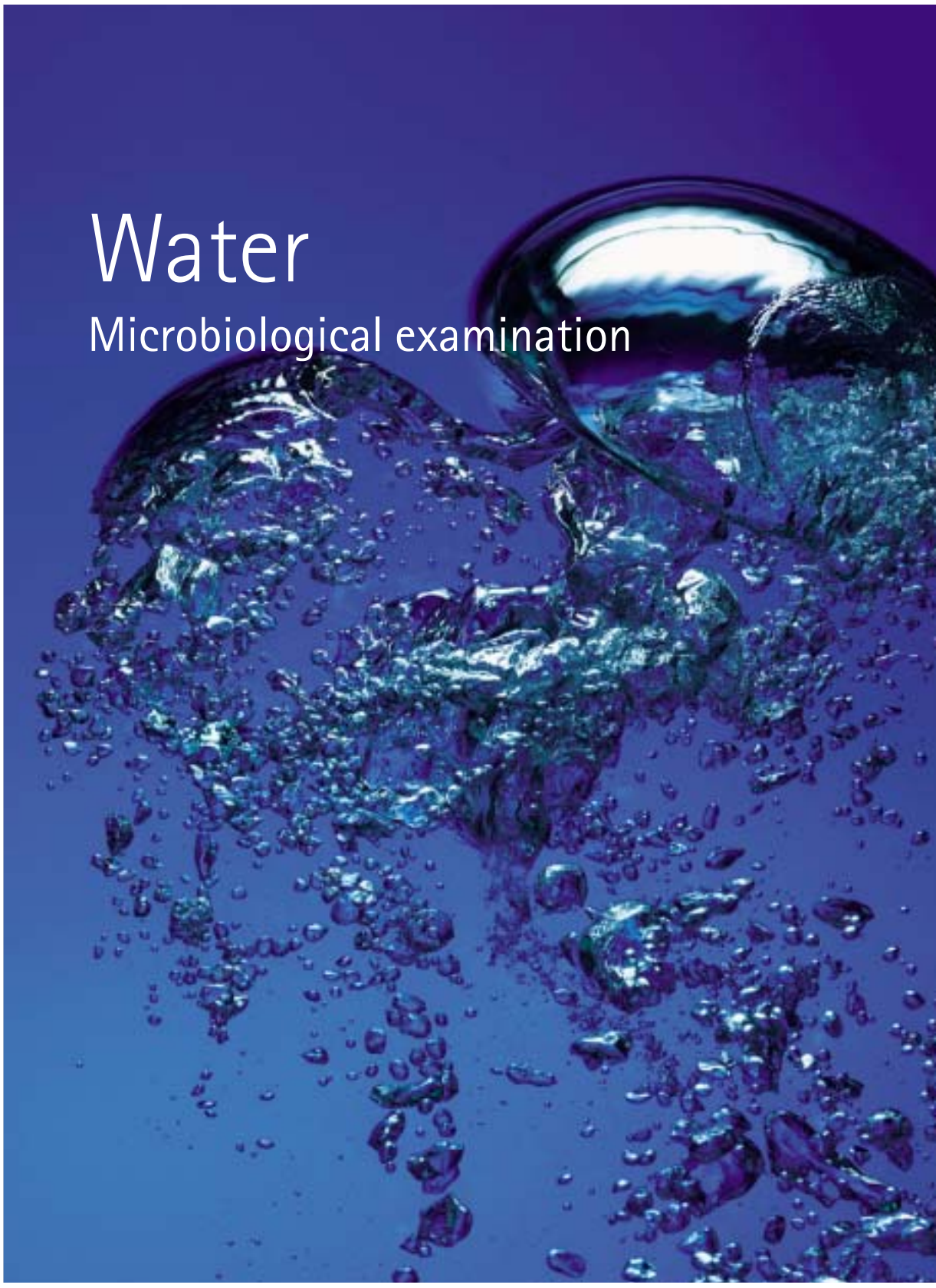
Water

Microbiological examination



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Content

Method protocols and recommended media

This brochure does not specify all details of the examinations.
Before starting an examination it is advised to read the reference documents.

The availability of clean, fresh water is one of the most important issues facing humanity today - and will be increasingly critical for the future, as growing demands outstrip supplies and pollution continues to conta-minate rivers, lakes and streams.

"Lack of access to water - for drinking, hygiene and food security - inflicts enormous hardship on more than a billion members of the human family", said United Nations Secretary-General Kofi Annan. "Water is likely to become a growing source of tension and fierce competition between nations, if present trends continue, but it can also be a catalyst for cooperation."

Water borne illness

Water borne infections still ravage the global community and are responsible for millions of deaths per year. Water that looks clear and pure may be sufficiently contaminated with pathogenic microorganisms to be a health hazard. A prime public health consideration is the continuous supply of drinking water which is free of pathogens and significant levels of toxic chemicals. Protection of drinking water from contamination by human or other animal excrement in sewage, food processing wastes and storm water run off is of paramount importance.

The majority of infections associated with the water cycle are those which cause gastroenteritidis. The symptoms vary with etiological agent.
A variety of etiological agents have been implicated in water borne diseases. The causative agents include: Bacteria, viruses and intestinal parasites. The causative agent varies with the geographical area climate, general level of sanitation, endemic persistence, as well as cultural and socio-economic characteristics of the population.

It is well established that unsatisfactory water supplies are related to ill health. Water contamination occurs generally due to seepage of sewage or surface contaminated water in aquifers and improperly protected wells, or is due to inadequately treated or distributed drinking water. Person to person contact has been documented in the transmission of *Legionella* to humans. *Legionella* is a common inhabitant of natural waters. The direct way of transmission is the inhalation of contaminated aerosolized contaminated water.

Some pathogens that may occur in drinking water:

Bacteria

- Vibrio cholerae
- Shigella dysentriae
- Enterotoxigenic Escherichia coli
- Salmonella spp. (e.g. Salmonella typhi)
- Campylobacter jejuni
- Aeromonas hydrophila
- Pseudomonas aeruginosa
- Legionella
- Yersinia enterocolitica

Viruses

- Enterovirus
- Poliovirus
- Coxsackievirus
- Echovirus
- Reovirus Adenovirus
- Hepatitius A virus, Norwalk like virus, Astro virus
- Calcivirus, Epidemic non A, non B hepatitus

Intestinal parasites

- Cryptosporidium
- Giardia
- Entamoeba histolytica

Microbiological monitoring of water

Water systems should be monitored at a frequency that is sufficient to ensure that the system is under control and continues to produce water of an acceptable quality. Samples should be taken from representative locations within the processing and distribution system. Established sampling frequencies should be based on system validation data and cover critical areas.

The sampling plan should take into consideration the desired attributes of the water being sampled. Sampling ports should be sanitized and thoroughly flushed before a sample is taken. Samples containing chemical sanitizing agents require neutralisation prior to microbiological analysis.

Microbiological examination of drinking water is an attempt to determine the relation of the possible transmission of water borne disease. It is usually not practical to examine water supplies for the various pathogens that may be present. Therefore, the routine monitoring of water is based on the testing of indicator organisms.

Samples for microbiological analysis should be tested immediately.
If this is not possible, samples should be protected (in line with local regulations/guidelines) to preserve them until they can be analysed.

Reliance on water quality determination alone is insufficient to protect public health. It is neither physically nor economically feasible to test for all drinking water quality parameters equally. Therefore monitoring efforts and resources should be carefully planned and directed at significant or key characteristics.

Heterotrophic Plate Count

The Heterotrophic Plate Count (Total Viable Count) is part of the routine monitoring of drinking water, which includes water in closed containers, mineral water and general application to the monitoring of all water types. Heterotrophic counts are also employed in the testing of water used in the preparation of food and drinks.

The Heterotrophic Plate Count (HPC/TVC) was formerly termed standard plate count or "total" plate count. HPC gives an indication of the integrity of ground water sources, the efficacy of water treatment processes, the cleanliness, the integrity of the water distribution system by measuring the re-growth or the after-growth-potential in treated drinking water.

Microorganisms will normally grow in water and also as biofilms on surfaces in contact with water. Growth following drinking water treatment is normally referred to as re-growth. Growth is typically reflected in higher HPC/TVC values measured in water samples.

HPC/TVC indicates the effectiveness of water treatment processes, thus it is an indirect indication of pathogen removal and a measure of the numbers of re-growth organisms that may or may not have sanitary significance. In long term routine monitoring, a deviation from the common heterotrophic colony counts signals changes in the microbial water quality. A sudden raise in the HPC gives an early warning of pollution and calls for immediate investigation.

The consumption of, or exposure to water containing large numbers of HPC/TVC organisms can lead to diseases, such as gastroenteritis, skin and mucous membrane infections, particularly in people whose immune system is already compromised. Opportunistic pathogens are naturally present in the environment and can be found in source water and treated drinking water. The heterotrophic plate count bacteria identified as opportunistic pathogens include: *Acinetobacter, Aeromonas, Flavobacterium, Klebsiella, Legionella, Moraxella, Mycobacterium, Serratia, Pseudomonas* and *Xanthomonas*.

