Microbiological Testing of Foods, Beverages and Pharmaceuticals
Introduction

The consumer’s steadily growing requirements for the quality and the longer shelf life of foods and beverages must be met by the manufacturer. He cannot limit quality assurance to inspection of the final product alone, such as a bottled beverage or a prepared food product. Instead, he continuously must inspect incoming raw materials and perform in-process quality control tests throughout production if he wants to avoid later losses and customer complaints. Microbiological and aseptic testing play a significant role in such quality assurance.

In the soft drink industry the microbiological and hygienic quality including the biological stability of the products are important criteria for their assessment. The reason: just a few microbes are often all it takes to spoil large quantities of a beverage.

Although the explosive technological development has reduced the risk of contamination by spoilage microbes, the issue of shelf life has taken on new dimensions as a result of the enormous production output now possible. Quality control of bottling and filling, in terms of chemical and, above all, biological stability, must be adapted to this development by state-of-the-art test methods.

The requirements for a practical microbiological test method are that it permit quantitative and reproducible detection of trace contamination and that it can be performed efficiently and economically under routine conditions. These requirements are fulfilled optimally by the membrane filter method.

The principle of this method is based on the concentration of microorganisms from relatively large samples on the surface of the membrane filter, and on culturing these microbes on a nutrient pad or an agar culture medium.

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The Membrane Filter Method

Description
The Membrane Filter Method
A membrane filter of the appropriate pore size is placed in a filter holder, and the sample is filtered. In this process microorganisms in the test sample are retained on the filter surface by the screening action of the membrane filter.

Growth inhibitors can be removed by flushing the membrane with sterile NaCl solution after filtration. Afterwards, the membrane filter is placed on a culture medium and incubated.

For the Monitor MF-Methode the monitor is ready to use due to a pre-assembled membrane and pad inside.

The nutrient media is added from the top and sucked into the pad by a short vacuum (<1 sec.) After removal of the funnel the lid and the base fit to a petri dish.

Nutrients and metabolites are exchanged through the pore system of the membrane filter. Colonies, which have developed on the membrane filter surface during incubation, are counted and related to the sample volume.

The advantages:
- Proofen accuracy
  Compared with the direct method, considerably larger sample volumes can be tested. This concentration effect increases the accuracy of microbial detection.
- Quantitative results
  The visible colonies can be related directly to the sample volume.
- Documentation
  The membrane filter with colony growth can be filed as a permanent record of the test.

No inhibitors
Inhibitors, such as essential oils or disinfectants, can be flushed from the membrane filter after filtration.

GMP quality
Sartorius Membrane Filters are manufactured under GMP conditions, ensuring consistent quality and high reproducibility from batch to batch and within each batch.

The Culture Media
Microorganisms can be detected by different methods.

Methods involving culturing techniques and the microscope are used to detect microbes, whereas biochemical and serological techniques are commonly applied to differentiate among such organisms.

For detecting microorganisms in cultures, liquid and solid culture media are employed. Microorganisms are concentrated by growth in or on these culture media.

Quantitative detection is only possible with solid culture media because the individually developing colonies can be evaluated and counted on the surface.

The following culture media can be used for microbiological testing:

- Nutrient Pad Sets
  Nutrient Pad Sets definitely optimize the membrane filter method. They standardize microbiological test procedures, making them much more efficient. The simplify laboratory work. They help to save time and money.

  These sets are described on the following pages and certainly offer the most convenient way to use the membrane filter method.

- Absorbent pads to be wetted with culture media

- Culture media with agar or gelatin as the solidifying agent
**Direct Method**
The test sample is pipetted into a petri dish ...

... then mixed with the culture medium and incubated.

**Membrane Filter Method**
The test sample is filtered through a membrane filter.

**Standard MF method**
The membrane filter is rinsed and then placed on a culture medium – a, b, or c – and incubated.

**Monitor MF method**
The nutrient media is given from the top after filtration. After a short vacuum (< 1 sec.), the monitor is closed with the plug at the bottom. Remove the funnel, fit lid and base to a petri dish.

For further information on Sartorius Biosart 100 Monitors, please refer to the publications SLD2003e and SLD2004e.
Nutrient Pad Sets

Sartorius Nutrient Pad Sets have been used successfully in the membrane filter method for 20 years. Practical and easy to handle, they reduce labor and simplify many microbiological testing procedures.

Nutrient pads are sterile, dehydrated culture media. Once they are moistened with 3.0–3.5 ml of sterile and demineralized (or distilled) water they are ready to use immediately.

The level of moisture is optimal when an excess ring of water surrounding the pad is visible.

All Nutrient Pad Set types are supplied with the appropriate membrane filters, which are also presterilized and individually packaged. The membrane filters tailored to meet the special requirements of microbial detection are available with 47 mm or 50 mm diameters.

Nutrient pad sets (NPS) are continuously enhanced as part of our development program to adapt our products to changing application requirements. Besides the new NPS types, we have also updated our packaging design. The standard NPS box contains 100 sterile nutrient pads, each of which is individually inserted in a petri dish and sterilized. Ten each of these petri dishes are sealed in an aluminum bag. This special packaging in bags protects the sensitive formula constituents of the nutrient pads during transport and storage from fluctuations in humidity and temperature. As a result, it guarantees the high quality of our NPS throughout their entire shelf life ranging from 18 to 24 months.

How to Use Nutrient Pad Sets

It’s so easy to use Nutrient Pad Sets: NPS and go

Desinfect the working area
Cut open the packaging and remove the number of nutrient pads needed
Wet the nutrient pads with 3.5 ml sterile and distilled or demineralized water

Open the vacuum tap.
Flame the frit and close the vacuum tap
Flame the stainless steel funnel
Open the vacuum tap and flame inside the funnel. Close the vacuum tap and flame the lid.
User Benefits

Economical
Eliminates time-consuming and labor-intensive preparation of culture media (sterilization and cleaning, among others).

• After wetting with 3.5 ml destilled water NPS are ready to use: NPS and go

Simple to use
Nutrient Pad Sets can also be used in laboratories which do not have extensive microbiological equipment. Sterile water for moistening the pads can be prepared easily with a Sartorius Dosing Syringe and an attached Syringe Filter Holder (0.2 µm) or with an ampoule with sterile water.

• Everyone can use NPS

Consistent quality
During manufacture, each type of Nutrient Pad Set is compared with the corresponding agar medium with respect to their growth-promoting properties. This QA procedure ensures consistent quality and reproducible results.

• NPS are validated. In comparison of agar which is done within different deviations of amount and height NPS give always constant results

Trouble-free storage
Nutrient Pad Sets have a shelf life of 18 to at least 24 months at room temperature.

• No waste or overproduction of prepared agar media

Highly versatile
Nutrient Pad Sets can be modified by additives in the solution used to wet them; for example, Wort or Orange Serum Nutrient Pads when wetted with 5 – 8 % ethanol promote the growth of acetic-acid bacteria.

• Advanced system

Flame the forceps, shortly cool down

Take off the membrane

Place the filter on the frit of the filter holder. In case of a yellow protective disc make sure to discard it before assembling the funnel or the top part of the filter holder.

Filter the sample. Then rinse the inside of the filter holder with sterile water or physiological saline solution

Place the filter on the pad without entrapping air bubbles

Incubate the nutrient pad in the petri dish with the lid right side up (do not invert)
General Directions

General Procedure.
To obtain reliable results for microbiological tests, it is necessary to work under conditions that rule out contamination by microorganisms which distort such results.

That is why you should work near the flame of a Bunsen burner in a room protected from drafts. Before beginning with the actual procedure, spray or wash down your work area with a disinfectant (e.g., 70% alcohol).

Before use, filter holders, forceps and scissors should be sterilized by one of the standard methods, such as flaming for routine tests.

How to Handle Microorganisms
Microorganism cultures must always be handled as carefully as if they contained pathogens.

Working with microorganisms is not dangerous if the following safety rules are observed:

Wash your hands thoroughly before and after working in a laboratory.

Do not eat or drink in a laboratory.

Do not touch bacterial matter with your hands.

Never pipet bacteria suspensions with your mouth. Always use mechanical aids for pipetting (e.g., Peleus ball).

Before and after use, inoculating loops and wires must be sterilized by flaming until they glow red-hot.

All laboratory equipment which has come in contact with bacteria must be sterilized.

To protect people and animals from contagious diseases or poisoning, living cultures have to be destroyed before cleansing or disposing of the containers. One method is to coat them thoroughly with disinfectants or to autoclave them in suitable containers.

