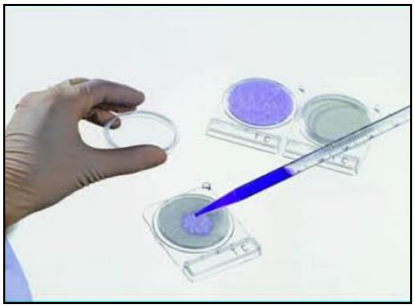


Self-contained anaerobic environment enabled by oxygen-scavenging technology and oxygen-barrier films. No gas packs, chambers, or CO_2 incubators needed. With the use of this system you can get true anaerobic results using standard aerobic incubation conditions. The self-contained anaerobic environment in this plate enhances the recovery of lactic acid bacteria, providing fast, accurate results for effective control of product quality and shelf life.

- DryPlates:

It is a microbial detection that does not require media preparation. That is why it has the advantages of a dehydrated media (long expiration term) and prepared medias (sterile, ready for immediate use, timer-saver). It cold self-diffuses the sample ml, thus avoiding the need for handles, and eliminating the agar's temperature cooling critical point to avoid burning microorganisms and increasing by 10 the plates detection limit.

- Compact-Dry plates:



They provide a convenient test method for counting microorganisms. The plates are supplied sterile and ready-to-use, and the media is dehydrated allowing for a long shelf life at room temperature. The incorporation of chromogenic agents in the media ensures that the plates are easy to interpret, making them an ideal alternative to the conventional pour plate method.

- Rapid YM agar:

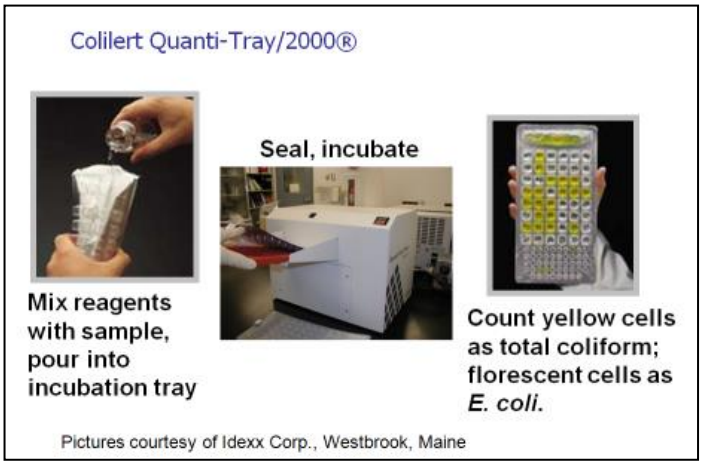
It is a medium used for selective isolation and enumeration of yeasts and moulds from food, water, and environmental samples. Peptones provide amino acids, carbon, nitrogen, vitamins and minerals for organisms growth. Glucose is the fermentable carbohydrate. Magnesium sulfate provides divalent cations and sulfur. Selective agents are incorporated into the medium to reduce colony diameters of spreading fungi and to inhibit the growth of accompanying microbiota.

- Quanti-P/A Clostricult:

Method for the quantification of *Clostridium perfringens* and its spores in 50-100 mL of water with TSC Agar medium. It does not need jars or anaerobic kits, which lowers its cost. Saves the oxygenating stress of the Membrane Filtration and its consequent 49% of false negatives (drastically increases the sensitivity). There is no alternative method by MPN.

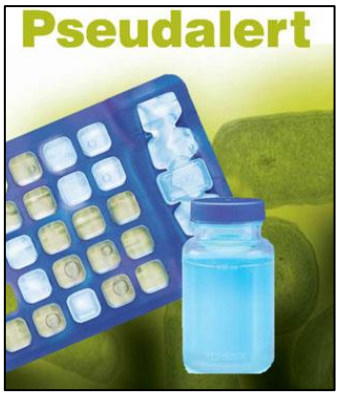
- Colilert-18 and Quanti-Tray:

Colilert-18 is a commercially available enzyme-substrate liquid-broth medium that allows the simultaneous detection of total coliforms and *E. coli*. It is available in the most-probable number (MPN) or the presence/absence (PA) format. The MPN method is facilitated by use of a specially designed disposable incubation tray called the Quanti-Tray.



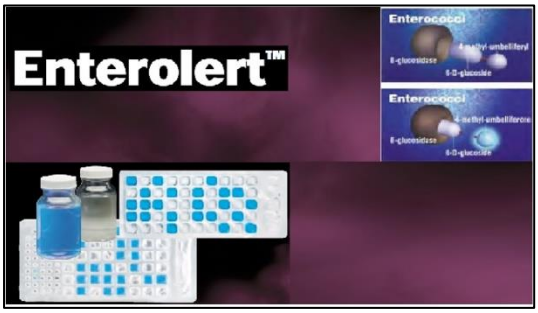
Two enzyme substrates are included in Colilert—a chromogen that reacts with the enzyme found in total coliforms (galactosidase), and a fluorogen that reacts with an enzyme found in *E. coli* (glucuronidase). After 18- or 24-hours incubation at 35ºC, a total- coliform-positive reaction turns the medium yellow; an *E. coli*-positive reaction causes the medium to fluoresce under a long-wave ultraviolet light (366 nm).

- Pseudalert:



The Pseudalert test detects the presence of *Pseudomonas aeruginosa* in bottled, pool, and spa water samples. The test is based on a bacterial enzyme detection technology that signals the presence of *P. aeruginosa* through the hydrolysis of a substrate present in the Pseudalert reagent. *P. aeruginosa* cells rapidly grow and reproduce using the rich supply of amino acids, vitamins, and other nutrients present in the Pseudalert reagent. Actively growing strains of *P. aeruginosa* have an enzyme that cleaves the substrate to produce a blue fluorescence under UV light. Pseudalert detects *P. aeruginosa* at 1 cfu in either 100 mL or 250 mL samples within 24 hours for non-carbonated water samples and within 26 hours for carbonated samples.

- Enteroalert-E:

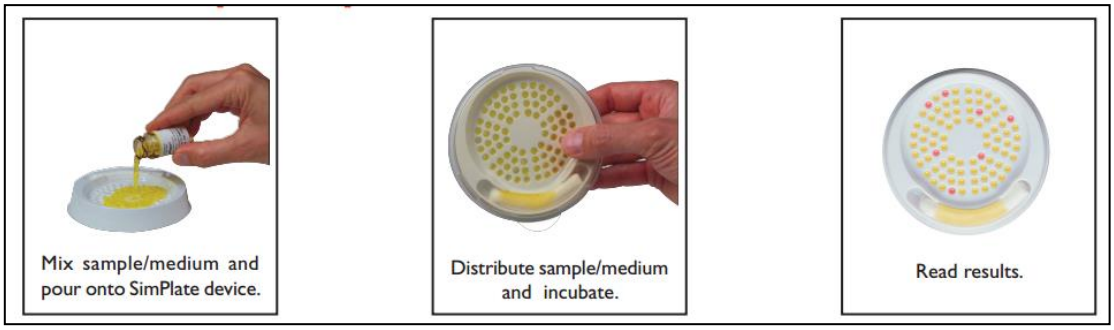


Enterolert-E detects enterococci, such as *E. faecium* and *E. faecalis*, in fresh and marine water. It is based on IDEXX's patented Defined Substrate Technology* (DST*). When enterococci utilize their ß-glucosidase enzyme to metabolize Enterolert-E's nutrient-indicator, 4-methyl-umbelliferyl ß-D-glucoside, the sample fluoresces. Enterolert-E detects enterococci at 1cfu per 100 mL sample within 24 hours.