Heterotrophic Plate Count bacteria require simple organic carbon rather than carbon dioxide for growth. HPCs vary with media composition, time of incubation, temperature of incubation and inoculation method (pour or surface plating). Heterotrophic counts are generally conducted at both 22°C and 35-37°C (see local regulations/guidelines).

Total coliform count

Total coliform count is used as indicator of the general sanitary quality of treated drinking water supplies. The term coliform bacteria represents a vaguely defined group of organisms which have a long history in water quality assessment.

Coliform bacteria are aerobic and facultative anaerobic, Gram-negative, non-spore forming bacilli. They ferment lactose, produce gas within 24h at 35°C. The enzyme ß-galactosidase is present in 94-96% of the coliform bacteria.

Coliform bacteria occur in the bowel of humans and warm-blooded animals, but also in soil and fresh surface water. Although many of these bacteria are of faecal origin, some are heterotrophic and able to multiply in various water environments. The presence of coliform bacteria is not always proof of faecal contamination. The presence in drinking water is usually a result of a problem with the treatment system or water pipes and indicates that the water may be contaminated with microorganisms that can cause disease. Coliform bacteria are detected by incubation of selective broths or plating agars at 35-37°C.

Faecal or thermotolerant coliform bacteria

Faecal coliform bacteria are bacteria that are associated with human or animal wastes as they usually live in human or animal intestinal tracts. Their presence in drinking water is a strong indication of recent sewage or animal waste contamination.

Faecal coliform bacteria are predominantly *Escherichia coli* and thermotolerant strains of *Klebsiella*.

The group of faecal or thermotolerant coliform bacteria is more closely related with faecal pollution than total coliform bacteria. The presence of faecal coliform bacteria in drinking water is generally not acceptable. The faecal or thermotolerant coliform bacteria are determined by incubation of selective broths or plating agars at elevated temperature (44 or 45.5°C) using the water bath.

Escherichia coli

Escherichia coli is a species belonging to the coliform group of bacteria that normally inhabits the bowel of humans and warm-blooded animals.
The presence of *E.coli* in water indicates a pollution of faecal origin as result of recent sewage or animal waste contamination. *E.coli* rarely multiplies in water environments.

During rainfalls, snowmelts or other types of precipitation, *E.coli* may be washed into creeks, rivers, streams, lakes and groundwater. When these waters are used as sources for drinking water and untreated or inadequately treated, *E.coli* may end up in the drinking water.

E.coli is a faecal coliform bacterium that ferments lactose, produces gas at 35°C and 44°C or 45.5°C (i.e. 90% of *E.coli*), is Indole-positive (i.e. 99% of *E.coli*) and characterised by ß-galactosidase and ß-glucuronidase (i.e. 96% of *E.coli*) activity.

E.coli is a thermotolerant coliform that is isolated or enumerated by incubation of selective broths or plating agars at an elevated temperature (44.5 or 45.5°C) using the water bath.

Faecal streptococci, intestinal enterococci, other enterococci

Formerly, all streptococci of faecal origin i.e. faecal streptococci or intestinal enterococci that produce group D antigen belonged to the group of Lancefield D streptococci. Since 1984 the classification of streptococci has been reorganised.

Streptococcus has been divided in three different genera: *Streptococcus*, *Enterococcus* and *Lactococcus*. The Lancefield D streptococci are grouped in the genus *Enterococcus*. *S. bovis* and *S. equinus* that belonged to the Lancefield D streptococci have been placed in a group of "other" streptococci.

Members of the genus *Enterococcus* are e.g. *E. avium*, *E. casseliflavus*, *E. durans*, *E. faecalis, E. faecium , E. gallinarium, E. hirae, E. malodoratus, E. mundtii, E. raffinosus, E. solitarius* and *E. faecalis* (variant). *Enterococcus* spp. are Gram-positive, Catalase-negative cocci and usually grow at 45°C in 6.5% NaCl and pH 9.6.

The species *E. faecalis, E. faecium, E. durans* and *E. hirae* occur frequently in faeces of humans and homeothermic animal's. *Enterococcus* spp. like *E. avium, E. cecorum, E. columbae* and *E. gallinarium* are of other faecal origin and occur rarely in environmental samples. It should be noted that some enterococci e.g. *E. casseliflavus* and *E. mundtii* are non-faecal species found in water that can also originate from plant material or some industrial effluents. *S. bovis* and *S. equinus* are of "other" faecal origin and occur rarely in environmental samples.

Enterococci occur less numerous than faecal coliforms and *E. coli* in human faeces. The low level or infrequent occurrence of enterococci in source water limits their use as indicators in drinking-water treatment processes. Enterococci rarely grow in the environment, are more resistant to various treatment and disinfection processes than coliform bacteria and possibly even coliphages and viruses. In the US, enterococci may be used as indicators when monitoring marine recreational water samples due to their ability to survive in high concentrations of salt as compared to *E. coli*.

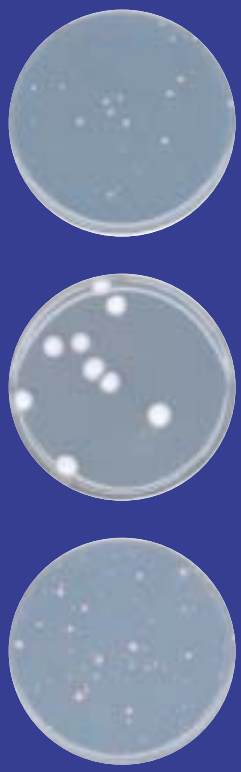
Enterococci are an indicator of water treatment efficiency. They are often employed as a secondary indicator for resampling after the detection of coliform bacteria or *E. coli* in distribution systems, as an indicator in the routine monitoring after new water mains are laid or after repairs to distribution systems. Enterococci tests are run to get supplementary data on the bacteriological quality of natural water systems, because they rarely multiply in water.

Clostridia or Clostridium perfringens

Clostridia and *Cl. perfringens* are suitable indicators for the survival of viruses and protozoan cysts in drinking water or oocysts in treated drinking water, when sewage is the suspected cause of contamination. The spores are largely of faecal origin and are always present in sewage. Vegetative cells appear not to reproduce in aquatic sediments.

Clostridia and *Cl. perfringens* are not recommended for the routine monitoring of distribution, as they survive and accumulate and may be detected long after pollution has occurred. The presence of *Clostridia* and *Cl. perfringens* in treated water suggests deficiencies in treatment, failure of disinfection processes or recontamination of the treated water.

A great advantage of *Clostridia* when used as indicator organisms is that the detection methods are relatively simple in comparison to the detection methods of viruses and cysts and a result is available within 24h. *Clostridia* spores are more resistent to disinfection than other pathogens. Particularly *Cl. perfringens* has a high specificity for faecal pollution.



Yeast Extract Agar

A medium rich in nutrients which permits the recovery of a wide spectrum of bacteria, yeast and moulds. The total count medium conforms with ISO 6222 .

Microbiological methods for examination of water

In the microbiological examination of water there are four different cultural methods routinely employed and recommended in standards. These include: Aerobic or Heterotrophic Plate Count (HPC), Presence - Absence (P-A) testing, Most Probable Number (MPN) method and Membrane Filtration (MF) method.

Heterotrophic Plate Count (HPC)

A plate count like the Heterotrophic Plate Count is commonly determined using the pour plate technique. 1ml of a water sample or a decimal dilution series is transferred to separate Petri dishes.15ml of liquified agar medium is then added to each Petri dish (no stacking of plates when pouring agar). The sample is thoroughly mixed by rotation (three times left, three times right and once through the centre). The agar is left to solidify (no stacking of plates during solidification) on a flat level, preferably cool, surface. After complete solidification (check by ticking the Petri dish on the side; solidification occurs latest in the centre) the plates are inverted and incubated (BAM requires 48 ±2h at 35°C). Plates showing 25 to 250 colonies (including pinpoint colonies) should be considered in determining the standard plate count. A count is designated as standard plate count at temperature of incubation. The incubation temperature can either be 20, 30 or 35-37°C. Depending on incubation temperature and atmosphere, the counts are termed psychrotrophic aerobic or anaerobic standard count (20°C) or mesophilic aerobic or anaerobic plate counts (30 or 35-37°C).

Presence-Absence Broth

Selective medium for the detection of coliform bacteria in water.

The medium conforms with the recommendations of Standard Methods for the examination of water and wastewater.

Lactose fermenting organisms produce acid which is identified by the indicator bromocresol purple with a color change from purple to yellow.

Presence-Absence (P-A) testing

The objective of the Presence-Absence testing is to obtain qualitative information on the presence and absence of the target organism or group of organisms. For the Presence-Absence test commonly a 100ml sample is transferred to a single flask. A double (acc. to ISO) or triple strength (acc. to Standard Method) liquid culture medium is then added. The use of a double or triple strength medium prevents that the sample dilutes in the culture medium and thereby reduces its selectivity.