Sartorius Nutrient Pads are participating regularly at official inter-laboratory tests for the microbiological investigation of drinking water according to the New European Drinking Water Guideline. This certificate of the "Niedersächsischen Landesgesundheitsamt" in Aurich (public health agency, Lower Saxony) quote a reference for the passed tests with good success.
**Description and Typical Growth Evaluation Results**

1. **Total colony count**

**Caso NPS**
Type 14063

Soybean-Casein Digest medium for isolating microorganisms and for determining the total CFU count. Dehydrated culture medium for cultivating microorganisms in pharmaceuticals, cosmetics, raw materials, water (general quality), waste water, foods and other products.

**References:**

**Incubation Conditions*: up to 5 days at 32.5±2.5 °C

**Evaluation and Typical Results:**
Predominantly bacteria of different sizes, shapes and colors. Remarks: Depending on the microbes to be detected, this medium can be converted into a selective one by mixing the wetting liquid with additives before moistening the pad. When 10% serum is added to the wetting liquid a number of fastidious pathogenic bacteria like the genera Pneumococcus, Neisseria, Streptococcus, Corynebacterium, Erysipelothrix and Brucella are able to grow on the medium.

**R2A NPS**
Type 14084

Low nutrient medium for the enumeration of heterophilic organisms in treated potable water and highly purified water. The optimal growth medium for bacteria which have adapted to the particular living conditions of water low in nutrients. Dehydrated culture medium for cultivating microorganisms in water for pharmaceutical purpose, water (general quality), waste water and other products.

**References:**
APHA (water), EP, ISO 7704 and Internal SOPs.

**Incubation Conditions*: 48–72 h at 35±2 °C; 5–7 days at 20±2 °C

**Evaluation and Typical Results:**
Predominantly bacteria grow on this medium. Their colonies are of different size and color, most of them are white or colorless. Remarks: Stressed and chlorine-tolerant bacteria are stimulated by this medium in combination with lower incubation temperatures and longer incubation time.

**Standard TTC NPS**
Type 14055; 14005

Meat extract-peptone medium for determining the total CFU count; formulated following the “APHA (water)”, 1998, and modified by the addition of TTC. Dehydrated culture medium for cultivating microorganisms in raw materials, water (general quality), waste water, beverages, beer, foods and other products.

**References:**
APHA (water), ISO 7704, VLB and Internal SOPs.

**Incubation Conditions*: 2–5 days at 30±2 °C

**Evaluation and Typical Results:**
Predominantly bacteria grow on this medium. The majority of their colonies are stained red by TTC reduction.

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* The incubation conditions are recommended by Sartorius. They may be varied according to the type of samples in compliance with the reference standard or customer’s requirements.
1. Total colony count

**Standard NPS**
Type 14064

Meat extract-peptone medium for determining the total CFU count; formulated following the “APHA (water)”, 1998. Dehydrated culture medium for cultivating microorganisms in raw materials, water (general quality), waste water, beverages, beer, foods and other products.

**References:**
APHA (water), ISO 7704, VLB and Internal SOPs.

**Incubation Conditions***:
2–5 days at 30±2°C

**Evaluation and Typical Results:**
Predominantly bacteria grow on this medium. The morphology and color of their colonies vary.

**NEW! TGE NPS**
Type 14076

Total count medium for isolating microorganisms and for determining the total CFU count. Dehydrated culture medium for cultivating microorganisms in raw materials, water (general quality), waste water, beverages, soft drinks, concentrates, foods and other products.

**References:**
APHA (dairy), APHA (food), APHA (water), API, ISO 7704 and Internal SOPs.

**Incubation Conditions***:
2–5 days at 30±2°C

**Evaluation and Typical Results:**
On this medium predominantly colonies of bacteria grow that can have different size and colors.

**NEW! Yeast extract NPS**
Type 14090

For the detection of the total count of aerobic heterotrophic bacteria. Dehydrated culture medium for cultivating microorganisms in water (general quality) and other products.

**References:**
EG 98/83, HMSO, ISO 6222, ISO 7704, ISO 8199 and Internal SOPs.

**Incubation Conditions***:
44±4 h at 36±2°C; 68±4 h at 22±2°C

**Evaluation and Typical Results:**
Predominantly bacteria grow on this medium. The majority of all colonies are colorless.

* The incubation conditions are recommended by Sartorius. They may be varied according to the type of samples in compliance with the reference standard or customer’s requirements.
2. E. coli and coliforms, Enterobacteria

**Chromocult NPS**
Type 14087

For the detection of total coliforms and *Escherichia coli*. Dehydrated culture medium for cultivating microorganisms in raw materials, water (general quality), waste water, beverages, foods and other products.

**References:**
ISO 7704, Journal Food Protection, ZenHyg (journal of hygiene) and Internal SOPs.

**Incubation Conditions**: 24 h at 36±1° C

**Evaluation and Typical Results:**
E. coli develops dark-blue to violet colonies, other coliforms red to pink colonies. Other gram-negative colonies are colorless, a few with β-Glucuronidase activity are light blue to turquoise. Remarks: To confirm E. coli give one drop of Kovacs indole reagent on each dark blue colony. Cherry red color after a few seconds is a positive reaction.

**ECD NPS**
Type 14082

Selective culture medium for detecting and identifying *Escherichia coli*. Bile salt inhibits the accompanying flora of microbes not living in the intestine.
Dehydrated culture medium for cultivating microorganisms in raw materials, water (general quality), waste water, beverages, foods and other products.

**References:**
APHA (water), DIN 10110, EG 98/83, ISO 7704, ISO 8199, ISO 9308-1 [2001], LMBG, USDA and Internal SOPs.

**Incubation Conditions**: 18–24 h at 37±1° C or acc. to ISO 9308-1

**Evaluation and Typical Results:**
Colonies that show light blue fluorescence under UV light indicate E. coli; confirmation with a drop of KOVÁCS indole reagent is required, a positive reaction is shown a by cherry color after a few seconds. Remarks: This medium can be used for the rapid detection of *Escherichia coli* acc. to ISO 9308-1.

**Endo NPS**
Type 14053; 14003

Selective medium for detecting and enumerating E. coli and coliform bacteria.
Dehydrated culture medium for cultivating microorganisms in raw materials, water (general quality), natural water, waste water, beverages, soft drinks, concentrates, fruit juice, sugar, sugar products, foods and other products.

**References:**
APHA (dairy), APHA (food), APHA (water), DGHM, ISO 7704, ISO 9308-1 [1990], MTVO, USDA and Internal SOPs.

**Incubation Conditions**: 24±2 h at 36±2° C or acc. to ISO 9308-2 [1990]

**Evaluation and Typical Results:**
E. coli form red colonies with a metallic sheen and a red dot at the underside of the membrane. Other coliforms grow as dark to light red colonies without metallic sheen. Colorless colonies of lactose-negative bacteria are not counted.

*The incubation conditions are recommended by Sartorius. They may be varied according to the type of samples in compliance with the reference standard or customer’s requirements.*
2. E. coli and coliforms, Enterobacteria

**MacConkey NPS**
Type 14097

For the isolation and differentiation of coliform bacteria and other enterobacteriaceae. Dehydrated culture medium for cultivating microorganisms in pharmaceuticals, cosmetics, raw materials, water (general quality), natural water, waste water, beverages, soft drinks, concentrates, fruit juice, foods and other products.

**References:**
APHA (dairy), APHA (food), APHA (water), AOAC, DAB, DIN 38411, DGHM, EP, ISO 7704, LMBG, MTVO, USDA, USP and Internal SOPs.

**Incubation Conditions**: 18–24 h at 36±2°C

**Evaluation and Typical Results:**
Escherichia coli forms large red or reddish colonies, coliform microbes form large pink, sometimes slimy colonies, lactose-negative enterobacteria form colorless colonies. Gram-positive microbes are inhibited.

**m FC NPS**
Type 14068

For the detection of E. coli and faecal coliform bacteria according to Geldreich et al. Dehydrated culture medium for cultivating microorganisms in raw materials, water (general quality), waste water, beverages, foods and other products.

**References:**
APHA (food), APHA (water), AOAC, FDA, ISO 7704, ISO 9308-1 [1990], USDA and Internal SOPs.

**Incubation Conditions**: 20±4 h at 36±2°C (44±1°C water bath)

**Evaluation and Typical Results:**
E. coli and coliform bacteria form blue colonies with a blue surrounding. This color is dark blue at faecal coliforms with strong lactose fermentation and lighter blue for non-faecal coliforms with weaker lactose fermentation. Lactose-negative bacteria grow with different colors and are not evaluated. Remarks: High incubation temperatures largely suppress the non-faecal coliforms.

**Remarks:**
High incubation temperatures largely suppress the non-faecal coliforms.

**Teepol NPS**
Type 14067

Lauryl Sulphate medium for the detection of E. coli and faecal coliform bacteria according to Burman, N.P. (1967). Dehydrated culture medium for cultivating microorganisms in water (general quality), waste water, beverages, foods and other products.