- Miliflex Quantum:

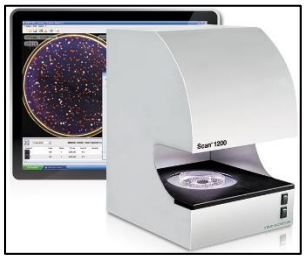
The Milliflex Quantum system is a rapid fluorescent-based technology designed for fast quantitative detection of microorganisms over a broad range of filterable matrices. This non-destructive method also allows you to identify any detected microorganisms using the current ID methodology.

- SimPlate:



SimPlate Total Plate Count utilizes proprietary Binary Detection Technology to provide quantitative results for total aerobic bacteria from food and environmental samples in just 24 hours. Proprietary chromogenic media and patented plating device with isolation wells combine to produce easily counted results and eliminate problems associated with food particle interference or liquefying of gels caused by certain bacterial enzymes.

- Scan 1200:



Scan 1200 is an HD automatic colony counter. It adapts itself to all media and counts in 1 click all colonies even the smallest ones. It ensures accuracy and excellent reproducibility. Images and results are saved automatically. Scan 1200 is also an inhibition zone reader.

- SphereFlash:

It is an Automatic Colony Counter that meets all the requirements for modern microbiology laboratories. With its Colony Lite software version, it performs colony counting in a simple and reliable way thanks to its world-class software and its patented built-in lighting system.

- Quantica 500:

The device makes it possible to count colonies on various plating types, and thanks to an efficient algorithm and the best-in-class camera resolution, it correctly sums up even the merged colonies. This feature puts the Bioavlee device in front of the existing standard devices that have difficulties separating this type of colonies.

1.3. Method for enumeration by miniaturized MPN

- TEMPO

The TEMPO instrument is the food industry's first automated quality indicator testing system for the enumeration of quality indicator organisms in food and environmental samples. TEMPO is based on the Most Probable Number (MPN) method. The principle of the MPN method is to allow any microorganism present in samples to grow in suitable conditions in tubes, using a minimum of three dilutions and three tubes per dilution. BioMérieux has automated and miniaturized this method, which was previously time-consuming and subject to errors due to the numerous steps involved.

2. Thursday

2.1. Environmental control procedures

- Count-Tact, Lock&Block and RODAC plates:

- Contact slides:

Contact slides used to assess the microbiological contamination of surfaces or fluids. They are double sided. Feature a hinged paddle that bends for easy sampling. It is used in total bacterial and enterobacterial counts with inactivation of disinfectants as they include neutralizing components.

- Quick Swab:

It is a ready-to-use environmental swab system to detect microbial contamination. For Dry Sampling:

1) Remove swab from tube and swab targeted area. Place swab back into tube and bring to the lab.
2) Bend red snap valve to transfer all the broth into the tube.
3) Shake or vortex tube vigorously for 10 seconds to release bacteria from swab.
4) Pour contents onto, for example, a Petrifilm Plate.

- Samplers Spin Air, MicroBio, Airwel, AIR IDEAL 3P and Coriolis COMPACT:





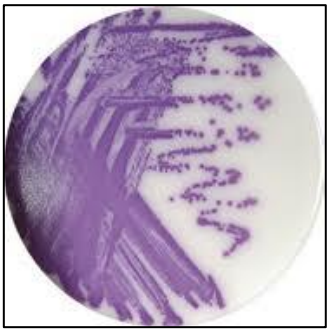
Since air can play a central role as a reservoir for microorganisms, in controlled environments such as food industry, regular microbial monitoring is useful to measure air quality and identify critical situations.

- Air sampler Coriolis:

The Coriolis Micro portable biological air sampler is a standard for testing air quality. Both indoors and outdoors, it's the most efficient biological air sampler: It can take just 10 minutes to collect airborne particles using its cyclonic technology paired with a high suction rate. By generating samples in the fluid phase, Coriolis µ is compatible with rapid microbiological analysis methods.

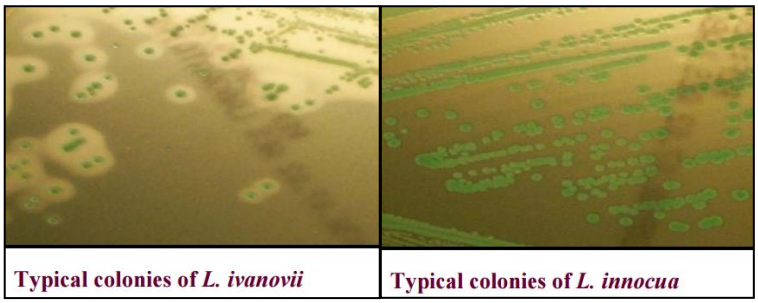
2.2. Description and preparation of chromogenic culture media

- SALMA One Day:



The use of chromogenic substrates for *Salmonella* detection in food samples has been described in different commercial methods. Most of the media are based on C8-esterase activity with a high selectivity, but were reported to miss some strains such as *S. enteritidis* Dublin, or *S. enteritidis* Paratyphi which express a weak enzymatic activity and/or may be stressed by selective agent. Furthermore, conventional chromogenic medium or XLD demonstrated some difficulties in recovering atypical strains, such as serotypes Seftenberg or Dublin. BioMerieux has developed a new medium for the detection of *Salmonella* in food samples. The high specificity was achieved, thanks to the combination of esterase substrates with a specific base. SALMA ONE DAY® method was validated according to ISO16140-2 standard and enabled a faster and easier detection of Salmonella spp. in food samples compared to the EN/ISO/6579 method.

- ALOA:



Typical colonies of *L. ivanovii* | Typical colonies of *L. innocua*

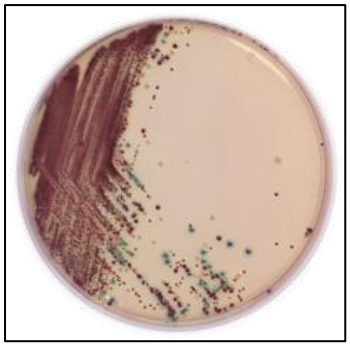
ALOA agar is a pre-prepared, selective and differential medium for the isolation of *Listeria* spp. from food samples and for the presumptive identification of *L. monocytogenes*. To minimize the growth of contaminating organisms, lithium chloride and a balanced antimicrobial and antifungal mixture is employed. The incorporation of the chromogenic substrate X-glucoside for the detection of beta-glucosidase demonstrates the presence of *Listeria* spp., whilst the detection of a specific phospholipase C enzyme produced by pathogenic *Listeria* spp. including *L. monocytogenes* is also achieved. *Listeria* spp. grow on this medium producing blue - green colonies, with pathogenic species (*L. monocytogenes* and *L. ivanovii*) producing similar coloured colonies surrounded by a characteristic opaque halo after 24 hours incubation at 37ºC. Non *Listeria* spp. produce white colonies.

- chromID Coli:

Detection and enumeration of *E. coli* at 44°C and simultaneous enumeration of *E. coli* and other coliforms at 37°C, in food products. The chromID Coli medium contains 2 chromogenic substrates, which enable the simultaneous detection of coliforms and identification of *E. coli*, without the use of additional reagents.