Most Probable Number or Multiple tube technique (MPN)

The Multiple tube testing is a modification of the Presence-Absence testing. Instead of adding the sample to a single tube, the sample is divided in portions and multiple tubes are inoculated with variable volumes of the same water sample. The reference methods employ different volumes and replicates for the Multiple tube testing. For detailed information please refer to ISO 8199, the general guide to the enumeration of microorganisms by culture or to Standard Methods 21st Edition, Method 9221 “Multiple tube fermentation” therein. For sample volumes less than 5 ml or 10 ml (Standard Methods) the sample is added to an equal volume of single strength media. For volumes of 10-100ml, double

strength media are commonly used. Standard Methods, however, recommend the use of triple strength media for volumes of 100ml and higher. Moreover, Standard Methods 21st Edition shows a table in Method 9221 for the preparation of Lauryl Sulfate Broth.

The positive and negative test results of each tube can be used for a calculation of the estimated number of counts of microorganisms. The Most Probable Number (MPN) is read from the MPN table found in Standard Methods.

Membrane Filtration (MF)

Membrane Filtration is simpler and yields numerical results quicker than the Multiple tube test. The membrane filter technique is useful in drinking water, but has its limitations when testing waters that are heavily contaminated or have a high non-coliform background.

A1-Medium

Selective medium for the detection of faecal coliform bacteria in water. The medium conforms with the recommendations of Standard Methods for the examination of water and wastewater.

With no or little experience with the Membrane Filtration technique it is advised to first run samples using Membrane Filtration in parallel with the more familiar Presence-Absence test.

The sample size should be chosen so that the yield on the membrane filter ranges from 20-60 colonies (e.g. for *faecal Streptococcus*/*Enterococcus* acc. to Standard Methods and acc. to ISO) or 20-80 colonies (e.g. for coliform bacteria acc. to Standard Methods, EP or USP). The usual sample size is 100 ml. Occasionally a larger volume is used: e.g. for the detection of *Pseudomonas aeruginosa* in natural water (200 ml) or up to 500 ml for swimming pool waters. The membrane filters commonly used have a mean pore diameter of 0.45 mm and about 50 mm in total diameter. The type of filter material is chosen so that the bacteria retaining efficiency is neither affected by the components of the filter nor by the components of the sample to be investigated.

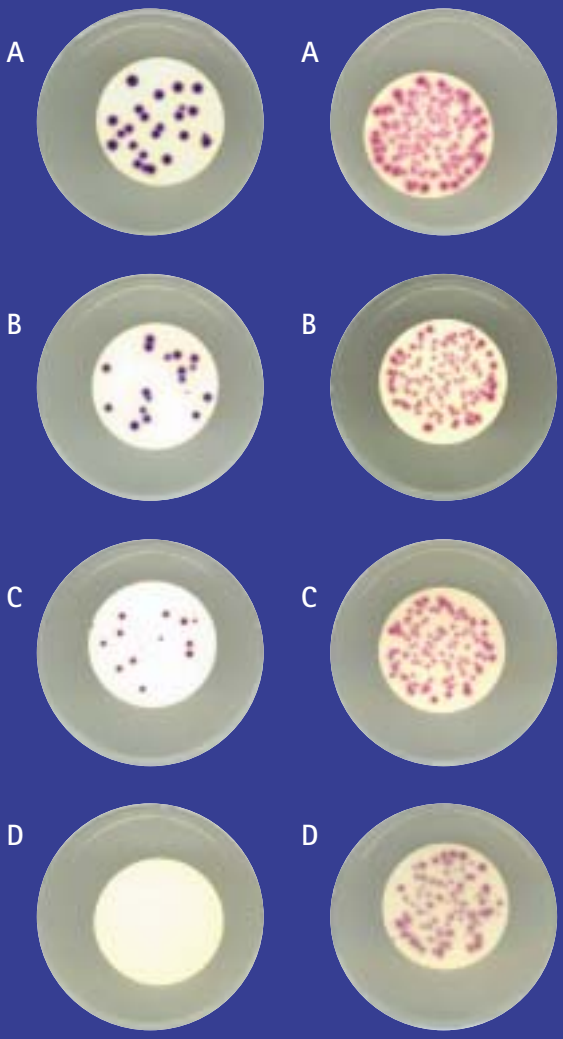
The Membrane Filtration technique is relatively simple. At the beginning of each filtration series, the filtration units are sterilised to avoid contamination. Using sterile tweezers, a sterile membrane filter is placed over the porous plate of the filtration unit, grid side up. The matched funnel unit is carefully placed over the receptacle and locked in place. A volume of water is then filtered through the membrane filter under partial vacuum. The membrane filter retains the organisms on the surface. The filter may be rinsed with three 30ml portions of sterile buffered water or membrane rinsing fluid (EP or USP). It is advised to validate the number of washes.

After the filtration, the funnel is unlocked and removed. The membrane filter is removed with sterile tweezers and placed on the agar medium with a rolling motion to avoid the entrapment of air. Agar plates used, should have a visually dry surface. The agar plate is incubated inverted. After the recommended incubation period the colonies are counted. The counts of typical colonies on a selective medium are presumptive counts. A confirmation of a square root of typical colonies gives the confirmative count. Counts are expressed as cfu per ml for a filtered sample.

Membrane Filtration

The type and brand of membrane filters can greatly affect the recovery of microorganisms as this figure illustrates for the recovery of coliform bacteria on ChromoCult® Coliform Agar.

The best performance on ChromoCult® Coliform Agar is obtained when using Cellulose-Mixed-Ester material e.g. Pall GN-6 or Schleicher and Schuell ME 25 (Ossmer et al. 1999).



A: *Pall GN-6 Mixed-Ester*

B: *Whatman 7141114 Cellulose-Nitrate*

C: *Schleicher & Schuell 405370 Cellulose-Nitrate*

D: *Schleicher & Schuell 405370 Cellulose-Acetate*

Rapid testing methods

Fluorogenic and chromogenic culture media

On a medium containing fluorogenic and/or chromogenic substrates a target organism is identified by the activity of an enzyme specific to that organism.

In fluorogenic culture media the enzyme characterizing the target organism splits a fluorogenic substrate e.g. 4-methylumbelliferyl-ß-D-glucuronide (MUG), into two separate components, a sugar or amino acid and a fluorogen. The mentioned fluorogen converts UV-light to visible light. The fluorescence produced is read in the dark under exposure to a UV light at 366 nm. An agar, containing a fluorogenic, should be read after 24 hours as these substrates have the tendency to migrate into the agar making it impossible to identify a single colony of the target organism. It is also important to mention that fluorogenic substrates are heat sensitive and pH dependent. Tests have shown that the fluorescence is most intense at a pH above 8.5.

In chromogenic culture media indolyl derivatives are quite commonly used. These chromogenic substrates are water soluble, heat stable and pH independent. As with fluorogenic culture media the enzyme that characterizes the target organism splits the chromogenic dye into two components. The chromogenic colors the broth and/or the colony. The color does not diffuse into the agar, therefore, only the target colonies are colored.

The presence of the target organism(s) in fluorogenic and chromogenic culture media is identified by the color and/or fluorescence. The high specificity of the differential system eliminates the need for subculturing and further biochemical tests and greatly improves identification. The use of combinations of different chromogenic substrates or fluorogenic and chromogenic substrates (e.g. FluoroCult® LMX Broth) allows the simultaneous testing and identification of different microorganisms or groups of microorganisms.

Rapid Presence - Absence (P-A) testing and Most Probable Number or Multiple tube technique (MPN)

FluoroCult® LMX and Readycult® Coliforms 50 and 100

FluoroCult® LMX Broth allows the simultaneous testing of total coliform bacteria and *E.coli*. It contains a fluorogenic substrate and a chromogenic dye. FluoroCult® LMX is US EPA approved as a slight modification of the already EPA approved Readycult® medium (self preparation for FluoroCult® LMX Broth versus ready-to-use snap pack format for Readycult® Coliform).

Readycult® Coliforms is the convenient and easy to open snap pack format of FluoroCult® LMX Broth. It contains pre-weighed sterile granulated FluoroCult® LMX medium and is available as Readycult® Coliform 50 for the preparation of 50ml broth or Readycult® Coliform 100, for the preparation of either 100 ml broth or 50 ml double strength broth.

Readycult® Coliforms 50 and 100 allow immediate on site testing. The content of one snap pack is directly added to the water sample.

FluoroCult® LMX and Readycult® Coliforms are convenient and cost-saving approved methods for testing *E.coli* and coliforms acc. to the EPA Total Coliform Rule. The overall testing time is reduced by 4 days compared to traditional EPA methods and 3 days compared to the ISO method.

Total coliform bacteria

When using FluoroCult® LMX Broth with the Presence-Absence or the Multiple tube (MPN) method, results of total coliform bacteria are available within 24 hours. A positive total coliform test is indicated by a blue-green color. This color change from yellow to blue-green is the result of the splitting of X-Gal by the enzyme ß-galactosidase, which is characteristic for 96-97% of coliform bacteria. A color comparator is not needed to read results in FluoroCult® LMX Broth or Readycult® Coliform. The distinct blue-green color of a positive test sample is easily distinguished even with colored water samples. FluoroCult® LMX Broth is also much easier and more reliable to read than the traditional gas production in inverted (Durham) tubes (Manafi & Rossmann 1999). X-Gal was proven to be a faster and more sensitive parameter for total coliforms than the gas production from lactose (Ossmer 1993).