**References:**
AFNOR, APHA (water), BS, FDA, ISO 7704, ISO 9308-1 [1990], USDA and Internal SOPs.

**Incubation Conditions**: 18–24 h at 36±1°C

**Evaluation and Typical Results:**
E. coli and coliform bacteria form 1–2 mm diameter yellow colonies surrounded by a yellow zone. Non-lactose fermenting bacteria develop red or colorless colonies without yellow zone.

*The incubation conditions are recommended by Sartorius. They may be varied according to the type of samples in compliance with the reference standard or customer’s requirements.*
3. Other Faecal bacteria

Tergitol TTC NPS
Type 14056; 14006

Selective and differential medium for the detection and enumeration of coliform bacteria and E. coli according to Pollard; modified acc. to Chapman. Dehydrated culture medium for cultivating microorganisms in raw materials, water (general quality), waste water, beverages, foods and other products.

References:

Incubation Conditions*:
21±3 h at 36±2° C

Evaluation and Typical Results:
E. coli forms yellow colonies with a yellow surrounding, Enterobacter orange colonies with a small yellow surrounding. Coliform colonies are red and have a yellow dot under the membrane filter. According to ISO 9308-1 all colonies that show yellow color under the membrane filter are counted as positive. Remarks: Tergitol 7 inhibits Gram positive colonies and minimizes the swarming of Proteus.

Azide NPS
Type 14051

For the detection and enumeration of intestinal enterococci according to Slanetz and Bartley. Dehydrated culture medium for cultivating microorganisms in raw materials, water (general quality), natural water, waste water, beverages, foods and other products.

References:
APHA (food), APHA (water), EG 98/83, HMSO, ISO 7704, ISO 7899-2, ISO 8199, LMBG, MTVO and Internal SOPs.

Incubation Conditions*:
44±4 h at 36±2° C

Evaluation and Typical Results:
Enterococci form red, pink or reddish brown colonies with a diameter of 0.5–2 mm. Remarks: Enterococci are considered to be indicator organisms of faecal contamination. They are less sensitive to chemical effects than are E. coli organisms and are therefore longer detectable, for instance in waste water and in chlorinated water.

Bismuth Sulfite NPS
Type 14057

Selective culture medium according to Wilson and Blair for isolating Salmonella thyphii and other salmonellae. Dehydrated culture medium for cultivating microorganisms in pharmaceuticals, cosmetics, raw materials, water (general quality), waste water, foods and other products.

References:
AFNOR, APHA (dairy), APHA (food), AOAC, DGHM, FDA, HMSO, ISO 6579 [1981], ISO 7704, USDA, USP and Internal SOPs.

Incubation Conditions*:
up to 48 h at 36±2° C

Evaluation and Typical Results:
Most salmonellae form light colored colonies with brown to black centers surrounded by a black zone with a metallic sheen ("fish eye"). Some Salmonella species develop uniformly dark brown to black colonies which may lack the typical zone. Remarks: If a very slight contamination with salmonellae is suspected, prepare a selective enrichment culture and subsequently streak the sample with an inoculation loop on a membrane filter that has been placed on the pre-wetted NPS.

* The incubation conditions are recommended by Sartorius. They may be varied according to the type of samples in compliance with the reference standard or customer's requirements.
4. Non-faecal, pathogenic bacteria

Cetrimide NPS
Type 14075

For the detection and enumeration of Pseudomonas aeruginosa according to Lowbury. Dehydrated culture medium for cultivating microorganisms in pharmaceuticals, cosmetics, raw materials, water (general quality), waste water, foods and other products.

References:
APHA (water), AOAC, ASM, DIN 38411, EG 98/83, EP, FDA, ISO 7704, ISO 8199, ISO 12780, USP and Internal SOPs.

Incubation Conditions*:
48 ±4 h at 37 ±1°C

Evaluation and Typical Results:
Pseudomonas aeruginosa forms blue, blue-green or yellow-green colonies with 1–2 mm diameter and blue zones. The colonies produce pyocyanin and fluorescein and show fluorescence in UV-light. Other Pseudomonads develop colonies with different colors. Remarks: Further tests are necessary for definitive identification of Ps. aeruginosa.

Chapman NPS
Type 14074

Mannitol salt medium according to Chapman, modified for detecting and isolating pathogenic Staphylococci. Dehydrated culture medium for cultivating microorganisms in pharmaceuticals, cosmetics, raw materials, water (general quality), waste water, foods and other products.

References:
APHA (food), AOAC, DGHM, FDA, HMSO, ISO 7704, USP and Internal SOPs.

Incubation Conditions*:
up to 3 days at 36±2°C

Evaluation and Typical Results:
Staphylococcus aureus forms yellow colonies with a yellow surrounding (mannitol-positive). Other Staphylococci grow without zones of color change. Most other bacteria are inhibited.

Lysine NPS
Type 14061

Selective medium for isolating and enumerating “wild yeasts” in breweries acc. to Morris and Eddy. Dehydrated culture medium for cultivating microorganisms in beer and other products.

References:
Journal Institute of Brewing, VLB and Internal SOPs.

Incubation Conditions*:
2–5 days at 25–28°C

Evaluation and Typical Results:
Only “wild yeasts” (not belonging to the genus Saccharomyces) which utilize lysine as sole source of nitrogen grow on this medium, they form white or cream colored colonies; brewery culture yeasts grow not at all or very poorly.

5. Yeasts and molds

Lysine NPS
Type 14061

Selective medium for isolating and enumerating “wild yeasts” in breweries acc. to Morris and Eddy. Dehydrated culture medium for cultivating microorganisms in beer and other products.

References:
Journal Institute of Brewing, VLB and Internal SOPs.

Incubation Conditions*:
2–5 days at 25–28°C

Evaluation and Typical Results:
Only “wild yeasts” (not belonging to the genus Saccharomyces) which utilize lysine as sole source of nitrogen grow on this medium, they form white or cream colored colonies; brewery culture yeasts grow not at all or very poorly.
5. Yeasts and molds

**Malt extrac NPS**
Type 14086
For the isolation and enumeration of yeasts and molds.
Dehydrated culture medium for cultivating microorganisms in beverages, wine, soft drinks, concentrates, fruit juice, foods and other products.

**Sabouraud NPS**
Type 14069
For the cultivation and enumeration of yeasts and molds.
Dehydrated culture medium for cultivating microorganisms in pharmaceuticals, cosmetics, raw materials, water (general quality), waste water and other products.

**Schaufus Pottinger (m Green yeast and mold) NPS**
Type 14070; 14072; 14080; 14083.
M Green Yeast and Mold medium for the detection of yeasts and molds according to Schaufus and Pottinger.
Dehydrated culture medium for cultivating microorganisms in wine, soft drinks, concentrates, sugar, sugar products and other products.

References:
APHA (food), AOAC, IFU and Internal SOPs.

Incubation Conditions*:
up to 3 days at 25±2° C or 7 days at 30±2° C

Evaluation and Typical Results:
Yeasts normally develop smooth white, rarely colored colonies. Molds generally form velvety or fluffy, cotton-like colonies that are white during the early growth phase and later, after conidiospore formation, of various colors. Remarks: The low pH of this medium suppresses the growth of most bacteria. This medium is available with two different types of membrane filters.

References:
APHA (food), AOAC, EP, USP and Internal SOPs.

Incubation Conditions*:
up to 5 days at 20–25° C

Evaluation and Typical Results:
Yeasts usually develop smooth white or colored colonies. Molds form velvety or fluffy, cotton-like colonies that are white in the early growth phase and may take various colors after conidiospore production.

Remarks: The low pH of this medium suppresses the growth of most bacteria. This medium is available with various types of membrane filters: 3 different pore sizes and 2 different colors.

References:
Internal SOPs.

Incubation Conditions*:
2–7 days at 25–30° C

Evaluation and Typical Results:
Molds develop velvety or fluffy whitish or greenish colonies which can get various colors after conidiospore production. Yeasts have a smooth surface. Acid forming sugar fermenters are whitish to yellow, non-acid formers are, by contrast, greenish to blue-green. Remarks: The low pH suppresses the growth of most bacteria. This medium is available with various types of membrane filters: 3 different pore sizes and 2 different colors.

* The incubation conditions are recommended by Sartorius. They may be varied according to the type of samples in compliance with the reference standard or customer’s requirements.
6. Product-spoiling microorganisms

**NEW! Wallerstein (WL Nutrient) Type 14089**

For the detection and enumeration of the microbiological flora of brewing and fermentation processes acc. to Green and Gray (1950). Dehydrated culture medium for cultivating microorganisms in beverages, beer, wine, soft drinks, concentrates, fruit juice and other products.