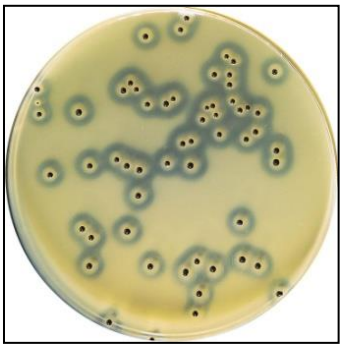
- β-GAL (+): presence of coliforms other than *E. coli* → blue colonies (β-galactosidase).
- β-GUR (+): presence *of E. coli* → rose colonies (β-glucuronidase).

- ChromID EHEC:



It enables the confirmation and presumptive identification of enterohemorrhagic Escherichia coli (EHEC) of serogroups O157:H7, O26, O103, O111, O145, O121 and O45 after an immunoconcentration step performed on the sample. These strains are resposnible for various diseases ranging from asymptomatic infection to hemorrhagic colitis, which can be complicated by the hemoytic uremic syndrome.

- Baird-Parker RPF agar:



Staphylococcus aureus is a Gram-positive coccus capable of producing enterotoxin which can induce food poisoning. The organisms may be present in small numbers in many foods, and, if allowed to multiply unchecked, may produce highly heat resistant enterotoxins. The ability of *S. aureus* to produce lecithinase and lipase has been recognised for many years, and the detection of these enzymes in egg yolk media has become a widely used procedure for the identification of this organism. Its ability to produce coagulase using a similar basal formulation enables confirmatory diagnosis with the incorporation of rabbit plasma into the base medium.

This medium is a modification of Baird-Parker Medium and is recommended for the selective isolation, enumeration and confirmation of *S. aureus* from food and other specimens. The reduction in potassium tellurite concentration in RPF Agar results in *S. aureus* strains forming white, grey or black colonies, which are surrounded by an opaque halo of precipitation, i.e. the coagulase reaction.

- *Brilliance* Salmonella agar (Oxoid *Salmonella* Precis method):

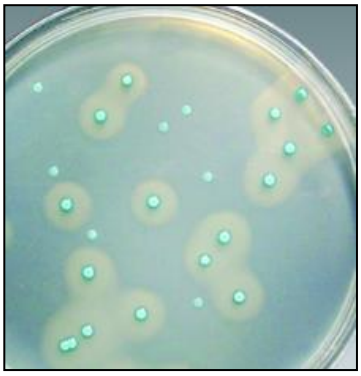
An Inhibigen compound is comprised of two components, combined together by a bond that can only be cleaved by a specific enzyme. When bound together, the inhibitor compound is not toxic and therefore can exist in a medium without harming microorganisms. Once inside the cell, the bond will be cleaved if the target enzyme is present. When the bond is cleaved, the inhibitor molecule is released and disrupts cell wall synthesis, causing death of the organism. As cells die and lyse, free inhibitor is released but cannot be taken up by other cells, resulting in targeted inhibition.

The Inhibigen in *Brilliance* Salmonella Agar targets *E. coli*. Novobiocin and cefsulodin, presented as a freeze-dried supplement, are added to the medium to inhibit the growth of other competing flora such as *Proteus* spp. and *Pseudomonas* spp.

Differentiation of *Salmonella* from the other organisms that grow on *Brilliance* Salmonella Agar is achieved through the inclusion of two chromogens that also target specific enzymes: caprylate esterase and ß-glucosidase. Caprylate esterase is an enzyme present in all *samonellae* as well as some species of *Klebsiella*, *Enterobacter* and *Proteus*. Organisms possessing caprylate esterase cleave the chromogen to

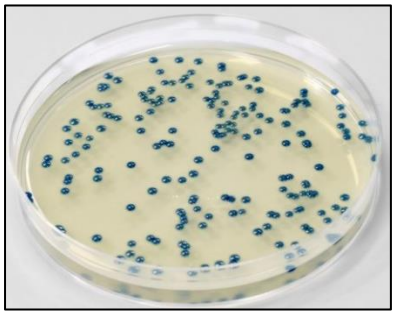
release an insoluble purple chromophore. As the cells grow, the chromophore builds up and produces a purple-coloured colony. Some Enterobacteriaceae, including *Klebsiella* and *Enterobacter* but not *Salmonella*, possess ß-glucosidase. If these organisms grow, they will form blue or dark blue colonies, even if they are esterase positive, which make them easy to differentiate from purple *Salmonella* colonies.

- *Brilliance* Listeria agar (Oxoid Listeria Precis method):



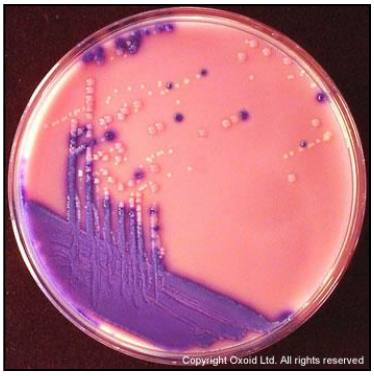
It uses the chromogen X-glucoside for presumptive identification of *Listeria* spp. This chromogen is cleaved by ß-glucosidase which is common to all *Listeria* species. Other organisms that possess this enzyme, such as enterococci, are inhibited by the selective agents within the medium; lithium chloride, polymyxin B and nalidixic acid, whilst amphotericin inhibits the growth of any yeasts and moulds present in the sample. *Listeria monocytogenes* and pathogenic *Listeria ivanovii* are then further differentiated by their ability to produce the phospholipase enzyme, lecithinase. This enzyme hydrolyses the lecithin in the medium, producing an opaque white halo around the colony. The medium is designed to identify *Listeria* spp. based on their utilisation of a chromogenic substrate. However, in this modification, the pathogenic *Listeria* spp. are then further differentiated by the detection of lecithinase (phosphotidylcholine phospholipase C (PCPLC) activity, rather than phosphotidylinositol phospholipase C (PIPLC) activity. Both enzymes, PCPLC and PIPLC, are associated with virulence in *Listeria* spp., and, therefore, the presence of either enzyme is a useful indicator of pathogenicity.

- *Brilliance* Staph 24 agar:



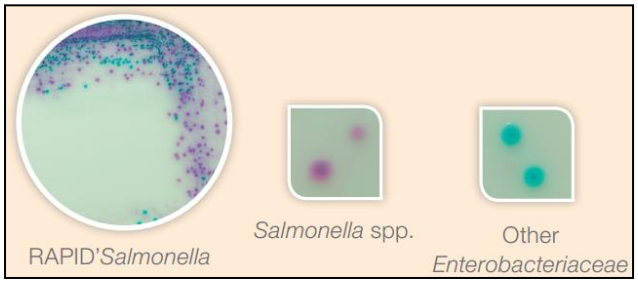
It is specifically designed to detect coagulase-positive staphylococci (CPS). With traditional media, such as Columbia CNA Agar, detection is not limited to staphylococci, so organisms like streptococci can also grow. However, Brilliance Staph 24 reduces non-target organism growth while allowing all strains of CPS to grow uninhibited, providing more accurate results and reducing the number of confirmatory tests required. Coagulase-positive staphylococci (CPS) grow as dark blue colonies on a clear background, making it much easier to read than existing Baird-Parker Agar formulations. A result is achieved in 24 hours, far quicker than the 48h required for Baird-Parker Egg Yolk Tellurite Agar (BP-EYT). Selective agents have been carefully designed to inhibit the growth of Gram-negative flora and nontarget Gram-positive organisms. Sodium pyruvate is added to improve the recovery of stressed cells, and a carefully formulated blend of peptones and growth factors ensures rapid growth of target organism. The chromogen is specifically activated by CPS which colours positive colonies dark blue, while coagulase-negative staphylococci are inhibited or remain colourless.