The FluoroCult® LMX Broth, Readycult® 50 and 100 total coliform methods are as sensitive as the traditional Standard Method or ISO reference methods and easier to interpret (Betts *et al.* 1994, Lee *et al.* 1995, Manafi 1995).

Aeromonas constitute a fraction of the heterotrophic population found in drinking water. It is well documented that in Colilert-18 aeromonads provoke a positive response (Covert et al. 1989, Landre et al. 1998, Edberg et al. 1998, Katamay 1990, Cowburn et al. 1994). *Aeromonas hydrophila* is a water borne pathogen that should not be present in finished waters. As with Colilert, *A. hydrophila* and *A. sobria* (a fish pathogen) can produce false positive test results in FluoroCult® LMX Broth and Readycult® Coliforms 50 and 100 (Manafi 1995, Manafi & Rossmann 2000).

FluoroCult® LMX Broth:

A fluorogenic and chromogenic enrichment broth for the simultaneous detection of coliform bacteria (total coliform testing) and E.coli. A distinct color change from yellow to blue green indicates a positive total coliform test. No need for a color comparator!

The medium comes in granulated form which reduces the health risk of inhaling dust particles and dissolves quickly. Results are easy to read and allow cost-cutting, since additional time consuming confirmation procedures are not needed.

The selectivity of FluoroCult® LMX Broth can be enhanced by the addition of E.coli/Coliform Selective-Supplement. Cefsulodin prevents the growth of aeromonads and allows the testing of non-treated water without the problematic interference of non coliforms.

Readycult® Coliforms:

Ready-to-use snap pack containing pre-weighted sterile granulated FluoroCult® LMX Broth for the preparation of single (50 or 100ml) or double strength (50 ml) media. Readycult® Coliforms is US EPA approved according to the Total Coliform Rule (40 CFR 141, 21f) and, as of today, the only EPA approved method which allows the Indole reaction to be performed directly in the broth to verify the presence of fluorescence positive confimed E.coli result.

Escherichia coli

When using FluoroCult® LMX Broth or Readycult® Coliforms the presence of *E.coli* in a positive total coliform sample is indicated by a blue fluorescence when exposed to UV light in the dark. A UV lamp with a minimum of 6 watts and a wavelength of 366nm should be used. Simultaneous blue-green color and fluorescence is a strong indication of the presence of *E.coli*. It is known (e.g. Manafi,1995) that there are a small number of bacteria other than *E.coli* capable of producing positive fluorescence. Therefore, an additional reaction, the Indole test, may be used to confirm the presence of *E.coli*.

Indole test:

This test using Kovàcs' reagent has been used for several decades and established by microbiologists worldwide as one biochemical reaction on the way to an identification of bacteria. The test is based on an enzymatic reaction where tryptophan is cleaved (tryptophanase hydrolysis) and transferred into Indole, pyruvic acid and ammonia. The presence of Indole is detected with Kovàcs' reagent and indicated by a cherry red color. 99% of *E.coli* are Indole-positive.

FluoroCult® LMX Broth and Readycult® Coliforms allow this Indole reaction to be performed directly in the broth, meaning the presence of *E.coli* is confirmed within 24 hours! False positive fluorescing samples are easily detected!

Kovàcs' reagents is added to an aliquot from a fluorescence (MUG) positive sample (shake the sample prior to taking the aliquot). When using the reagent, a red ring, confirms the presence of *E.coli*.

E.coli 0157

ß-D-glucuronidase is used as an indicator for *E.coli*. Other *Escherichia spp*. do not produce this enzyme (Rice et al. 1991). Some pathogenic strains of *E.coli* such as typical *E.coli 0157:H7* however do not posses ß-D-glucuronidase either (Frampton and Restaino, 1993). So, these pathogenic strains do not show positive fluorescence, however, they do produce a positive Indole reaction. When using FluoroCult® LMX Broth or Readycult® Coliforms, blue-green colored samples (total coliform positive) showing no fluorescence (negative MUG reaction) can be checked for *E.coli 0157* using the Indole reaction as described above. A positive Indole reactions in such water samples indicates presumptive *E.coli 0157* and further tests for confirmation should be performed.

E.coli 0157 confirmation

The presence of pathogenic *E.coli* strains can be confirmed in less than 20 minutes by using the new Singlepath® *E.coli 0157* lateral flow test (WQTC poster, Philadelphia, November 2003; AOAC approval pending). An aliquot from a blue-green colored, fluorescence negative and Indole-positive water sample is directly transferred to the lateral flow test. No further handling step is necessary. The appearance of red bands at the test "T" level and the control "C" level are considered positive for E.coli 0157. A signal only at the control "C" level is considered negative.

ChromoCult® Enterococci Broth

A selective enrichment medium for the detection of faecal (intestinal) streptococci/enterococci in water samples.

The advantages of FluoroCult® LMX, Readycult® Coliform and ChromoCult® Coliform Agar

More effective	• *Simultaneous detection of total coliforms and E.coli*
Faster	• *Two confirmed results in one test within 24h allows a water municipality to take corrective actions*
More specific	• *Distinct color reaction eases routine reading with high confidence level for personnel* • *ß-galactosidase reaction (99%) is more specific than gas reaction (95%) and 99% of E.coli's are Indole - positive, whereas only 90% of E.coli's produce gas and acid from lactose at 44°C*
Easier	• *No Durham tube necessary* • *No further confirmation required* • *Less false positives* • *Naturally yellow-colored water samples are not a problem*
Economical	• *Cost reduction, because less material and less workload in the lab*
Convenient	• *Readycult® easy-to-use snap pack format can be used in the field and incubated during transportation to the lab*

EPA approval

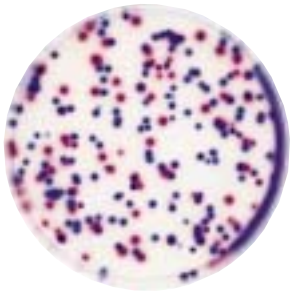
ChromoCult® Coliform Agar is a US EPA approved method to test drinking water for total coliforms and E.coli using the Membrane Filtration method.

The advantages of ChromoCult® Enterococci Broth / Agar and Readycult® Enterococci

More specific	• *Clear color changes*
Faster	• *MPN / P-A: generally within 24 h* • *Membrane Filtration (MF): within 24-48 h*
Economical	• *Less false positive results* • *No further confirmation of faecal (intestinal) streptococci required*
Convenient	• *Readycult® easy-to-use snap pack format*

ChromoCult® Enterococci Broth and Readycult® Enterococci 100

ChromoCult® Enterococci Broth uses for the suppresion of non enterococci similar to Azide Dextrose Broth an azide selective system. ChromoCult® Entero-cocci Broth includes additional the chromogenic substrate X-Glu. The use of the X-Glu diagnostic system improves the accuracy and simplifies the reading. The enzyme ß-D glucosidase is characteristic for faecal streptococci/enterococci and splits the chromogenic substrate X-Glu resulting in a distinct color change from yellow to blue-green. The blue-green color of the broth confirms largely the presence of faecal streptococci within 24h. *S.bovis* and *S.equines* both grow in ChromoCult® Enterococci Broth/Readycult® Enterococci 100 producing
the blue-green color, whereas *Aerococcus viridans* grows but does not produce the typical blue-green color. Faecal streptococci do not require additional testing. The presence of *Enterococcus* spp. should be verified by confirming the growth in Brain Heart Infusion (BHI with 6.5% salt) at 45°C.



ChromoCult® Coliform Agar

is a selective agar for the simultaneous detection of total coliform bacteria and E.coli in drinking water.
E.coli: dark-violet
Coliform bacteria: pink-red
Non-coliforms: colorless or green

Membrane Filtration (MF)

ChromoCult® Coliform Agar

ChromoCult® Coliform Agar contains 2 chromogenic substrates, Salmon-Gal and X-Glu. The agar allows not only the distinguished differentiation between total coliforms, E.coli and non-coliform bacteria but also the enumeration of these organisms within 24 hours.

Total coliform bacteria

Coliform bacteria appear as pink to red colonies on ChromoCult® Coliform Agar. The color is the result of the splitting of Salmon-Gal by ß-D-galactosidase, an enzyme, which is produced by coliform bacteria.

Escherichia coli

E.coli bacteria appear as dark violet colonies on ChromoCult® Coliform Agar. This color is the result of the splitting of Salmon-Gal by ß-D galactosidase and X-Glu by ß-D-glucuronidase, which is produced by *E.coli*. Further confirmation of typical *E.coli* colonies can be achieved by the addition of a drop of Kovàcs' reagent to the dark pink colonies. This Indole reaction produces a red halo around the dark violet colonies. It has been shown that the ChromoCult® Coliform Agar performs better than other commercial products and the traditional Standard Methods (Manafi & Rosman 1999, Lee et al.1995).