**References:**
ISO 7704 and Internal SOPs.

**Incubation Conditions**: Up to 14 days at 25–30°C aerobic or anaerobic depending on the target of the investigation.

**Evaluation and Typical Results:**
Yeasts usually grow as yellowish green colonies. Molds generally form velvety or fluffy cotton-like colonies that look white in the early growth phase and may take various colors after conidiospore production. Bacteria grow very slowly and their colonies are of different size and color. Remarks: The addition of 0.004 g/l cycloheximide to the wetting solution make the medium selective for lactic acid bacteria.

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**Wort NPS Type 14058; 14008**

For the detection and determination of yeasts and molds. Dehydrated culture medium for cultivating microorganisms in raw materials, beverages, beer, wine, soft drinks, concentrates, foods and other products.

**References:**
VLB and Internal SOPs.

**Incubation Conditions**: 2–5 days at 25–30°C

**Evaluation and Typical Results:**
Yeasts usually develop smooth white or colored colonies. Molds generally form velvety or fluffy cotton-like colonies that look white in the early growth phase and may take various colors after conidiospore production.

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**Glucose Tryptone NPS Type 14066**

For the enumeration of mesophilic and thermophilic bacteria, especially "flat-sour" microorganisms in canned foods. Tight-fitting, special petri dishes for microaerophilic incubation. Dehydrated culture medium for cultivating microorganisms in fruit juice, sugar, sugar products, foods and other products.

**References:**
APHA (dairy), APHA (food), AOAC, ICUMSA, IFU, ISO 7704, NCA and Internal SOPs.

**Incubation Conditions**: 48h at 55±2°C or up to 3days at 31±1°C

**Evaluation and Typical Results:**
Microorganisms that ferment glucose and produce acid grow as yellowish green colonies. "Flat-sour" colonies have a diameter of 2–5 mm, a yellowish-green color and are surrounded by a yellow zone. Remarks: For the incubation at 55 degrees Celsius the petri dishes must be placed into a moist chamber.

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**Saccharomyces cerevisiae**

**Saccharomyces cerevisiae**

**Bacillus coagulans**, the "flat sour" colony

**Lactobacillus plantarum**

**Yeast and molds from spoiled beer**

**Mixed culture from canned vegetables**

*The incubation conditions are recommended by Sartorius. They may be varied according to the type of samples in compliance with the reference standard or customer’s requirements.*
6. Product-spoiling microorganisms

**Jus de Tomate (Tomato Juice) NPS**
Type 14079

For the detection of product spoiling lactic acid bacteria especially Oenococcus oeni acc. to Dubois, Bindan and Lafon-Lafourcade. Tight-fitting, special petri dishes for micro-aerophilic incubation. Dehydrated culture medium for cultivating microorganisms in wine, fruit juice and other products.

References:
ISO 7704, Lanaridris & Lafon-Lafourcade and Internal SOPs.

**Incubation Conditions**: 4–6 days (up to 8 days) at 28–30° C

**Evaluation and Typical Results**:
Lactobacilli form compact, whitish to slightly yellowish colonies with 1–3 mm diameter. Pediococci develop somewhat smaller colonies with approx. 1 mm diameter that later get a whitish to slightly brownish color. Oenococcus oeni grows as colorless to whitish colonies with a diameter smaller than 1 mm. Remarks: This medium must be incubated under anaerobic to micro-aerophilic conditions.

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**Orange Serum NPS**
Type 14062; 14096

For the isolation and enumeration of acid-tolerant microorganisms. Dehydrated culture medium for cultivating microorganisms in raw materials, water (general quality), waste water, wine, soft drinks, concentrates, fruit juice, foods and other products.

References:
APHA (water), IFU, ISO 7704, MPP (packaging staff) and Internal SOPs.

**Incubation Conditions**: up to 3d at 30±2° C aerobic or anaerobic

**Evaluation and Typical Results**:
Only acid-tolerant microorganisms can grow on this medium such as lactic acid bacteria (Lactobacillus, Pediococcus etc.), acetic acid bacteria, yeasts and molds. Remarks: This medium is available with pH 5,5 and with pH 3,2.

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**VLB-S7-S NPS**
Type 14059

For the detection of pediococci and lactobacilli according to Emes; modified acc. to Rinck and Wackerbauer. Dehydrated culture medium for cultivating microorganisms in beer and other products.

References:
EBC, ISO 7704, MEBAC, VLB and Internal SOPs.

**Incubation Conditions**: 5–7 days at 25–28° C, anaerobic

**Evaluation and Typical Results**:
Pediococci ("Sarcina") develop round pale green colonies with smooth peripheries and approx. 1 mm in diameter. Lactobacilli grow as slightly rounded, irregularly lobed colonies with approx. 2 mm in diameter which are initially light green and later dark green. Remarks: This medium must be incubated under anaerobic to microaerophilic conditions.

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* The incubation conditions are recommended by Sartorius. They may be varied according to the type of samples in compliance with the reference standard or customer's requirements.
Troubleshooting Guide

Failure to follow the directions may lead to unsatisfactory results listed below:

1. Inhibited growth, tiny colonies
   - pad too dry: not enough water used

2. Colonies run
   - pad too wet, water film on the membrane filter: too much water used.
   - Colonies of motile microbes (such as Bacillus or Proteus) tend to run even though the water dosage is correct. To prevent this, add NaCl or an emulsifier.

3. Contamination from underneath
   Inhibited colony growth, excess ring of liquid cloudy, often including discoloration of the pad:
   - membrane placed with grid facedown on the pad instead of faceup
   - contamination during rehydration (by airborne microbes, by contact or by contaminated water)
   - contamination during preparation
   - microbes rinsed off the membrane filter by incomplete vacuum filtration of the sample or rinse liquid or by tilting the prepared petri dish
   - contaminated filter support
   - contaminated forceps

4. Growth on one side only
   - petri dish slanted in the incubator

5. Too profuse or too sparse growth (optimum microbial number between 20 and 200 per filter)
   - wrong dilution selected or sample inadequately mixed with the diluent.

6. Non-uniform growth
   - sample volume less than 5 ml filtered without adding sterile NaCl-buffersolution as a diluent or sample volume inadequately mixed with the diluent.

Membrane Filters for Use on Agar Plates or on Adsorbent Pads

If agar plates or absorbent pads to be wetted with liquid culture medium are used instead of Nutrient Pad Sets, we recommend Sartorius cellulose nitrate (cellulose ester) membrane filters. These membranes are offered in a choice of three different colors to suit your specific test application, and provide a high-contrast background. For simple evaluation of the results, a grid divides the filtration area into 130 squares, each measuring 3.1x3.1 mm. Naturally, the membrane filters must be free of microbes. For this purpose, they can be boiled or autoclaved. However, it is more convenient to order the membrane filters individually packed and sterilized. The certificate included in every package documents the quality assurance tests as well as the compliance of the 0.45 µm membrane filters with ISO 7704.

Cellulose nitrate prefilters

11301, a white membrane filter with a pore size of 8 µm is used as a prefilter in a special prefilter attachment (16807) for bacteriological analyses. It retains coarse suspended particles, whereas it allows microorganisms to pass through. These microbes are trapped on the surface of the underlying bacteria-retentive membrane filter.

Order no.: 11301-047ACN and 11301-050ACN

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Weman NPS
Type 14065

For the detection and determination of slime-forming mesophilic bacteria according to Weman, modified acc. to Lorenz. Dehydrated culture medium for cultivating microorganisms in soft drinks, concentrates, sugar, sugar products and other products.

References:
ICUMSA, ISO 7704 and Internal SOPs.

Incubation Conditions*:
2–3 days at 25–30° C

Evaluation and Typical Results:
The colonies of slime-forming mesophilic bacteria are smooth, round, usually colorless and transparent or translucent. Some have a diameter greater than 5 mm.

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Mixed culture from sugar syrup

Leuconostoc mesenteroides
Membrane Filters for Use on Agar Plates or on Absorbent Pads

For detection of bacteria in dyed media.

- White membrane with black grid
  - Pore size: 0.45 µm
    - Pkg. size: 47, 100
    - Order no.: 11406-47-ACN*
    - 47, 1,000
    - 11406-47-ACR*
    - 50, 100
    - 11406-50-ACN*
    - 50, 1,000
    - 11406-50-ACR*

- 0.65 µm
  - Pkg. size: 47, 100
  - Order no.: 11405-47-ACN*
  - 50, 100
  - 11405-50-ACN*

- 0.8 µm
  - Pkg. size: 47, 100
  - Order no.: 11404-47-ACN*
  - 50, 100
  - 11404-50-ACN*

- 1.2 µm
  - Pkg. size: 47, 100
  - Order no.: 11403-47ACN*
  - 50, 100
  - 11403-50ACN*

For detecting yeasts and molds.