- *Brilliance* coliform agar:



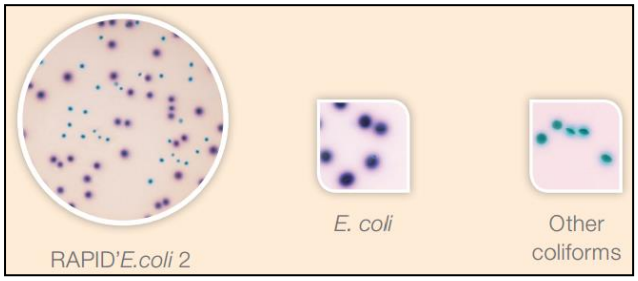
It is a differential agar used for the presumptive identification of *Escherichia coli* and coliforms from food and environmental samples. The agar base uses two enzyme substrates to differentiate between *E. coli* and other coliforms. One chromogenic substrate is cleaved by the enzyme glucuronidase, which is specific for *E. coli* and produced by approximately 97% of strains. The second chromogenic substrate is cleaved by galactosidase, an enzyme produced by the majority of coliforms. This results in purple *E. coli* colonies, as they are able to cleave both chromogenic substrates, and pink coliform colonies, as they are only able to cleave the galactosidase chromogen.

- RAPID' *Salmonella*:



The principle of RAPID 'Salmonella chromogenic medium relies on the demonstration of two enzymatic activities. *Salmonella* spp. takes the form of readily identified typical magenta colonies (detection of C8 esterase). Counter selection based on β-D-Glucosidase is used to reveal other bacteria with a different colour. As expected in the regulations, RAPID' Salmonella permits detection of motile and non-motile *Salmonella*, as well as lactose-positive *Salmonella*, *Salmonella* Typhi and *Salmonella* Paratyphi.

- RAPID' *E.coli* 2:



RAPID' *E.coli* 2 is a selective chromogenic medium used for direct enumeration without confirmation, of coliforms including *Escherichia coli*. The principle of the medium relies on simultaneous detection of two enzymatic activities: β-D-Glucuronidase (GLUC) and β-D-Galactosidase (GAL). Coliforms (GAL+/GLUC-) form blue to green colonies, *E. coli* (GAL+/GLUC+) forms violet to pink colonies.

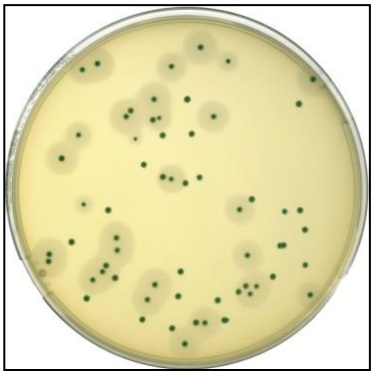
- RAPID'*L.mono*:

The RAPID' L.mono chromogenic medium specifically detects the phospholipase of *L. monocytogenes* and its inability to metabolize xylose. After 24 hours of incubation, *L. monocytogenes* forms characteristic blue colonies without a yellow halo.

- IRIS *Salmonella* agar:

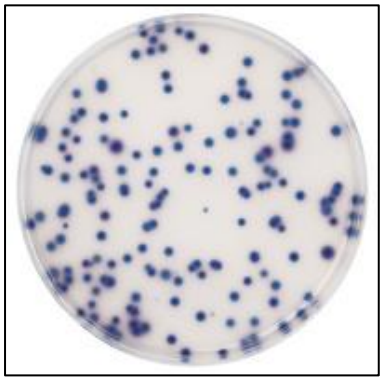
IRIS *Salmonella* Agar shows a high specificity for the detection of *Salmonellae* including atypical species and serovars, which is a source of confusion on other medium. Indeed, the detection of *Salmonella* Typhi and Paratyphi, lactose-positive *Salmonellae* (*Salmonella* Senftenberg and subspecies *S. arizonae* and *S. diarizonae*), saccharose-positive strains are ensured. The media allows the detection of non-motile serovars (*S.* Pullorum and *S.* Gallinarum) or monophasic strains. IRIS Salmonella Agar allows also the detection of strains which show a light or absence of esterasic activity on other medium (*Salmonella* Bongori, *Salmonella* Dublin and Atento, certain strains of *S. houtenae* and *S. diarizonae* subspecies). The selective agents permit the inhibition of Gram-positive and some Gram-negative bacteria. IRIS Salmonella Agar may be used in the standard methods for the detection of *Salmonellae* as second isolation medium.

- COMPASS *Listeria* agar:



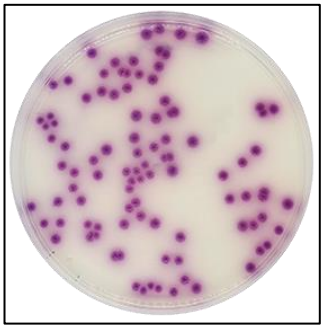
It allows the detection of blue colonies surrounded by an opaque halo, typical of *Listeria monocytogenes*. The peptones and growth factors (yeast extract, sodium pyruvate and magnesium sulfate) favor the excellent growth of *Listeria monocytogenes*. Yeast extract is also a source of vitamin B complex. Sodium chloride maintains the osmotic equilibrium of the media. *Listeria* hydrolyzes the 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside (or X-β-glucoside). The resulting product is subjected to an oxidative dimerization that forms a blue precipitate in the center of the colonies. Phosphatidyl-inositol is used as a substrate for the detection of phospholipase C of *Listeria monocytogenes*. When it is degraded, an opaque precipitate is formed around the colonies. Secondary microflora is inhibited by the association of lithium chloride and a judicious mixture of selective agents that include several antibiotics and an antifungal agent.

- Chromocult coliform agar:



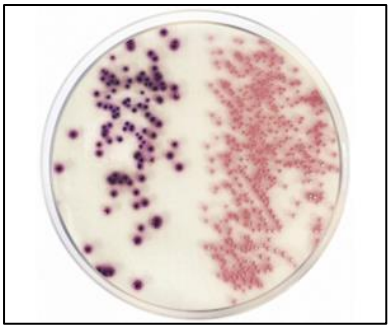
It is a selective and differential chromogenic culture medium for the microbiological analysis of water samples. Within 24 hours this medium enables the simultaneous detection, differentiation and enumeration of *E. coli* and coliform bacteria in drinking water. Counting of coliform bacteria is based on the ability of ß-D-galactosidase, an enzyme which is characteristic of coliform bacteria, to cleave the substrate Salmon-GAL. The reaction results in salmon red colored coliform bacteria colonies. Counting of *E. coli* is based on the cleavage of both the substrates X-glucuronide by ß-D-glucoronidase and Salmon-GAL by ß-D-galactosidase, an enzyme combination, which is characteristic of *E. coli*. In the presence of *E. coli* both substrates are cleaved, resulting in colonies that take on a dark blue to violet color as opposed to the salmon red of other coliform bacteria colonies. Non-coliform bacteria appear as colorless or in rare cases as turquoise colonies. The CCA formulation contains sodium heptadecylsulfate (e.g. Tergitol 7) as an inhibitor of Gram-positive bacteria with no negative effect on the growth of the targeted coliform bacteria / *E. coli*.