ChromoCult® Enterococci Agar

is a selective agar for the detection of faecal (intestinal) streptococci/enterococci in water samples.

Faecal (intestinal) streptococci/enterococci: red
Aerococcus spp.: blue

ChromoCult® Enterococci Agar

To test for faecal streptococci (intestinal enterococci)/*Enterococcus*, Chromocult® Enterococci Agar contains a mixture of chromogenic substrates. The selective system is an azide selective system, similar to that of Slanetz and Bartley Agar and m-Enterococcus Agar for streptococci. The splitting of the chromogenic substrates by enzymes that characterize enterococci results in red colonies which are easily distinguished from the blue-violet or turquoise colored colonies of non-enterococci colonies like *Aerococcus* spp. The ChromoCult® Enterococci Agar is more specific than most non chromogenic streptococci/enterococci agars as e.g. *Aerococcus* and *S.dysgalactiae* produce typical colonies on KAA agar but do not produce typical red colonies on ChromoCult® Enterococci Agar.

Merck´s culture media

Merck has more than 100 years of experience as a manufacturer of products for culturing microorganisms. Already in 1878 Merck produced peptones that were initially used as food supplements. In 1885 Merck started to sell peptones, gelatine, agar-agar specifically for culturing microorganisms and began in 1892 to manufacture peptones on industrial scale specifically for culture media preparation.

Merck is a pharmaceutical company and this is unique in culture media manufacturing. Merck started in1910 with the manufacturing of dehydrated culture media and is the oldest manufacturer of this product. After having identified the hazards of working with powdered culture media, Merck pioneered as early as 1950s the manufacturing of granulated culture media and is until now the only manufacturer. Merck takes great care to ensure the quality of its products and the service to its customers.

Merck laboratory products for microbiology have always set the highest standards for quality. Our reliability is the result of painstaking quality control. Merck's internal laboratories involved in pharmaceutical, chemical, analytical and diagnostic research also keep a watchful eye to ensure that the quality of Merck's microbiology products remains flawless.

Merck's customers can rest assured that our products fully comply with all statutory requirements. Merck gives the assurance, for instance, that only TSE low risk substances are used in all our culture media. Laboratory staff can always refer to certificates of analysis and detailed safety data sheets. In addition, Merck's sophisticated ChemDat database, accessible via the Internet and our Health and Safety data CD-ROM provide an invaluable service to all our customers.

As one of the global market leaders in analytical reagents, Merck is a company whose products are found in virtually all of the world's scientific laboratories. Merck's innovative strength stems from a thorough knowledge of the market and of product applications but it is also its close cooperation with the customers that guarantees special user-targeted features in the new products. Merck undertakes everything itself: research and development, manufacture and also supply of complete product ranges of culture media and tests for microbiology, food and environmental analysis and hygiene monitoring.

Safety

- All culture media are manufactured from TSE (BSE) - "low risk" ingredients
- Certificates of suitability obtained for all animal based materials
- All culture media comply with the recommendationes of EDQM and the European Pharmacopoeia
- The granular form reduces the exposure to powder and hazardous/toxic chemicals

Quality standards

- Culture media manufactured by a pharmaceutical company
- Culture media with the highest quality of peptones
- A batch to batch consistent high performance
- Culture media quality controlled acc. to ISO 11133
- Clear and meaningful Certifications of Analysis
- A pharmaceutical Regulatory Documentation support

Economical aspects

- Cost saving, because granulation allows the incorporation of selective components in the dehydrated base medium: fewer purchase of additional supplements
- Large batches and longer shelf life reduce cost on quality control

Convenience

- Ease of handling due to no sticking and quick dissolution of the granules

Service

- Safety data sheets obtainable via Chemdat online, www.chemdat.info, or on CD-ROM
- Certificates of Analysis obtainable via internet www.merck.de
- Batch specific TSE (BSE) certificates
- Brochures with technical information

Rapid testing methods

The introduction of fluorogenic and chromogenic culture media has shortened the time to test results by days. The protocols including FluoroCult® LMX / Readycult® Coliform and chromogenic culture media, like ChromoCult® Coliform Agar and Enterococci Broth media, are rapid cultural testing methods.

The use of combinations of different chromogenic substrates or fluorogenic and chromogenic substrates allows the simultaneous testing of different microorganisms or groups of microorganisms.

FluoroCult® LMX Broth and ChromoCult® Coliform Agar are approved by the Environmental Protection Agency (EPA) for the simultaneous detection of coliform bacteria and *Escherichia coli*.

Membrane Filtration (MF)

Presence–Absence / Multiple tube test (P–A / MPN)

* *Method is approved by Environmental Protection Agency (EPA) for monitoring of drinking water quality in accordance with Standard Method 9223*

US EPA approved[1] acc. to Standard Method 9223 MF

ChromoCult® Coliform Agar[1], Chromoplate® Coliform Agar
Detection and enumeration of coliform bacteria and *Escherichia coli*

Preparation

Sample size resulting to 20–60 cfu per filter
Filter up to 100 ml water

Membrane Filtration

Detection — day 1

- Transfer membrane filter to ChromoCult® Coliform Agar
- Incubate at 36 ±1°C for 24h

Positive on ChromoCult® Coliform Agar

Confirmation — day 2

Confirmed result: Total coliforms and E.coli

- Count[2] salmon to red colonies on filter as coliform bacteria
- Count dark blue violet colonies as E.coli

Optional:

Verification — day 2

Add Kovàcs' reagent to dark blue violet colonies or transfer biomass to Bactident® Indole

Verified result: E.coli

Dark blue violet colonies with red halo or positive Bactident® Indole test are verified as E.coli

Product list

Stage	Culture medium / Reagent	Merck Cat.No.
Detection	ChromoCult® Coliform Agar[1]	1.10426.0500
	Chromoplate® Coliform Agar	1.00074.0020
Verification	Kovàcs' indole reagent	1.09293.0100
	Bactident® Indole	1.11350.0001

[1] USEPA: 40 CFR Part 141 (sec. 141.21) Federal Register/Vol 67, No. 209, Tuesday October 29, 2002/Rules and Regulations

[2] Count filters with 20-60 colonies / filter

Chromogenic Method MF

ChromoCult® Enterococci Agar
Detection and enumeration of faecal streptococci[1] and *Enterococcus spp.*

Sample size resulting to 20–60 cfu per filter
Filter up to 100 ml water

Membrane Filtration

- Transfer membrane filter to ChromoCult® Enterococci Agar
- Incubate at 36 ±1°C for 24 – 44 ±4h

Positive on
ChromoCult® Enterococci Agar

Confirmed result: Enterococcus spp.

Count all raised red-maroon or pink typical colonies as enterococci / faecal streptococci

day 1 — Preparation

day 2–3 — Detection

day 2–3 — Confirmation

Product list

Culture medium / Reagent	Merck Cat.No.	Stage
ChromoCult® Enterococci Agar	1.00950.0500	Detection

[1] Intestinal enterococci

US EPA approved[1] acc. to Standard Method 9223 P-A/MPN

ReadyCult® Coliforms 100[1] and FluoroCult® LMX Broth[1] Detection and enumeration of coliform bacteria and *Escherichia coli* in drinking water

Prep.

Sample
(e.g. 1 x 100ml or 5 x 20ml or 10 x 10ml)

Detection — day 1

- Add sample to equal volume of Readycult®/FluoroCult® LMX Broth (100ml sample to 100ml triple strength and 10ml to 10ml double strength)
- Incubate at 36 ±1°C for 24h

Positive reaction in Readycult®/FluoroCult® LMX Broth

Confirmation — day 2

Confirmed result: Coliforms

Coloration from yellow to blue green denotes coliforms present

Confirmed result: E.coli

- Blue green color and
- Fluorescence (365-366nm)

Optional:

Verification — day 2

Verified result: E.coli

- Blue green color and
- Fluorescence (365-366nm) and
- Positive Kovàcs - or Bactident® Indole test gives a verified E.coli result

Product list

Stage	Culture medium / Reagent	Merck Cat.No.
Detection	ReadyCult® Coliforms 50	1.01295.0001
	ReadyCult® Coliforms 100	1.01298.0001
	FluoroCult® LMX Broth modified according to Manafi and Ossmer[1]	1.10620.0500
Verification	Kovàcs' indole reagent	1.09293.0100
	Bactident® Indole	1.11350.0001

[1] USEPA: 40 CFR Part 141 (Sec. 141.21) Federal Register/Vol. 67, No. 209, Tuesday, October 29, 2002/Rules and Regulations

ReadyCult® Enterococci 100 and ChromoCult® Enterococci Broth
Detection and enumeration of faecal streptococci[1] and *Enterococcus spp.* in drinking water

Prep.