- Green membrane with dark green grid
  - Pore size: 0.45 µm
    - Pkg. size: 47, 100
    - Order no.: 13806-47-ACN*
    - 47, 1,000
    - 13806-47-ACR*
    - 50, 100
    - 13806-50-ACN*
    - 50, 1,000
    - 13806-50-ACR*

- 0.65 µm
  - Pkg. size: 47, 100
  - Order no.: 13805-47-ACN*
  - 50, 100
  - 13805-50-ACN*

- 0.8 µm
  - Pkg. size: 47, 100
  - Order no.: 13804-47-ACN*
  - 50, 100
  - 13804-50-ACN*

- 8 µm
  - Pkg. size: 47, 100
  - Order no.: 13001-47-N (non-sterile)
  - 50, 100
  - 13001-50-N (non-sterile)

For detection of yeasts.

- Grey membrane with white grid
  - Pore size: 0.45 µm
    - Pkg. size: 47, 100
    - Order no.: 13006-47-ACN*
    - 47, 1,000
    - 13006-47-ACR*
    - 50, 100
    - 13006-50-ACN*
    - 50, 1,000
    - 13006-50-ACR

- 0.65 µm
  - Pkg. size: 47, 100
  - Order no.: 13005-47-ACN*
  - 50, 100
  - 13005-50-ACN*

- 0.8 µm
  - Pkg. size: 47, 100
  - Order no.: 13004-47-ACN*
  - 50, 100
  - 13004-50-ACN*

- 8 µm
  - Pkg. size: 47, 100
  - Order no.: 13001-47-N (non-sterile)

NEW! HighFlow

The special pore structure of the new 0.45 µm HighFlow membrane filters allow shorter filtration times due to higher flow rates and throughputs.

As every Sartorius 0.45 µm membrane filter lot these membranes are also tested and released according to ISO 7704.

* Also available as a non-sterile version.

To order boxes with 100 pcs, replace ACN with N and for boxes of 1,000 pcs, replace ACR with R.
### Typical Application Examples

<table>
<thead>
<tr>
<th>Product</th>
<th>Detection and enumeration of...</th>
<th>Nutrient pad type</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beer</strong></td>
<td>Lactobacilli and Pediococci and other beer spoiling organisms</td>
<td>VLB-S7-S</td>
</tr>
<tr>
<td>Total colony count</td>
<td>Standard, Standard TTC</td>
<td></td>
</tr>
<tr>
<td>Wild yeasts</td>
<td>Lysine</td>
<td></td>
</tr>
<tr>
<td>Yeasts and molds</td>
<td>Malt Extract*, Wallerstein, Wort</td>
<td></td>
</tr>
<tr>
<td><strong>Foods</strong></td>
<td>Acid-tolerant microorganisms</td>
<td>Orange Serum</td>
</tr>
<tr>
<td>Enterobacteria, E. coli and coliforms</td>
<td>Chromocult, ECD, Endo, (MacConkey), m FC, Teepol, Tergitol TTC</td>
<td></td>
</tr>
<tr>
<td>Enterococci, Streptococcus faecalis</td>
<td>Azide</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Cetrimide</td>
<td></td>
</tr>
<tr>
<td>Salmonellae</td>
<td>Bismuth Sulfite</td>
<td></td>
</tr>
<tr>
<td>Staphylococci, Staphylococcus aureus</td>
<td>Chapman</td>
<td></td>
</tr>
<tr>
<td>Thermophilic spore formers and mesophilic bacteria</td>
<td>Glucose Tryptone</td>
<td></td>
</tr>
<tr>
<td>Total colony count</td>
<td>Caso, Standard, Standard TTC, TGE</td>
<td></td>
</tr>
<tr>
<td>Yeasts and molds</td>
<td>Malt extract, Wort</td>
<td></td>
</tr>
<tr>
<td><strong>Fruit juice</strong></td>
<td>Enterobacteria, E. coli and coliforms</td>
<td>Endo, (MacConkey), Tergitol TTC</td>
</tr>
<tr>
<td>Oenococcus and other product spoiling organisms</td>
<td>Jus de Tomate (Tomato Juice), Orange Serum, Wallerstein</td>
<td></td>
</tr>
<tr>
<td><strong>Milk</strong></td>
<td>E. coli and coliforms</td>
<td>Endo</td>
</tr>
<tr>
<td>Enterococci, Streptococcus faecalis</td>
<td>Azide</td>
<td></td>
</tr>
<tr>
<td>Salmonellae</td>
<td>Bismuth Sulfite</td>
<td></td>
</tr>
<tr>
<td><strong>Pharmaceuticals, WFI, raw materials and cosmetics</strong></td>
<td>Enterobacteria, E. coli</td>
<td>MacConkey</td>
</tr>
<tr>
<td>Enterococci, Streptococcus faecalis</td>
<td>Azide</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Cetrimide</td>
<td></td>
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<tr>
<td>Salmonellae</td>
<td>Bismuth Sulfite</td>
<td></td>
</tr>
<tr>
<td>Staphylococci, Staphylococcus aureus</td>
<td>Chapman</td>
<td></td>
</tr>
<tr>
<td>Total colony count</td>
<td>Caso, R2A</td>
<td></td>
</tr>
<tr>
<td>Yeasts and molds</td>
<td>Malt extract, Schaufus Pottinger (m Green yeast and mold), Wallerstein</td>
<td></td>
</tr>
<tr>
<td><strong>Soft drinks, concentrates</strong></td>
<td>Acid-tolerant microorganisms, Lactic-acid bacteria</td>
<td>Orange Serum, VLB-S7-S</td>
</tr>
<tr>
<td>Enterobacteria, E. coli and coliforms</td>
<td>Endo, MacConkey</td>
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<tr>
<td>Mesophilic slime-forming bacteria, Leuconostoc</td>
<td>Weman</td>
<td></td>
</tr>
<tr>
<td>Total colony count</td>
<td>Standard*, Standard TTC*, TGE</td>
<td></td>
</tr>
<tr>
<td>Yeasts and molds</td>
<td>Malt extract, Schaufus Pottinger (m Green yeast and mold), Wallerstein</td>
<td></td>
</tr>
<tr>
<td><strong>Sugar, sugar products</strong></td>
<td>E. coli and coliforms</td>
<td>Endo</td>
</tr>
<tr>
<td>Mesophilic slime-forming bacteria, Leuconostoc</td>
<td>Weman</td>
<td></td>
</tr>
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<td>Thermophilic spore formers and mesophilic bacteria</td>
<td>Glucose Tryptone</td>
<td></td>
</tr>
<tr>
<td>Yeasts and molds</td>
<td>Malt extract*, Schaufus Pottinger (m Green yeast and mold), Wort*</td>
<td></td>
</tr>
<tr>
<td><strong>Water (general quality), mineral water, natural water, waste water</strong></td>
<td>Acid-tolerant microorganisms, Lactic-acid bacteria</td>
<td>Orange Serum</td>
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</tr>
<tr>
<td><strong>Wine</strong></td>
<td>Acetobacter</td>
<td>Orange Serum (both wetted with 3-5% ethanol), Wort</td>
</tr>
<tr>
<td>Acid-tolerant microorganisms, Lactic-acid bacteria</td>
<td>Jus de Tomate (Tomato Juice)</td>
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</tbody>
</table>

* These NPS types are suitable for the determination of the mentioned microorganisms, although the media are not explicitly declared in the references described in this publication.
The principle of the membrane filter method is based on the concentration of microorganisms from relatively large samples on the surface of a membrane filter. Nutrients and metabolites are exchanged through the pore system of the membrane filter. The pore size alone is not a meaningful criterion. Due to the variance in allocation of the pores, not all membranes guarantee sufficient nutrient supply.

A comparison of Sartorius cellulose nitrate (cellulose ester) membranes with competitive mixed ester membranes reveals significant differences in growth.

**Growth comparison**

**Growth of E. coli on Endo NPS**

E. coli forms red colonies with a metallic sheen. Other coliforms would grow as dark to light red colonies without metallic sheen.

**Growth of Pseudomonas aeruginosa on Cetrimide NPS**

Pseudomonas aeruginosa forms blue, blue-green or yellow-green colonies with 1–2 mm diameter and blue zones. The colonies produce pyocyanin and fluorescein and show fluorescence in UV-light. Other Pseudomonads would develop colonies with different colors.

E. coli shows no metallic sheen on this mixed esters membrane. Therefore it is very difficult to differentiate between E. coli and coliforms without any further test. A quantitative statement is difficult due to the fact of running colonies on the mixed esters membrane surface.

On this mixed esters membrane grow less colonies and without the blue zone. Due to the variance in the allocation of the pores, here the mixed esters membrane did not guarantee a sufficient nutrient supply. This may cause in false negative results.
Accessories

Combisart® 6-branch manifold
Made of high-grade stainless steel (B.S. 304S31| AISI 304); accommodates any type of vacuum funnel. Stainless steel three-way valves allow the vacuum for each filter station to be individually controlled and each holder to be steriley vented. This rules out secondary contamination of the underside of the filter. The material and the design meet the requirements of the current European Pharmacopoeia and ISO 8199.