- CondaChrome agar for *Salmonella*:



CondaChrome culture media have in their composition a colorless chromogenic substrate, which thanks to the specific enzymatic activity of each microorganism, is degraded releasing a part of it, called chromophore, giving the colony an intense and specific color that makes it possible to identify the bacteria at first sight.

- CondaChrome agar for *E. coli*-coliforms:



The interaction of ingredients in the medium, such as peptone, sorbitol and pyruvate, grants a quick colony growth, including infectious coliforms and also permits the recovery of sublethal thermally injured coliforms. Tergitol-7 inhibits Gram positive bacteria and some Gram negative without affecting the coliform bacteria. Selectivity is enhanced by the cefsulodine and vancomycin, supplied by the supplement *E. coli*-coliformes, act against *Pseudomonas* and Gram negative, oxidase positive bacteria, enterococci and other Gram positive bacteria. Sodium chloride maintains the osmotic balance and phosphate salts act as a buffer system.

Detection of ß-glucuronidase is widely used to differentiate *E. coli*, as the enzyme is present in *E. coli* but not in another member of coliform group. The chromogenic mixture contains chromogenic substrates: Salmon-GAL and X-glucuronide. Coliform enzymes produced, ß-D-galactosidase and ß-D-glucuronidase, cleave these substrates resulting in the different coloration of bacteria colonies. The ß-D-galactosidase cleaves Salmon-GAL substrate and gives a salmon-red colour to the coliform colonies. The ß-D-glucuronidase, enzyme characteristic of *E. coli*, cleaves X-glucuronide, giving a blue colour to these colonies. *E. coli* has the two enzymes and cleaves both chromogenic substances giving dark blue to violet colonies. Total coliforms are the sum of *E. coli* colonies plus salmon-red colonies. The addition of tryptophan to the medium allows the performance of the Indole test for further *E. coli* confirmation.

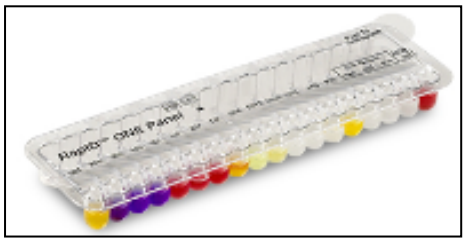
2.3. Preparation of diagnostic kits

- API (kits and reader):

The API (**A***ppereils et* ***P****rocedes d'****I****dentification*) microbial identification test system consists of an array of around 20 e.g. API 20E. Each of the mini-test tubes contains dehydrated media and reagents that are designed to detect the presence or, absence of selected biochemical pathways production, in the microbe of concern.

To perform an API test, pure microbial growth e.g. a colony from an agar plate, is mixed with sterile saline solution to form a microbial inoculum of the correct density; this is manually dispensed into each of the mini-test tubes using a sterile pipette. Some of the mini-test tubes are filled completely, while others are filled partially and overlaid with sterile mineral oil to provide anaerobic conditions. After inoculation, the strip is placed in a plastic incubation box along with some water, to prevent moisture loss from the mini-test tubes during incubation. After incubation at an appropriate temperature e.g. 37°C, for an appropriate length of time e.g.18 to 24 hours, growth and reagent utilisation is assessed by either by colorimetric, fluorescent or turbidity means - sometimes this requires the addition of extra reagents. For example, one drop of Kovac's reagent has to be added to develop the indole (IND) reaction; one drop of TDA reagent (10% ferric chloride) to develop the tryptophan deaminase (TDA) reaction; one drop of VP1 (40% potassium hydroxide) followed by one drop of VP2 (6% alpha naphthol) for the Voges Proskaueur reaction; and one drop of oxidase reagent for the cytochrome oxidase (OX) reaction. The reactions of each test tube are recorded with the aid of the manufacturer's interpretation.

- RapID ONE:



The RapID ONE System is a qualitative micromethod employing conventional and chromogenic susbtrates for the identification of medically important *Enterobacteriaceae* and other selected oxidase-negative, gram-negative bacilli isolated from food, water, human clinical specimens, etc.

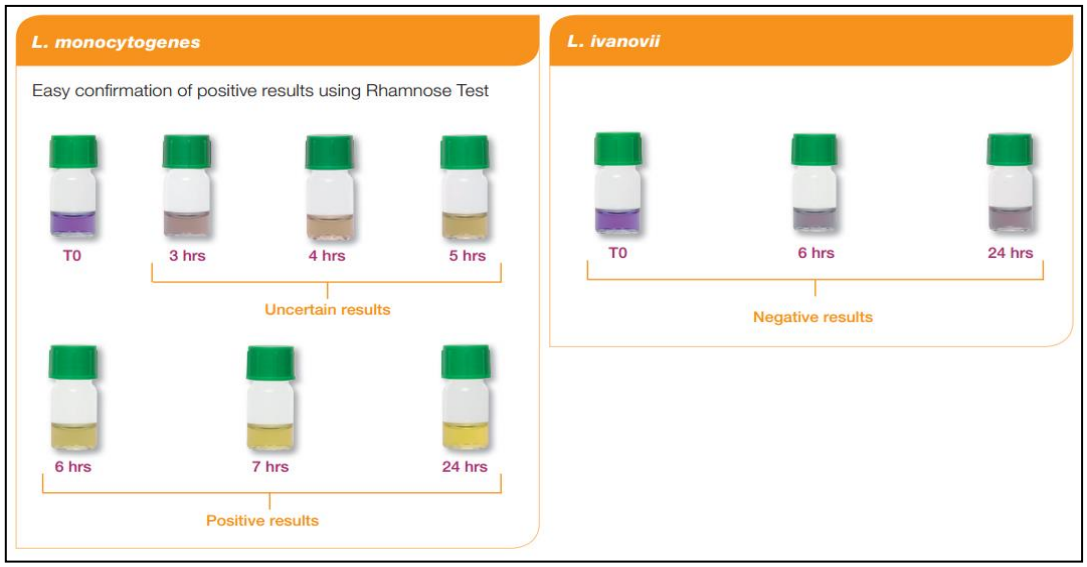
A clear plastic tray contains reagent impregnated wells, allowing simultaneous inoculation of each cavity with a predetermined volume of inoculum. A suspension of test organism in RapidID inoculation fluid is used as the inoculums which rehydrates and initiates test reactions. After incubation, each cavity is examined for reactivity by noting the development of a color. In some cases, reagents must be added to the cavities to provide a color change. The resulting pattern of positive and negative scores is used as the basis for identification of the test isolate by comparison of the test results to reactivity patterns stored in a database or through the use of a computer-generated code compendium (ERIC).

- Microbact:

The Microbact range offers a selection of self-contained, biochemical-based tests for the identification of key pathogenic bacteria, including Gram-negative bacteria, *Listeria* species and *Staphylococcal* species.