Sample
(e.g. 1 x 100ml or 5 x 20ml or 10 x 10ml)

day 1 — Detection

- Add sample to equal volume of Readycult®/ChromoCult® Enterococci Broth (100 ml sample to 100ml triple strength and 10ml to 10ml double strength)
- Incubate at 36 ±1°C for 24 h

Positive reaction in
Readycult®/ FluoroCult® Enterococci Broth

day 2 — Conf.

Confirmed Result: Enterococcus spp.

Coloration from yellow to blue green

Product list

Culture medium / Reagent	Merck Cat.No.	Stage
Readycult® Enterococci 100	1.01299.0001	Detection
ChromoCult® Enterococci Broth	1.10294.0500	

[1] Intestinal enterococci

ISO

International Standardisation Organisation (ISO)

The International Organisation for Standardisation (ISO) is a network of the national standards institutes of 147 countries. There is one member per country, with a Central Secretariat in Geneva, Switzerland, that coordinates the system. ISO is a non-governmental organization; its members are not delegations of national governments as this is the case in the United Nations system.

International Standards are developed by ISO technical committees (TC) and subcommittees (SC).

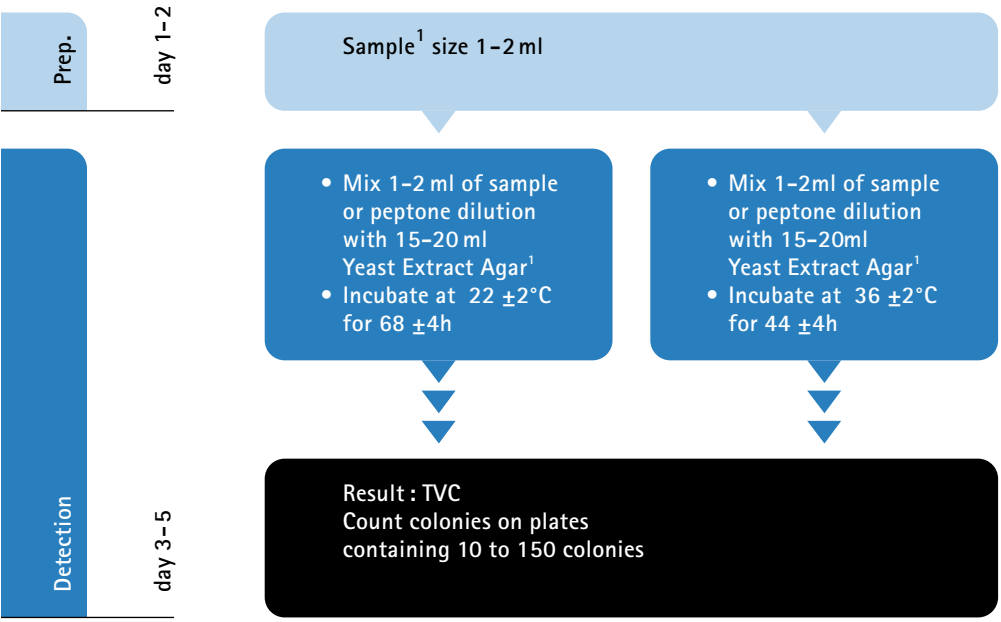
Most standards require periodic revision and therefore ISO has established the general rule that all ISO standards should be reviewed at intervals of not more than five years. If required, a revision of a standard can occur earlier.

Plate Count (TVC)

Membrane Filtration (MF)

Presence-Absence - Multiple tube test (P-A/MPN)

Enumeration of culturable microorganisms
Colony Count by inoculation in a Nutrient Agar culture medium



Product list

Stage	ISO 6222 1999 product description	MERCK product description	Merck Cat.No.
Detection	Yeast extract agar	Yeast extract agar acc. to ISO 6222 and Swedish Standard SS 028171	1.13116.0500
Dilution	Peptone diluent (ISO 8199)	Peptone from casein, pancreatically digested, granulated	1.07213.1000/2500

Other products for enumeration of culturable microorganisms

Stage	ISO or DEV product description	MERCK product description	Merck Cat.No.
Detection	Deutsches Einheitsverfahren	DEV nutrient agar	1.11471.0500/5000
		Merckoplate® DEV-Nutrient agar	1.00075.0020
		DEV gelatin agar	1.10685.0500
Dilution	Peptone water (1 %)	Peptone water (buffered); acc. to ISO 6579	1.07228.0500
	Peptone diluent (1 %)	Peptone from casein, pancreatically digested, granulated	1.07213.1000/2500
	Peptone saline solution	Maximum recovery diluent	1.12535.0500
	Ringer's solution	RINGER tablets	1.15525.0001
	Phosphate buffer solution	Potassium dihydrogen phosphate (ISO,Reag. Ph Eur)[2]	1.04873.0250/1000/5000
		Magnesium chloride[2]	8.14733.0100/0500

[1] Sampling acc. to ISO 5667-1, 2 and 3; Sample preparation, dilution and inoculation acc. to ISO 8199, 5667-3 and 6887

[2] For preparation of phosphate buffer solution

ISO 11731 1998 – 2 2004[1] MF

Detection and enumeration of Legionella

Sample size 1 l
(in presence of biocide add inactivating agent)

Low count

Membrane Filtration

Centrifugation
(6000 g for 10 min. or
3000 g for 30 min.)

High count

- Direct plating
  if sample contains
  > 10^5 Legionella[2] cfu/ml
- Dilution in Pages saline or
  Ringers, if samples contain
  high cfu non Legionella

Without treatment[3]

Heat treatment[3]

1 ± 0.5 ml of sample
at 50 °C for 30 ± 2 min.

Acid treatment[3,4]

1 – 10 ml of sample
- Centrifuged (6000 g
  for 10 min. or 3000 g
  for 30 min.)
- Remove half of
  supernatans volume
- Add acid buffer and
  resuspend
- Let stand 5 +0.5 min.

day 1 – Preparation

- Spread plate 0.1 – 0.5 ml on to GVPC Agar[5]
- Incubate at 36 ± 1 °C and read plates after 2, 4, 10 d

Presumptive result: Legionella
- Examine[6] for at least three occasions at 2d intervals plates with binocular microscope
- Count each colony with an entire edge and typical ground glass appearance and colored:
  white-grey-blue-purple, brown, pink, lime green or deep red

day 2 – Detection

- Select three[7] colonies characteristic of Legionella
- Subculture each onto BYCE and BCYE-cys[8] (alternative to BCYE is Nutrient Agar or Blood Agar)
- Incubate at 36 ± 1 °C for 48 h

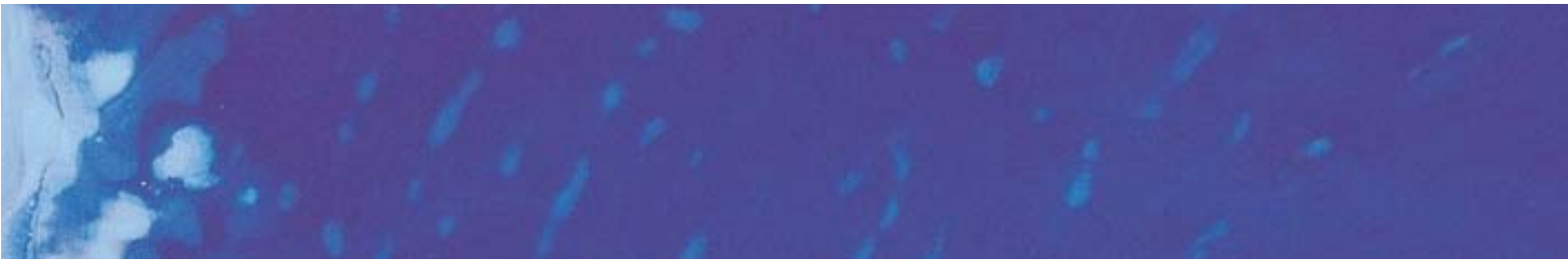
Confirmed result: Legionella

Legionella are colonies that grow on BCYE but fail to grow on BCYE-cys[9]

day 3 – 5 – Confirmation

[1] New ISO 11731-2 First Edition 2004-05-01 Detection and Enumeration of Legionella – Direct Membrane Filtration Method for waters with low bacterial counts. **Following foot notes 2-8 concern changes to ISO 11731-2 2004:** [2] > 2 x 10^4 cfu/ml; [3] > Membrane Filtration of 10 – 1000 ml after filtration add 20 ml of acid buffer on top of membrane and leave for 5 min., remove buffer by filtration and wash with 10 ml sterile water; [4] Each sample is further handled via Without treatment, Heat treatment and Acid treatment; [5] or BCYE Agar; [6] Examine with binocular microscope; [7] At least five colonies; [8] *L.oakrigenesis* and *L.spiritensis* require *L-cysteine* and iron; [9] If *Legionella spp.* or serogroups are to be reported use at least 3 colonies for identification by serology, immuno assay or chromatography