16843 6-branch manifold
16842 3-branch manifold
16840 Stainless steel single base for adapting Biosart 100 or 250 or stainless steel funnels onto the Combisart manifold.

Combisart® 3-branch manifold plus Biosart® 250 Funnels
The Biosart 250 Funnel has been designed for microbiological quality assurance in industry. The sterile 250 ml (50 ml graduations) plastic funnel guarantees fast filtration and high sample throughputs during routine testing. Its large inner diameter allows high flow rates, and the tapered inner wall permit thorough flushing of the funnel, after filtration.

16407-25-ALK Biosart 250 Funnels, 50 units, sterile-packaged
16407-25-ACK Biosart 250 Funnels, 50 units, individually sterile-packaged

Vacuum pumps, water traps and vacuum hose
The vacuum pumps are neoprene membrane pumps with low noise level, oil- and maintenance-free, reliable sources of vacuum. The water traps are preventing an overflow of filtrate into the vacuum pump.

16612 For multiple filtration runs, 13 mbar final vacuum, 26 l/min, 220 V, 50 Hz
16615 For multiple filtration runs, 13 mbar final vacuum, 26 l/min, 110 V, 60 Hz
16692 For individual filtration run, 100 mbar final vacuum, 20 l/min, 220 V, 50 Hz
16695 For individual filtration run, 100 mbar final vacuum, 20 l/min, 110 V, 60 Hz
17804-M Vacusart, 3 individually sterile-packaged PTFE filter
16610 Wulff's bottle, 500 ml, with stop cock
16623 Rubber vacuum hose, 1 m

Stainless steel prefilter attachment
For removal of coarse particulate substances from samples in a single step along with bacteria-retainive filtration for subsequent microbiological testing. Clips between a filter support (16840 or 16841) and a stainless steel funnel (as show at the photo) or Biosart 250 Funnel. Autoclavable and can be flamed.

16607 Prefilter attachment

Combisart® individual systems and filter holders
For low number of samples to test, the individual system is ideal to use. In this equipment set-up, you simply use a silicone stopper and a single base to fit your choice of funnel type on a suction flask.

16841 Stainless steel single base
17575-ACK Minisart SRP 25, 50 sterile venting filters
6981065 Stainless steel funnel, 100 ml
6981002 Stainless steel funnel, 500 ml
17173 Silicone stopper
16672 Suction flask
Alternatively to position 1–4 you can use 16219 as 100 ml filter holder or 16201 as 500 ml filter holder.

Combisart® 3-branch manifold plus Biosart® 100 Monitors
Biosart 100 Monitors are sterile disposables with an incorporated membrane filter and cellulose pad. They are ready-to-use and after filtration, the funnel will be removed, so the lid and the base fit to a petri dish. Each box contains 48 units with 47 mm, gridded membrane filters.

16401-47-07-ACK Biosart 100 Monitor, individually sterile-packaged, 0.2 µm white|black grid
16401-47-06-ACK Biosart 100 Monitor, individually sterile-packaged, 0.45 µm white|black grid
16402-47-06-ACK Biosart 100 Monitor, individually sterile-packaged, 0.45 µm green|dark green grid
16403-47-06-ACK Biosart 100 Monitor, individually sterile-packaged, 0.45 µm grey|white grid
16414 Biosart 100 Adapter
AirPort MD8
AirPort MD8 uses the gelatin membrane filter method guaranteeing reliable and exact measurement results. It is battery-powered and portable for universal use.

16757 AirPort MD8, 100-240 V, 47-63 Hz, complete with holder and battery charger
17528-80-ACD Gelatin membranes, individually sterile-packaged, each in 1 bag
17528-80-BZD Gelatin membranes, individually sterile-packaged, each in 3 bags

Dosing Syringe
The most convenient way to moist the NPS with water is to use a dosing syringe with an adapted Minisart syringe filter. Simultaneous sterilization and dosing of demineralized water in 3.5 ml steps is easy done by dropping the sinker at the end of the suction tubing into the water, and the dosing syringe filled and dosed by operating the twigger automatically.

16685 Dosing syringe
17597K Minisart, 0.2 µm, individually sterile-packaged

MD8 airscan®
Together with disposable gelatin filter units the system is routinely used for the quantitative detection of air-borne organisms, mainly in sterile areas of class A and B, isolators and blow-fill-seal machines. The very high, adjustable air flow rate enables short, isokinetic sampling times.

16746 MD8 airscan, 230 V, 50 Hz
16747 MD8 airscan, 115 V, 60 Hz
16748 MD8 airscan, 100 V, 50-60 Hz
17801 Holder for disposable gelatin filter units
17528-80-ACD Gelatin membranes, individually sterile-packaged, each in 1 bag
17528-80-BZD Gelatin membranes, individually sterile-packaged, each in 3 bags

Colonies Counter | Anaerobic Container
Compact battery operated colony counter, is as simple to use as a ball-point pen, and has a 4-digit LCD-display. The counter is supplied with an additional marker refill.

Stainless steel container with a metal insert for convenient insertion and removal of petri dishes. For holding up to fourteen 60 mm or six 90 mm petri dishes, DN 6 hose nipple on the inlet and outlet, with two taps and a vacuum gauge.

17649 Colony Counter
16671 Anaerobic Container

Absorbent Pads
The 1.4 mm thick absorbent pads are wetted with the appropriate liquid culture medium before a membrane filter is placed on. Each box contains 1,000 absorbent pads in 10 tubes, each with 100 pads, and with manual dispensing device, all presterilized.

15410-47-ALR Absorbent pads, 47 mm, each approx. 3 ml absorbent capacity
15410-50-ALR Absorbent pads, 50 mm, each approx. 3.5 ml absorbent capacity
13906-47-APR Absorbent pads, 47 mm, including membrane filters 0.45 µm, white|green grid, individually sterile-packaged

arium® Laboratory Water Systems
arium® – the name of the flexible Sartorius family of laboratory systems for reagent grade water: arium® 613, the powerful reverse osmosis system and the arium® 611 series, ultrapure (Type 1) laboratory water purification system. Whether it’s reagent grade water for routine analysis or pyrogen free water for sensitive cell lines, there’s a model to suit your application.

611DI all critical lab applications
611UV low TOC applications e.g. HPLC
611UF low endotoxin applications
611VF for low TOC and low endotoxin applications
61315060 F05M1A Includes arium 61315, 60 l Tank, 2 x RO modules, 2 x Pre-treatment cartridges + sanitizing syringes for RO module & storage tank
## References and Technical Data of Nutrient Pad Sets