- Rhamnose test:

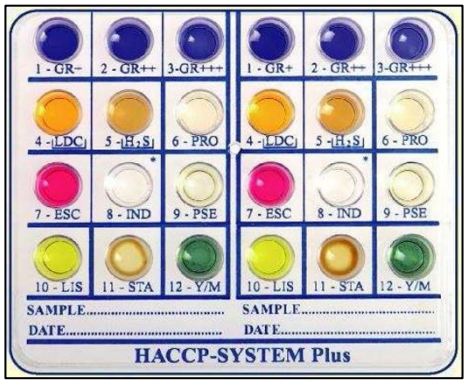
L. monocytogenes is able to produce acid from rhamnose, whereas *L. innocua, L. welshimeri*, and *L. grayi* show variable rhamnose utilization, and strains *of L. ivanovii, L. seeligeri,* and *L. marthii* are negative. Rhamnose is a naturally occurring L-6-deoxy hexose. It is present as a substituent of pectin in plant cell walls where it is periodically attached via α-1,2-glycosylic linkages to galacturonic acid. In *L. monocytogenes* serovar 1/2 strains, rhamnose is not only used as a carbon source, but also found as a decoration of the cell wall teichoic acids, and required for adsorption of A118 like bacteriophages.



- EnteroPluri-*Test*:

EnteroPluri-Test makes possible the identification of the *Enterobacteriaceae* and other gram negative, oxidase negative bacteria isolated from non-clinical samples. The identification is based on biochemical tests performed on culture media containing specific substrates. The combination of positive and negative reactions allows building up a code number that permits to identify bacteria by using the Codebook.

- HACCP System *Plus*:



It is a 24-well system containing desiccated biochemical substrates and culture media for determination of the total microbial count and for the search for and presumptive identification of microorganisms from work surfaces and equipment with flat faces. Specifically, the system provides for the search for and presumptive identification of: *Salmonella* spp., *Citrobacter* spp., *Proteus* spp., *Pseudomonas* spp., *E. coli*, *Listeria* spp., *S. aureus*, yeasts and moulds. The system is inoculated with a suspension of samples obtained from swabs of the work surfaces and equipment and is incubated at 36 °C ± 1 °C for 18-24 hours. The tests for determination of the total microbial count, and for the search for and presumptive identification of the micro-organisms present in the sample, are interpreted by assessing the change in colour of the various wells and performing a microscope examination.

2.4. Other environmental control procedures

2.4.1. Luminescence

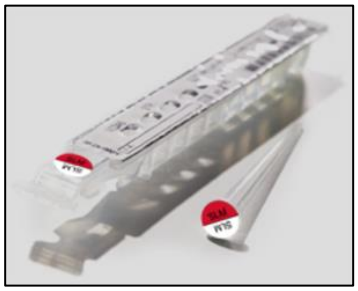
- Luminometer EnSURE (UltraSnap, AquaSnap, MicroSnap):

MicroSnap *E. coli* is a rapid test for detection and enumeration of *E. coli* bacteria. The test uses a novel bioluminogenic test reaction that generates light when enzymes that are characteristic of E. coli bacteria react with specialized substrates. The light generating signal is then quantified in the EnSURE luminometer. Depending on the required level of detection, MicroSnap *E. coli* is able to give results in the same day.

2.5. Immunological detection methods

2.5.1. ELFA

- VIDAS SPT:

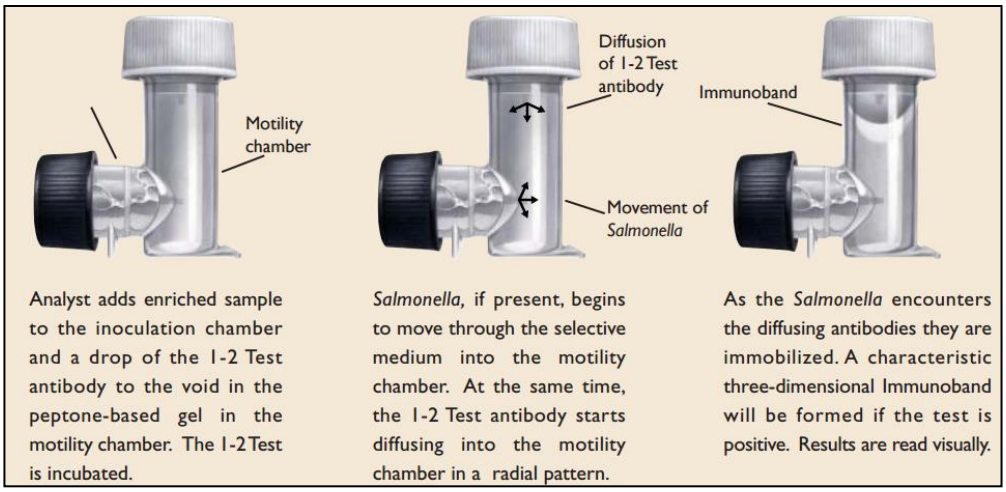


The VIDAS Up Salmonella (SPT) is a specific enzyme-linked fluorescent immunoassay performed in the automated VIDAS instrument. VIDAS SPT combines the cutting-edge phage recombinant protein technology and the ease-of-use of the VIDAS system. Providing reliable results in less than 19 hours and validated for samples of up to 375g, this innovative solution is both time-saving and cost-effective, as its speed can accelerate corrective action plans in case of contamination.

The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device. The interior of the SPR is coated with anti-salmonella antibodies adsorbed onto its surface. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips. All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.

2.5.2. Immunoprecipitation

- 1-2 Test for *Salmonella*:



The 1-2 Test is highly sensitive and specific. It uses a unique combination of a built-in selective enrichment, the immunodiffusion principle, and a proprietary preparation of antibodies to detect *Salmonella*.

2.5.3. Lateral immunomigration

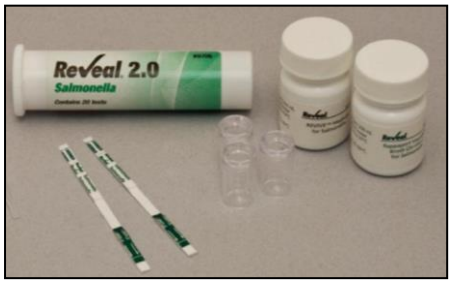
- Singlepath:

Singlepath L'mono is an immunological screening and an extremely fast confirmation test for the specific detection of *L. monocytogenes*, based on the immune flow principle and is designed in such a way that time-consuming and personnel intensive working steps for the application and interpretation of the tests are avoided.

- VIP Gold:

VIP Gold is a single-step visual immunoassay for the detection of pathogens in food and environmental samples. Each device contains a proprietary reagent system, which forms a visually apparent antigen-antibody-chromogen complex if the pathogen is present. Detects all species of *Listeria* providing a true indication of product and surface quality.

- Reveal 2.0:



Reveal 2.0 for *Salmonella* is a simple yet sophisticated lateral flow technology that is easy to perform with minimal sample touch time. This robust lateral flow technology lends itself to multiple food matrices and is scalable to any operation—from low volumes to a theoretically unlimited throughput. Reveal 2.0 for *Salmonella* continues the tradition of convenient unitized-irradiated media, which eliminates need for autoclaving. The simple assay procedure produces clear results in 15 minutes following enrichment.