▶ Product list Next page 30



Product list: ISO 11731 1998

Stage	ISO 11731 1998 and ISO 11731-2 2004 product description	MERCK product description	Merck Cat.No.
Detection	Buffered Charcoal Yeast extract Agar Base Medium (BCYE) Buffered Charcoal Yeast extract Agar Base Medium with selective supplements (GVPC Medium)	Legionella Combi-Pack for the preparation of 6 x 500 ml Legionella-GVPC-Selective-Agar	1.10425.0001
	Buffered Charcoal Yeast extract Agar Base Medium (BCYE)	Merckoplate® Legionella BCYE agar	1.10097.0020
	Buffered Charcoal Yeast extract Agar Base Medium with selective supplements (GVPC Medium)	Merckoplate® Legionella GVPC-selective agar	1.10097.0020
	Buffered Charcoal Yeast extract Agar Base Medium (BCYE)	Legionella CYE Agar Base	1.10242.0500
	Buffered Charcoal Yeast extract Agar Base Medium with selective supplements (GVPC Medium)	Legionella GVPC selective supplement	1.10241.0001
		Legionella BCYE a-growth supplement	1.10240.0001
Confirmation	BCYE-Cys	-	-
Dilution	Diluted Ringer's solution	RINGER tablets[1]	1.15525.0001
	Phosphate buffered saline	Potassium dihydrogen phosphate (ISO,Reag. Ph Eur)	1.04873.0250/1000/5000
		di-Sodium hydrogen phosphate (ACS,Reag. Ph Eur)	1.06586.0500/2500
		Sodium chloride (ACS,ISO,Reag. Ph Eur)	1.06404.0500/1000/5000
		Potassium chloride (GR for analysis)	1.04936.0500/1000/5000
	Pages saline	Potassium dihydrogen phosphate (ISO,Reag. Ph Eur)	1.04873.0250/1000/5000
		Magnesium chloride	8.14733.0100/0500
		Calcium chloride dihydrate (ACS,Reag. Ph Eur)	1.02382.0250/0500/5000
		di-Sodium hydrogen phosphate (ACS,Reag. Ph Eur)	1.06586.0500/2500
		Sodium chloride (ACS,ISO,Reag. Ph Eur)	1.06404.0500/1000/5000
	Formol saline	-	-

[1] To prepare 1:10 dilution of Ringers solution quarter strength

Product list: ISO 9308-1 2000

Stage	ISO 9308-1 2000 product description	MERCK product description	Merck Cat.No.
Detection	Lactose TTC agar with sodium heptadecylsulfate	Lactose TTC Agar with Tergitol® 7	1.07680.0500
		Merckoplate® Lactose TTC Agar with Tergitol® 7	1.00074.0020
	Tryptophan broth	DEV tryptophan broth	1.10694.0500
	Tryptone soy agar	Tryptic soy agar (USP)	1.05458.0500
	Tryptone Bile Agar	Chromocult® TBX (Tryptone Bile X-glucuronide) Agar	1.16122.0500
Confirmation	Kovacs' reagent for indole test	KOVACS' indole reagent	1.09293.0100
		Bactident® Indole	1.11350.0001
	Oxidase reagent	Bactident® Oxidase	1.13300.0001

Products for dilution

Stage	ISO product description	MERCK product description	Merck Cat.No.
Dilution	Peptone water, buffered (1%)	Peptone water (buffered); acc. to ISO 6579	1.07228.0500
	Peptone diluent (1%)	Peptone from casein, pancreatically digested, granulated	1.07213.1000/2500
	Peptone saline solution	Maximum recovery diluent	1.12535.0500
	Ringer's solution	RINGER tablets	1.15525.0001
	Phosphate buffer solution	Potassium dihydrogen phosphate (ISO,Reag. Ph Eur)[2]	1.04873.0250/1000/5000
		Magnesium chloride[2]	8.14733.0100/0500

[a1] Sampling acc. to ISO 5667-1, 2 and 3; Sample preparation, filter, dilution and inoculation acc. to ISO 8199, 6887-1

[2] For preparation of Phosphate buffer solution

Detection and enumeration of *Escherichia coli* and coliform bacteria

Sample[1] size 100 ml or more (e.g. 250 ml for bottled water) resulting to 20 - 80 cfu per filter

Membrane Filtration[1]

day 1 — Preparation

Standard test

- Transfer membrane filter to Lactose TTC Agar with Tergitol® 7
- Incubate at 36 ±2°C for 21 ±3h if negative reincubate to 44 ±4h

Positive on Lactose TTC Agar with Tergitol® 7

Presumptive result: Coliform bacteria Count yellow lactose positive colonies

Rapid test (optional)

- Transfer membrane filter to Tryptic Soy Agar (TSA)
- Incubate at 36 ±2°C for 4-5h

- Transfer membrane filter from TSA to Trypton Bile Agar (TBA)
- Incubate at 44 ±0.5°C for 19-20h

day 2 - 3 — Detection

Subculture (> 10 to all) on:

- Tryptic Soy Agar (TSA)
- Incubate at 36 ±3°C for 21 ±3h

- Tryptophan Broth
- Incubate at 44 ±0.5°C for 21 ±3h

Oxidase test

Indole test

Indole test (Kovàcs' reagent and UV irradiation)

Confirmed result: Coliform bacteria

Count Oxidase (-) colonies (TSA) as coliform bacteria

Confirmed result: E.coli

Count Oxidase (-) and Indole (+) as colonies as E.coli

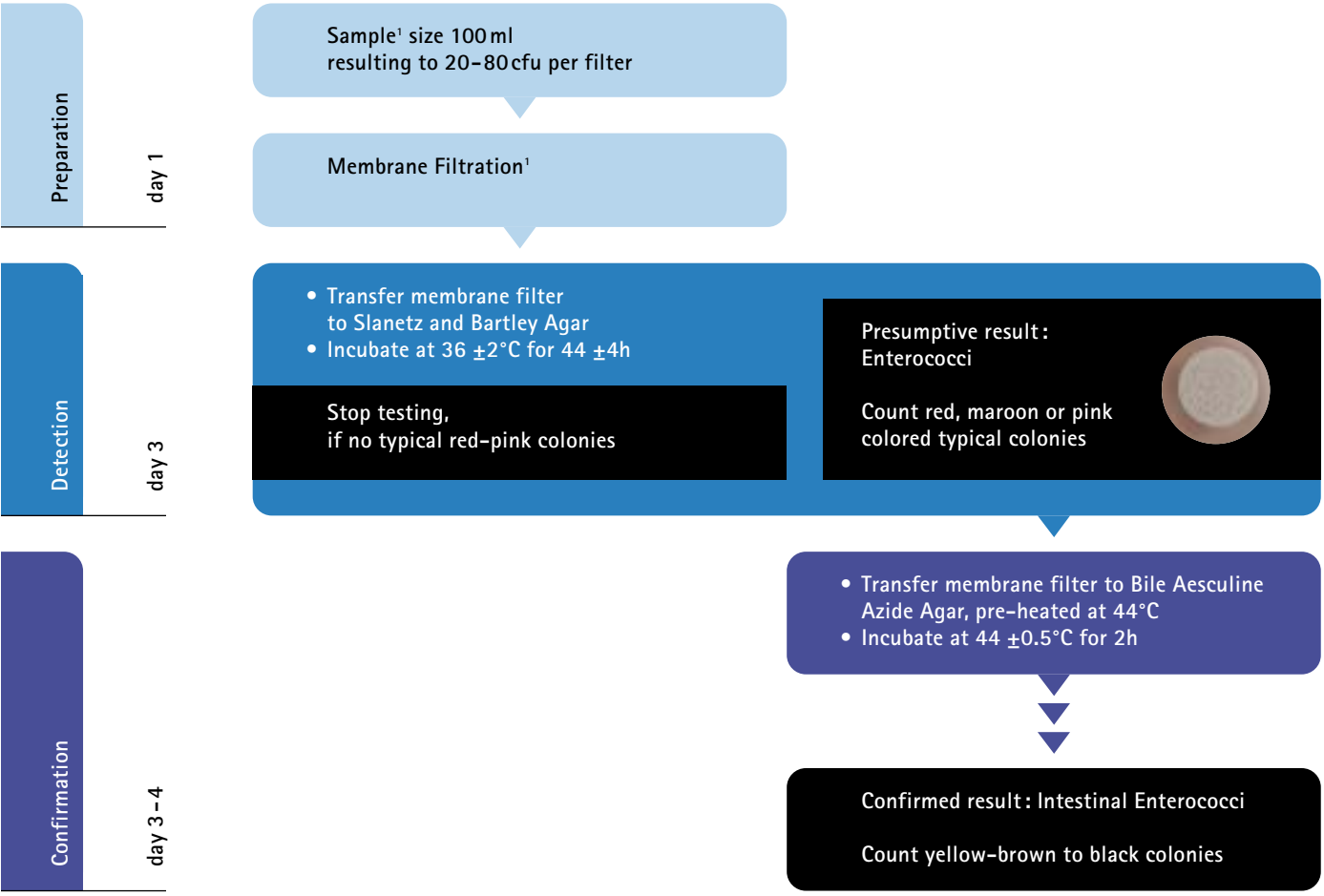
Confirmed result: E.coli

Count all Indole (+) as colonies as E.coli

day 4 — Confirmation

Product list
Previous page 30

Detection and enumeration of intestinal enterococci



Product list

Stage	ISO 7899-2 2000 product description	MERCK product description	Merck Cat.No.
Detection	Slanetz and Bartley medium (basal medium)	Membrane-filter enterococcus selective agar acc. to SLANETZ and BARTLEY (base)	1.05289.0500
		Merckoplate® Membrane-filter Enterococcus selective agar acc. to SLANETZ and BARTLEY	1.0076.0020
	Complete medium (Slanetz and Bartley medium + TTC)	Membrane-filter enterococcus selective agar acc. to SLANETZ and BARTLEY	1.05262.0500
Confirmation	Bile-aesculin-azide-agar	Bile aesculin azide agar, acc. to ISO 7899-2	1.00072.0500
		Merckoplate® Bile-aesculin-azide-agar	1.00077.0020

[1] Sampling acc. to ISO 5667-1, 2 and 3; Sample preparation, filter, dilution and inoculation acc. to ISO 8199, 5667-3 and 6887-1

Detection and enumeration of the spores of sulphite-reducing anaerobes (clostridia)

- Sample[1] size resulting to 20-80 cfu per filter
- Heat at 75 ±5°C for 15 min.