<table>
<thead>
<tr>
<th>NPS Type</th>
<th>References</th>
<th>Order #</th>
<th>pH (±0.2)</th>
<th>Recommended Incubation Conditions</th>
<th>Membrane Type: Order #, pore size, filter color</th>
<th>Shelf life [month]</th>
<th>Test Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azide</td>
<td>APHA (food), APHA (water), EG 98/83, HMSO, ISO 7704, ISO 7899-2, ISO 8199, LMBG, MTVO and Internal SOPs.</td>
<td>14051</td>
<td>7.2 (±0.1)</td>
<td>44 ±4h at 36 ±2°C</td>
<td>13806 (0.45µm, green</td>
<td>dark green)</td>
<td>18</td>
</tr>
<tr>
<td>Bismuth Sulfite</td>
<td>APHA (food), AOAC, DGMM, FDA, HMSO, IFD, ISO 6587 [1981], ISO 7704, USDA, USP and Internal SOPs.</td>
<td>14057</td>
<td>7.6</td>
<td>up to 48 h at 36 ±2°C</td>
<td>13806 (0.45µm, green</td>
<td>dark green)</td>
<td>18</td>
</tr>
<tr>
<td>Caso</td>
<td>APHA (food), APHA (water), AOAC, DAB, EG 98/83, EP, FDA, IDF, ISO 7704, ISO 8199, ISO 9308-1 [1990], ISO 9308-1 [2001], USDA, USP and Internal SOPs.</td>
<td>14063</td>
<td>7.3</td>
<td>up to 5d at 32.5 ±2.5°C</td>
<td>13806 (0.45µm, green</td>
<td>dark green)</td>
<td>18</td>
</tr>
<tr>
<td>Cetrimide</td>
<td>APHA (food), AOAC, ASM, DAB, DIN 38411, EG 98/83, EP, FDA, ISO 7704, ISO 8199, ISO 12780, USP and Internal SOPs.</td>
<td>14075</td>
<td>7.1</td>
<td>48 ±4 h at 37 ±1°C</td>
<td>13906 (0.45µm, white</td>
<td>green)</td>
<td>18</td>
</tr>
<tr>
<td>Chapman</td>
<td>APHA (food), AOAC, DGMM, FDA, HMSO, ISO 7704, USP and Internal SOPs.</td>
<td>14074</td>
<td>7.4</td>
<td>up to 3d at 36 ±2°C</td>
<td>13906 (0.45µm, white</td>
<td>green)</td>
<td>18</td>
</tr>
<tr>
<td>Chromocult</td>
<td>ISO 7704, Journal Food Protection, ZenHyg (journal of hygiene) and Internal SOPs.</td>
<td>14087</td>
<td>6.8</td>
<td>24h at 36 ±1°C</td>
<td>11406 (0.45µm, white</td>
<td>black)</td>
<td>18</td>
</tr>
<tr>
<td>ECD</td>
<td>APHA (water), DIN 10110, EG 98/83, ISO 7704, ISO 8199, ISO 9308-1 [2001], LMBG, USDA and Internal SOPs.</td>
<td>14082</td>
<td>7.0</td>
<td>18–24 h at 37 ±1°C or acc. to ISO 9308-1</td>
<td>13906 (0.45µm, white</td>
<td>green)</td>
<td>18</td>
</tr>
<tr>
<td>Endo</td>
<td>APHA (food), APHA (water), DGMM, ISO 7704, ISO 9308-1 [1990], MTVO, USDA and Internal SOPs.</td>
<td>14053; 14003</td>
<td>7.2</td>
<td>24±2 h at 36x2°C or acc. to ISO 9308-2 [1990]</td>
<td>13906 (0.45µm, white</td>
<td>green)</td>
<td>18</td>
</tr>
<tr>
<td>Glucose Tryptone</td>
<td>APHA (food), AOAC, ICUMSA, IFU, ISO 7704, NCA and Internal SOPs.</td>
<td>14066</td>
<td>6.8</td>
<td>48h at 55 ±2°C or up to 3d at 31 ±1°C</td>
<td>13906 (0.45µm, green</td>
<td>white)</td>
<td>18</td>
</tr>
<tr>
<td>Jus de Tomate (Tomato Juice)</td>
<td>ISO 7704, Laranidris Laffon-Lafourcade and Internal SOPs.</td>
<td>14079</td>
<td>4.4</td>
<td>4–6 days (up to 8d) at 28–30°C</td>
<td>13806 (0.45µm, green</td>
<td>dark green)</td>
<td>18</td>
</tr>
<tr>
<td>Lysine</td>
<td>Journal Institute of Brewing, VLBI and Internal SOPs.</td>
<td>14061</td>
<td>5.0</td>
<td>2–5 days at 25–28°C</td>
<td>13005 (0.65µm, gray</td>
<td>white)</td>
<td>24</td>
</tr>
<tr>
<td>MacConkey</td>
<td>APHA (food), APHA (water), AOAC, DAB, DIN 38411, DGMM, EP, ISO 7704, LMBG, MTVO, USDA, USP and Internal SOPs.</td>
<td>14097</td>
<td>7.1</td>
<td>18–24 h at 36 ±2°C</td>
<td>13906 (0.45µm, white</td>
<td>green)</td>
<td>18</td>
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<tr>
<td>Malt extract</td>
<td>APHA (food), AOAC, IFU and Internal SOPs.</td>
<td>14086</td>
<td>3.5 (±0.5)</td>
<td>up to 3d at 25 ±2°C or 7d at 30 ±2°C</td>
<td>--N: 13004 (0.8µm, gray</td>
<td>white); CCN: 13006 (0.45µm, gray</td>
<td>white)</td>
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<tr>
<td>m FC</td>
<td>APHA (food), APHA (water), AOAC, EPA, FDA, ISO 7704, ISO 9308-1 [1990], USDA and Internal SOPs.</td>
<td>14068</td>
<td>7.4</td>
<td>20 ±4 h at 36 ±3°C (44 ±1°Cwater bath)</td>
<td>13906 (0.45µm, white</td>
<td>green)</td>
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<tr>
<td>Orange Serum</td>
<td>APHA (water), IFU, ISO 7704, MPP (packaging staff) and Internal SOPs.</td>
<td>14062</td>
<td>5.5</td>
<td>up to 3d at 30 ±2°C aerobic or anaerobic</td>
<td>13806 (0.45µm, green</td>
<td>dark green)</td>
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<tr>
<td>Orange Serum</td>
<td>APHA (water), IFU, MPP (packaging staff) and Internal SOPs.</td>
<td>14096</td>
<td>3.2</td>
<td>up to 3d at 30 ±2°C aerobic or anaerobic</td>
<td>13004 (0.8µm, gray</td>
<td>white)</td>
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<td>R2A</td>
<td>APHA (water), EP, ISO 7704 and Internal SOPs.</td>
<td>14084</td>
<td>7.2</td>
<td>48–72 h at 35 ±2°C; 5–7 days at 20 ±2°C</td>
<td>13806 (0.45µm, green</td>
<td>dark green)</td>
<td>18</td>
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<tr>
<td>Sabouraud</td>
<td>APHA (food), AOAC, EP, USP and Internal SOPs.</td>
<td>14069</td>
<td>5.6</td>
<td>up to 5 days at 20–25°C</td>
<td>13005 (0.65µm, gray</td>
<td>white)</td>
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<tr>
<td>NPS Type</td>
<td>References (reference guide on page 26)</td>
<td>Order #</td>
<td>pH (±0.2)</td>
<td>Incubation Type: Order #, life conditions</td>
<td>Recommended Membrane Type: Order #, pore size, filter color</td>
<td>grid color</td>
<td>Shelf life [month]</td>
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<td>Schaufus Pottinger (m Green yeast and mold) Internal SOPs.</td>
<td>14070</td>
<td>4.4</td>
<td>2-7 days at 25-30°C</td>
<td>13905 (0.65µm, white</td>
<td>green)</td>
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<td>3, 5, 20, 23, 24</td>
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<td>Schauffus Pottinger (m Green yeast and mold) Internal SOPs.</td>
<td>14072</td>
<td>4.4</td>
<td>2-7 days at 25-30°C</td>
<td>13903 (1.2µm, white</td>
<td>green)</td>
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<td>Schauffus Pottinger (m Green yeast and mold) Internal SOPs.</td>
<td>14080</td>
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<td>2-7 days at 25-30°C</td>
<td>13004 (0.8µm, gray</td>
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<td>Schauffus Pottinger (m Green yeast and mold) Internal SOPs.</td>
<td>14083</td>
<td>4.4</td>
<td>2-7 days at 25-30°C</td>
<td>13005 (0.65µm, gray</td>
<td>white)</td>
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<td>3, 5, 20, 23, 24</td>
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<tr>
<td>Standard APHA (water), ISO 7704, VLB and Internal SOPs.</td>
<td>14064</td>
<td>7.2</td>
<td>2-5d at 30 ±2°C</td>
<td>13806 (0.45µm, green</td>
<td>dark green)</td>
<td>24</td>
<td>3, 7, 9, 18, 26</td>
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<td>Standard TTC APHA (water), ISO 7704, VLB and Internal SOPs.</td>
<td>14055; 14005</td>
<td>7.2</td>
<td>2-5d at 30 ±2°C</td>
<td>13806 (0.45µm, green</td>
<td>dark green)</td>
<td>24</td>
<td>3, 7, 9, 18, 26</td>
</tr>
<tr>
<td>Standard TTC 1 mod. APHA (water), ISO 7704, VLB and Internal SOPs.</td>
<td>14085</td>
<td>7.2</td>
<td>2-5d at 30 ±2°C</td>
<td>13806 (0.45µm, green</td>
<td>dark green)</td>
<td>18</td>
<td>3, 7, 9, 18, 26</td>
</tr>
<tr>
<td>Teepol AFNOR, APHA (water), BS, FDA, ISO 7704, ISO 9308-1 [1990], USDA and Internal SOPs.</td>
<td>14067</td>
<td>7.2</td>
<td>18–24 h at 36 ±1°C</td>
<td>13906 (0.45µm, white</td>
<td>green)</td>
<td>18</td>
<td>6, 9, 11, 21, 26</td>
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<tr>
<td>Tergitol TTC APHA (food), EG 98/83, ISO 7704, ISO 8199, ISO 9308-1 [1990], ISO 9308-1 [2001] and Internal SOPs.</td>
<td>14056; 14006</td>
<td>8.0</td>
<td>21±3 h at 36 ±2°C</td>
<td>13906 (0.45µm, white</td>
<td>green)</td>
<td>18</td>
<td>6, 9, 11, 21, 26</td>
</tr>
<tr>
<td>TGE APHA (dairy), APHA (food), APHA (water), API, ISO 7704 and Internal SOPs.</td>
<td>14076</td>
<td>7.0</td>
<td>2-5d at 30 ±2°C</td>
<td>13806 (0.45µm, green</td>
<td>dark green)</td>
<td>24</td>
<td>9, 18, 26</td>
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<td>VLB-S7-S EBC, ISO 7704, MEBAC, VLB and Internal SOPs.</td>
<td>14059</td>
<td>5.5</td>
<td>5-7 days at 25-28°C, anaerobic</td>
<td>13906 (0.45µm, white</td>
<td>green)</td>
<td>18</td>
<td>12, 13, 15, 19</td>
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<td>Wallerstein ISO7704 and Internal SOPs.</td>
<td>14089</td>
<td>5.5</td>
<td>up to 14 days at 25-30°C, aerobic or anaerobic</td>
<td>13906 (0.45µm, white</td>
<td>green)</td>
<td>24</td>
<td>5, 12, 20, 23, 24</td>
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<td>Weman ICUMSA, ISO 7704 and Internal SOPs.</td>
<td>14065</td>
<td>5.5</td>
<td>2-3 days at 25-30°C</td>
<td>13806 (0.45µm, green</td>
<td>dark green)</td>
<td>18</td>
<td>14, 16, 17</td>
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<td>Wort VLB and Internal SOPs.</td>
<td>14058; 14008</td>
<td>4.4</td>
<td>2-5 days at 25-30°C</td>
<td>13005 (0.65µm, gray</td>
<td>white)</td>
<td>24</td>
<td>5, 20, 23, 24</td>
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<td>Yeast extract EG 98/83, HMSO, ISO 6222, ISO 7704, ISO 8199 and Internal SOPs.</td>
<td>14090</td>
<td>7.2</td>
<td>44 ± 4h at 36 ±2°C; 68 ±4h at 22 ±2°C</td>
<td>13806 (0.45µm, green</td>
<td>dark green)</td>
<td>24</td>
<td>3, 7, 9, 18, 26</td>
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</tbody>
</table>