3. Friday

3.1. Diagnostic kits

- O·B·I·S· *L. monocytogenes*:

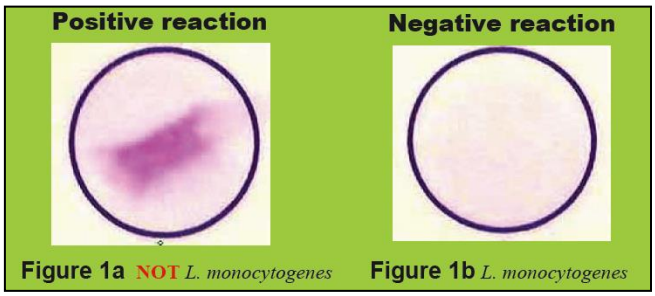
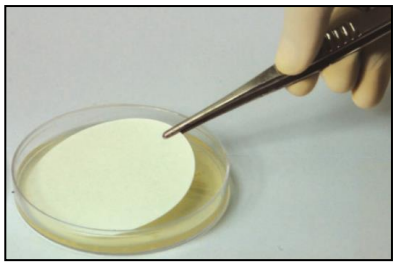


Figure 1a NOT *L. monocytogenes* Figure 1b *L. monocytogenes*

The O.B.I.S. mono test offers a rapid screening method for differentiation of *L. monocytogenes* from other *Listeria* species. This reduces the need for full biochemical identification of all suspect colonies. *Listeria* species, with the exception of *L. monocytogenes*, possess the enzyme D-alanyl aminopeptidase. Oxoid has developed a new system for aminopeptidase testing which uses a non-carcinogenic substrate. This is in

response to health concerns associated with amino acid conjugates of ß-naphthylamine4,5 as these are potent carcinogens. D-alanyl-7-amido-4-methylcoumarin (DALA) is provided as a suspension. An acidic solution of dimethylaminocinnamaldehyde is used as a colour developer. If the substrate is hydrolysed by DALAase, free 7-amino-4-methylcoumarin (7AMC) combines with the developer to produce a purple Schiff's base. Pick colonies which have typical *Listeria* morphology from selective *Listeria* isolation media such as Oxford Agar, PALCAM Agar and chromogenic Listeria Media, and streak onto a purity plate.

- ID membranes:



These membranes are for economical and rapid identification and confirmation of microorganisms in water, food, environmental and clinical samples. They find their application in various sectors in food and dairy industry, water industry, etc.

The membranes contain chromogenic substrates such as ONPG, X-Gal, or X-Glu and other substrates and indicators which serve as the basis for the differentiation by color. The target organisms are characterized by enzyme systems that metabolize the substrates and initiate the color change. The colors can then be visually detected on the membranes and show the enzymatic activity of the microorganisms which is a helpful tool for the identification of genus and species.

3.2. Other environmental control procedures

3.2.1. Luminescence

- N-Light *Listeria monocytogenes*:

It is a qualitative test method for rapid detection within 24 hours of the foodborne bacterial pathogen Listeria monocytogenes in food processing areas and equipment as part of an environmental monitoring program.
The NEMIS method is the first chemiluminescence detection system based on a novel, patented, dioxetane compound.

- Luminometers

Clean-Trace:

Single-use test that contains a swab for the collection of a sample from a surface. The swab is pre-moistened to aid in sample collection and processing. Once the test is activated, the chemical reacts with the sample collected on the swab to produce light. The amount of the light produced is proportional to the degree of potential contamination. Measurement of the light requires the use of a 3M™ Clean-Trace™ LM1 Hygiene Monitoring and Management System and the results are displayed in Relative Light Units (RLU). The higher the RLU number, the more contaminated the sample. With its unique liquid stable enzyme, the test offers superior levels of repeatability. This aspect of its performance and its incredible simplicity means customers throughout the world have put Clean-Trace at the heart of their hygiene monitoring programs.

AccuPoint Advanced:

The AccuPoint Advanced ATP Hygiene Monitoring System is a handheld device that accurately detects ATP from surfaces and rinse water samples. The three colour-coded samplers with liquid-stable chemistry are unrivalled in their accurate recovery of ATP from surfaces and rinse waters. Unlike traditional swabs utilised by other manufacturers, our samplers cover a larger surface area to extract ATP more consistently.

MVP ICON:

The MVP ICON is the first system to provide true HACCP management capabilities. Featuring advanced photon counting sensor (PCS) technology, the MVP ICON provides superior accuracy, sensitivity, and reproducibility for ATP, pH, temperature, conductivity, and concentration. The innovative design is sleek, lightweight and features an easy to use touch-screen interface.

EnSURE Touch:

The ENSURE TOUCH introduces the first luminometer with touch screen display and android style icons and interface which will feel very familiar to anybody that has used a smartphone. The easy to use interface enable the user to quickly set-up new users, test locations and test plans.

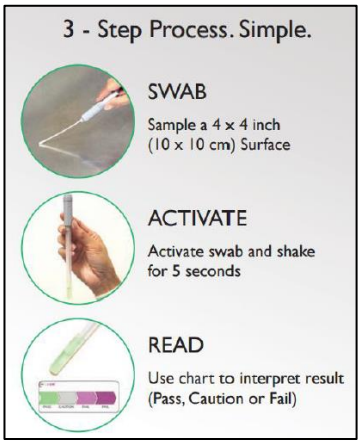
3.2.2. Colorimetry

- Contam Swab:

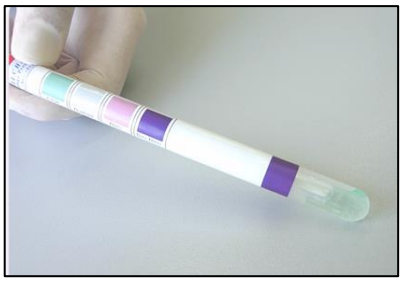
It is a swab containing a culture medium used for the detection of *Listeria* spp. directly from surfaces.

- Flash:

FLASH is a total protein visual test that rapidly detects protein residues left on food contact surfaces after cleaning. Protein is a difficult food residue to remove. Most allergens are proteins, so quick verification of surface hygiene helps minimize the risk of cross-contamination to allergen-free products. Using FLASH regularly also helps reduce the opportunity for biological contamination.



- Clean Test:

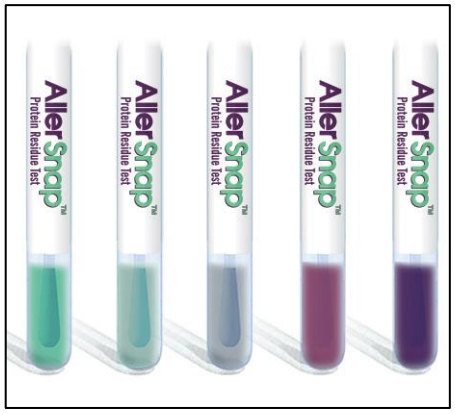


The test highlights the presence of proteinic residues and of other reducing substances on the analyzed surface. The method, based on the reaction of bicinchoninic acid (reagent A) with cupric sulfate (reagent B) in alkaline conditions, produces the complexation of Cu ions with the peptidic bonds of proteins. Such complex takes on a purple colouring directly proportional to the concentration of proteins, fats and sugars which are present on the checked surface. A pale green, colourless or clear light grey colouring assures the proper cleaning of the analysed surface. In case of a dark grey solution it is suggested to rinse the surface and/or repeat the test. However, if the solution turns to light or dark purple, a new cleaning cycle of the surface with suitable detergents is needed.

- InSite *Salmonella*:

InSite *Salmonella* is an easy-to-use, self-contained, environmental *Salmonella* test. Each device contains a liquid medium formulated with growth enhancers and chromogenic compounds selective for *Salmonella* species. Simply swab the test area and incubate! A change in color after 24-48 hours of incubation is considered presumptive positive for *Salmonella* species.