**Test Strains [ATCC No.], [DSM No.]**

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>Aspergillus niger 16404, 1988</td>
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<td>2</td>
<td>Bacillus cereus 11778, 345</td>
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<td>3</td>
<td>Bacillus subtilis 6633, 347</td>
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<td>4</td>
<td>Brevundimonas diminuta 19146, 1635</td>
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<td>5</td>
<td>Candida albicans 10231, 1386</td>
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<td>6</td>
<td>Enterobacter aerogenes 13048, 30053</td>
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<td>7</td>
<td>Enterococcus faecalis 29212, 2570</td>
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<td>8</td>
<td>Enterococcus faecium 35667, 6177</td>
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<td>9</td>
<td>Escherichia coli 8739, 1576</td>
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<td>10</td>
<td>Geobacillus stearothermophilus 7953, 5934</td>
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<td>11</td>
<td>Klebsiella pneumoniae 13883, 30104</td>
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<td>12</td>
<td>Lactobacillus linhardi DSM 20690</td>
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<td>13</td>
<td>Lactobacillus plantarum 14917, 20174</td>
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<td>14</td>
<td>Leuconostoc mesenteroides 8293, 20343</td>
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<td>15</td>
<td>Oenococcus oeni 23279, 20252</td>
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<td>16</td>
<td>Mixed culture from honey</td>
</tr>
<tr>
<td>17</td>
<td>Mixed culture from raw sugar</td>
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<tr>
<td>18</td>
<td>Mixed culture from tap water</td>
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<td>19</td>
<td>Pediococcus damnosus 29358, 20331</td>
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<td>20</td>
<td>Penicillium commune 10428, 2211</td>
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<td>21</td>
<td>Proteus mirabilis 14153, 788</td>
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<td>22</td>
<td>Pseudomonas aeruginosa 9027, 1128</td>
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<td>23</td>
<td>Rhodotorula mucilaginosa DSM 70404</td>
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<td>24</td>
<td>Saccharomyces cerevisiae 9763, 1334</td>
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<td>25</td>
<td>Salomonella choleraesuis DSM 554</td>
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<td>26</td>
<td>Staphylococcus aureus 6538P, 346</td>
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<td>27</td>
<td>Staphylococcus epidermidis 12228, 1798</td>
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<td>Abbreviation</td>
<td>Title</td>
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<tr>
<td>AFNOR</td>
<td>Association Francaise de Normalisation</td>
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<tr>
<td>APHA (dairy)</td>
<td>American Public Health Association: Standard Methods for the examination of dairy products</td>
</tr>
<tr>
<td>APHA (food)</td>
<td>American Public Health Association: Compendium of methods for the microbiological examination of foods</td>
</tr>
<tr>
<td>APHA (water)</td>
<td>American Public Health Association, American Water Works Association (AWWA) and Water Environment Federation (WEF): Standard Methods for the Examination of Water and Wastewater</td>
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<tr>
<td>AOAC</td>
<td>Association of Official Analytical Chemists</td>
</tr>
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<td>API</td>
<td>American Petroleum Institute: Recommended practice for biological Analysis of Subsurface Injection waters</td>
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<td>ASM</td>
<td>American Society for Microbiology</td>
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<tr>
<td>BS</td>
<td>British Standards</td>
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<td>DAB</td>
<td>Deutsches Arzneimittelbuch (German Pharmacopoeia, replaced by EP)</td>
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<tr>
<td>DIN 10110</td>
<td>Deutsches Institut für Normung: Mikrobiologische Fleischuntersuchung. Bestimmung der E. coli. (Microbial detection of E. coli on meat)</td>
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<td>DIN 38411</td>
<td>Deutsches Institut für Normung: Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung (German standard for water, waste water and sludge analysis)</td>
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<td>DGHM</td>
<td>Deutsche Gesellschaft für Hygiene und Mikrobiologie (German Association of Hygiene and Microbiology)</td>
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<tr>
<td>EBC</td>
<td>European Brewery Community</td>
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<td>EG 98/83</td>
<td>European Guideline 98/83: Water Quality for human purpose</td>
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<td>EP</td>
<td>European Pharmacopoeia</td>
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<td>EPA</td>
<td>U.S. Environmental Protection Agency: Laboratory standards for equipment and materials</td>
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<td>FDA</td>
<td>U.S. Federal Drug Administration</td>
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<td>ICUMSA</td>
<td>International Commission for Uniform Methods of Sugar Analysis</td>
</tr>
<tr>
<td>IDF</td>
<td>International Dairy Federation</td>
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<tr>
<td>IFU</td>
<td>International Federation of Fruit Juice Producers</td>
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<tr>
<td>Internal SOP</td>
<td>Internal Standard Operation Procedure of individual requests</td>
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<tr>
<td>ISO 7004</td>
<td>International Organization for Standardization: Water Quality, Evaluation of membrane filters used for microbiological analysis</td>
</tr>
<tr>
<td>ISO 9308-1</td>
<td>International Organization for Standardization: Water Quality – Detection and enumeration of E. coli and coliform bacteria</td>
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<tr>
<td>ISO 12780</td>
<td>International Organization for Standardization: Water Quality – Detection and enumeration of Ps. aeruginosa</td>
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<tr>
<td>JFoodP</td>
<td>Journal of Food Protection</td>
</tr>
<tr>
<td>JIBrew</td>
<td>The Journal of the Institute of Brewing</td>
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<tr>
<td>LLL</td>
<td>Method described by Lanaridris &amp; Lafon-Lafourcade</td>
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<tr>
<td>LMBG</td>
<td>Amtliche Sammlung von Untersuchungsverfahren nach dem §35 des Lebensmittel- und Bedarfsgegenständegesetzes des BGA (testing procedures for food stuffs and articles of daily use)</td>
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<tr>
<td>MEBAK</td>
<td>Methodensammlung der Mitteleuropäischen Brauereitechnischen Analysenkommission (methods of the Central European brewery commission)</td>
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<td>MPP</td>
<td>Merkblätter für die Prüfung von Packmitteln (Testing procedures for packaging stuff)</td>
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<td>MTVO</td>
<td>Verordnung über natürliches Mineralwasser, Quellwasser und Tafelwasser (Mineral/Table Water Guideline)</td>
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<td>NCA</td>
<td>National Canners Association: A Laboratory manual of the canning industry</td>
</tr>
<tr>
<td>USDA</td>
<td>U.S. Department of Agriculture</td>
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<td>USP</td>
<td>United States Pharmacopoeia</td>
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<tr>
<td>VLB</td>
<td>Versuchs- und Lehranstalt für Brauerei in Berlin (institute of brewery)</td>
</tr>
<tr>
<td>ZenHyg</td>
<td>Zentralblatt für Hygiene (Journal of Hygiene)</td>
</tr>
</tbody>
</table>

DIN standards and the "Amtliche Sammlung von Untersuchungsverfahren nach dem §35 des Lebensmittel- und Bedarfsgegenständegesetzes des BGA" are available through the German publisher Beuth-Verlag, Burggrafenstr. 6, 10787 Berlin
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