- AllerSnap:



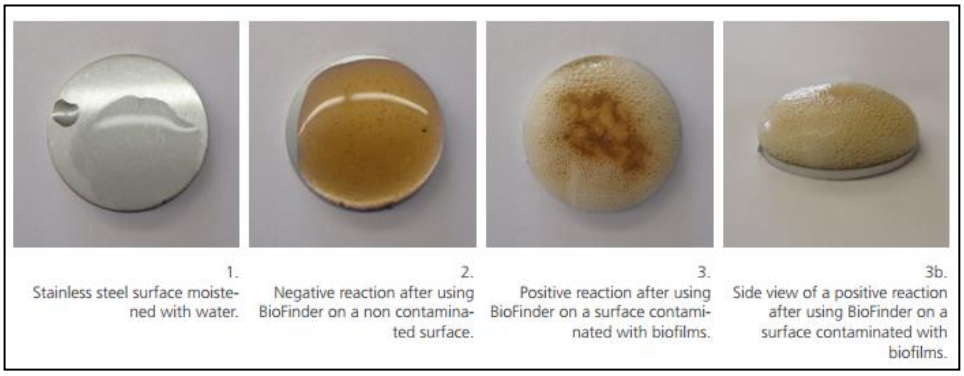
AllerSnap is an easy-to-use, quick and affordable test for verifying allergens have been removed from surfaces. By detecting all protein residues, AllerSnap screens for a broad range of allergens. This saves time and eliminates additional costs associated with running specific allergen tests.

3.2.4. Others

- BioFinder

It reacts to detect the group of microorganisms attached to surfaces, called Biofilms. It immediately reveals contaminated areas by simple visual inspection. It can be applied to the most commonly used surfaces in the food industry, such as stainless steel, polypropylene and epoxy-coated surfaces. Biofinder has advantages over other methods, with time and cost reduction being the most significant ones. It simplifies monitoring surface hygiene of industrial processes. Its formula and packaging type make it possible to treat large areas.

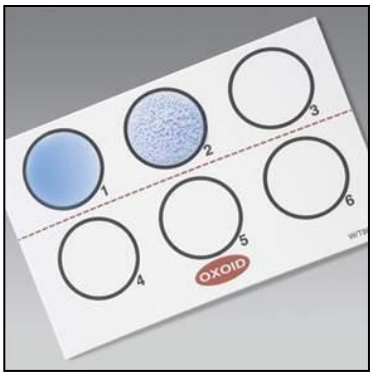
It doesn't stain or leave residues on surfaces due to its high water solubility which aids in rinsing. Thanks to its simple application and response type, technical staff is not required for handling. It is not considered hazardous to the environment, according to current legislation.



1. Stainless steel surface moistened with water.
2. Negative reaction after using BioFinder on a non contaminated surface.
3. Positive reaction after using BioFinder on a surface contaminated with biofilms.
3b. Side view of a positive reaction after using BioFinder on a surface contaminated with biofilms.

3.3. Immunological detection methods

3.3.1. Confirmation by latex agglutination



- Oxoid latex test:

A loop full of material is taken from the top of a positive indicator tube, for example, *Salmonella* Rapid Test and it is mixed with a drop of the latex test reagent. If agglutination of the latex test reagent occurs, then *Salmonella* is present in the material. Similarly, presumptive *Salmonella* colonies can be taken from an agar plate, mixed with the latex test reagent and agglutination will occur if *Salmonella* is present.

3.3.2. Lateral immunomigration

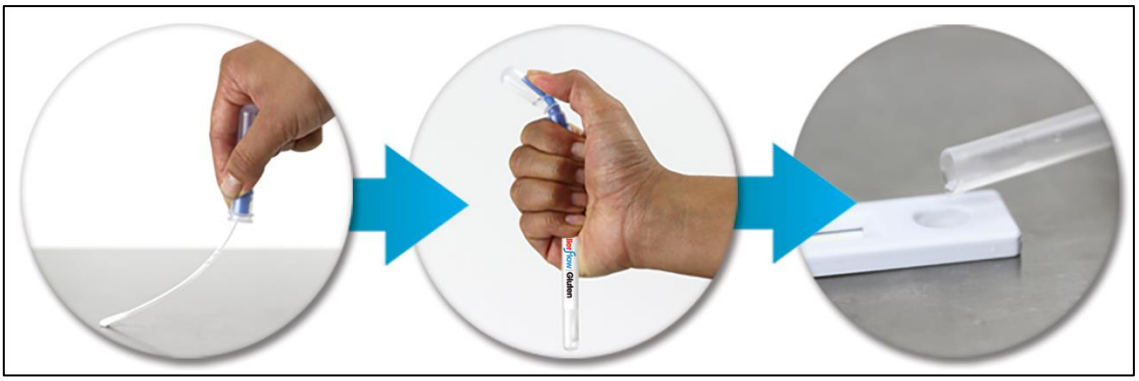
- Reveal Q+ for DON:

Reveal Q+ for DON is a single-step lateral flow immunochromatographic assay based on a competitive immunoassay format intended for the quantitative testing of DON in corn, barley, DDGS, malted barley, oats and wheat products.

- Reveal 3D for allergens (milk):

Neogen Reveal 3-D food allergen kits are easy-to-use and interpret strip tests that screen samples for the detection of trace levels of specific milk residue (casein or whey) in 10 minutes or less. The unique Reveal 3-D tests allow for rapid screening for the presence of low levels of allergen in CIP rinses and environments swabs virtually anywhere. The Reveal 3-D allergen tests utilize a 3-line readout: a control line confirms the method has been performed successfully and two further lines differentiate low & high levels of detection.

- AllerFlow Gluten:



AllerFlow Gluten is a rapid and convenient test for detection of gluten residue on surfaces as part of an allergen control program. Unlike other kits that contain several components, AllerFlow Gluten only consists of two parts – a sample collection device and a lateral flow cassette. The convenient sample collection swab device contains pre-measured extraction buffer for consistent results with minimal handling. After collection, the sample is poured directly into the lateral flow cassette fill well and results appear within 10 minutes.

- DipSensor:

One step test for the detection of beta-lactams (including cefalexin) and tetracyclines in milk

- Extenso:

In 13 minutes, EXTENSO can simultaneously detect 100+ antibiotic residues and toxins discriminated into 17 specific channels and covering all main contaminant groups such as ß-lactams, tetracyclines, aminoglycosides, lincosamides, macrolides, (fluoro) quinolones, sulfonamides, chloramphenicol, trimethoprim, colistin, melamine and even aflatoxin M1.

3.4. Molecular detection methods for pathogens (alternatives to PCR)

- Molecular Detection System (MDS):

The 3M Molecular Detection System was conceived as a molecular microbiology approach that could detect pathogens from food samples and samples taken from processing environments. The system is based on a combination of two technologies: Isothermal DNA amplification and bioluminescence detection. These two “pillar” technologies work together to provide a molecular detection method that is pure and simple.

The principle behind is that it uses multiple primers to recognize distinct regions of the genome and Bst DNA polymerase to provide continuous and rapid amplification of genetic material. Pyrophosphate ions (PPi), a by-product of the targeted DNA amplification reaction, and APS, are enzymatically converted into ATP by ATP-Sulfurylase. ATP reacts with luciferase to produce light which is detected indicating the presence of target organism DNA. Both amplification and detection occur simultaneously and continuously during the exponential phase providing real time results and a short run time.