Membrane[1] Filtration

Transfer membrane filter
- to Nutrient Agar with sulphite and iron (II) sulphate or
- to Tryptose Sulphite Agar

- Incubate at 37 ±2°C for 20 ±4 and 44 ±4h Anaerobic conditions

Confirmed result:
Sulphite reducing clostridia

Count typical white colonies with black halo

day 1 — Preparation

day 2-3 — Detection

Product list

ISO 6461-2 1986 product description	MERCK product description	Merck Cat.No.	Stage
Tryptose sulfite agar	Tryptose sulfite cycloserine agar (base)	1.11972.0500	Detection
	Merckoplate® TSC Agar	1.00078.0020	
Sulfite iron agar	Standard II nutrient agar	1.07883.0500	
	Sodium sulfite (Ph Eur, BP, E 221)	1.06652.1000	
	Iron (II) sulfate heptahydrate (ACS, ISO, Reag. Ph Eur)	1.03965.0100 / 0500	
Anaerobic incubator	Anaerobic jar 2,5 l-volume	1.16387.0001	

Other products for anaerobic incubation

ISO 6461-2 1986 product description	MERCK product description	Merck Cat.No.	Stage
Anaerobiosis	Anaerocult® A for microbiology (Reagent for the generation of ananaerobic medium in anaerobic jars)	1.13829.0001	Detection
	Anaerocult® A mini Gas generating system for the incubation of one to four petri dishes in an anaerobic atmosphere	1.01611.0001	
	Anaerocult® P for microbiology for generating an anaerobic atmosphere in the single Petri dish	1.13807.0001	
	Petri-dish rack for up to 12 petri dishes	1.07040.0001	
	Anaeroclip®	1.14226.0001	
	Anaerotest®	1.15112.0001	

[1] Sampling acc. to ISO 5667-1, 2 and 3; Sample preparation, filter, dilution and inoculation acc. to ISO 8199, 5667-3 and 6887-1

ISO/WD 6461 - 2 2002 MF

Detection and enumeration of *Clostridium perfringens*

Preparation — day 1

- Sample[1] size resulting to 20-80 cfu per filter
- For potable water 100 ml Heat at 60 ±2°C for 15 ±1 min.

Membrane Filtration[1]

Detection — day 2

- Transfer membrane filter to Tryptose Sulphite Cycloserine Agar without egg yolk (TSC Agar) or m-CP Agar[2]
- Incubate at 44 ±1°C for 21 ±3h Anaerobic conditions

Presumptive result: Clostridium perfringens

Count all black colonies

Confirmation — day 3 - 4

- Subculture (>10 or all) typical well isolated colonies to two plates of Columbia Blood Agar
- Incubate at 36 ±2°C for 21 ±3h one plate aerobically and one plate anaerobically

Nitrate-Motility test:
- Inoculate Buffered Nitrate-Motility Medium
- Incubate at 36 ±2°C for 21 ±3h

Acid-Gelatin liquification:
- Inoculate Lactose Gelatin Medium
- Incubate at 36 ±2°C for 21 ±3h

Confirmed result: Clostridium perfringens

Count black colonies that
- are non-motile
- reduce nitrate to nitrite
- produce acid from lactose
- liquefy gelatin within 44 ±4 h

Product list

ISO 6461-2 2002 product description	MERCK product description	Merck Cat.No.	Stage
Tryptose sulfite cycloserine agar without egg yolk	Tryptose sulfite cycloserine agar (base)	1.11972.0500	Detection
	Merckoplate® TSC Agar	1.0078.0020	
Buffered nitrate motility medium	-	-	Confirmation
Blood agar (with 5% horse blood)	Columbia agar base	1.10455.0500/5000	
Lactose Gelatin Medium	DEV nutrient gelatin[3]	1.10691.0500	
	Nutrient gelatin[3]	1.04069.0500	
	Lactose monohydrate[3]	1.07657.1000/5000	
	Phenol red (ACS)[3]	1.07241.0005/0025/0100	
Nitrate reagent A and B	Griess-Ilosvay's reagent for detection of nitrite	1.09023.0500	
Anaerobic conditions	Anaerobic jar 2,5 l-volume	1.16387.0001	

Other products for anaerobic incubation

ISO 6461-2 1986 product description	MERCK product description	Merck Cat.No.	Stage
Anaerobiosis	Anaerocult® A for microbiology (Reagent for the generation of ananaerobic medium in anaerobic jars)	1.13829.0001	Detection
	Anaerocult® A mini gas generating system for the incubation of one to four petri dishes in an anaerobic atmosphere	1.01611.0001	
	Anaerocult® P for microbiology for generating an anaerobic atmosphere in the single Petri dish	1.13807.0001	
	Petri-dish rack for up to 12 petri dishes	1.07040.0001	
	Anaeroclip®	1.14226.0001	
	Anaerotest®	1.15112.0001	

[1] Sampling acc. to ISO 5667-1, 2 and 3; Sample preparation, filter, dilution and inoculation acc. to ISO 8199, 5667-3 and 6887-1

[2] In accordance with 98/83/EC Annex III

[3] Merck product is termed DEV Gelatin to which 10 g Lactose (Cat.No. 1.07657.1000) and 12.5 ml phenol red (0.4%) w/v Solution (Cat.No. 1.11748.0005) is added

[4] m-CP-Agar acc. to 98/83/EC Annex III is not available from Merck KGaA

Detection and enumeration of *Pseudomonas aeruginosa*

Preparation — day 1

Sample[1] size to give 20 – 80 cfu per filter

Membrane Filtration[1]

Detection — day 2 – 3

- Transfer membrane filter to Pseudomona Selective (CN Agar)
- Incubate at 36 ±2°C for 44 ±4h

Pyocianinforming colonies

Confirmed result:
Pseudomona aeruginosa

Count colonies that
- are fluorescent with blue green (pyocyanin) pigment

Fluorescent colonies

Presumptive result:
Pseudomona aeruginosa

Count fluorescent non blue green (pyocyanin) colonies

Confirmation — day 4 – 9

- Transfer isolates to Acetamide Broth
- Incubate at 36 ±2°C for 44 ±4h

Confirmed result:
Pseudomona aeruginosa

Count colonies that
- are fluorescent without blue green pigment
- produce ammonia in Acetamide Broth

Red brown colonies

Presumptive result:
Pseudomona aeruginosa

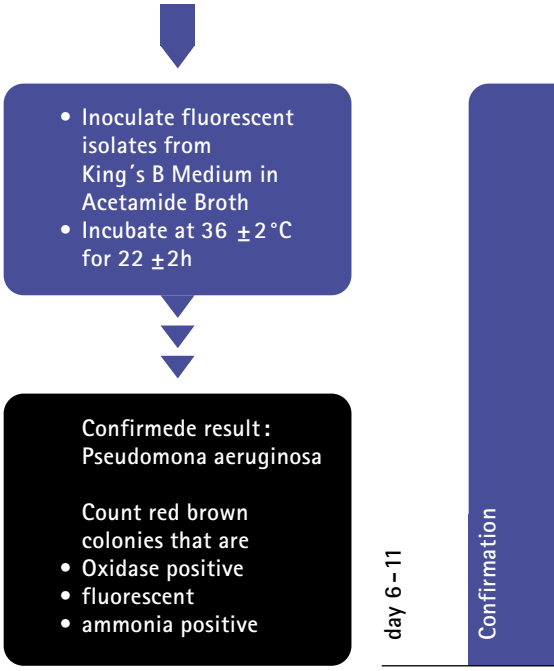
Count non fluorescent or red brown colonies

- Pure culture red brown colonies on Nutrient Agar
- Incubate at 36 ±2°C for 22 ±2h

Oxidase test

- Subculture oxidase positive and red brown colonies on King's B Medium
- Incubate at 36 ±2°C for up to 5 days

Go to next page



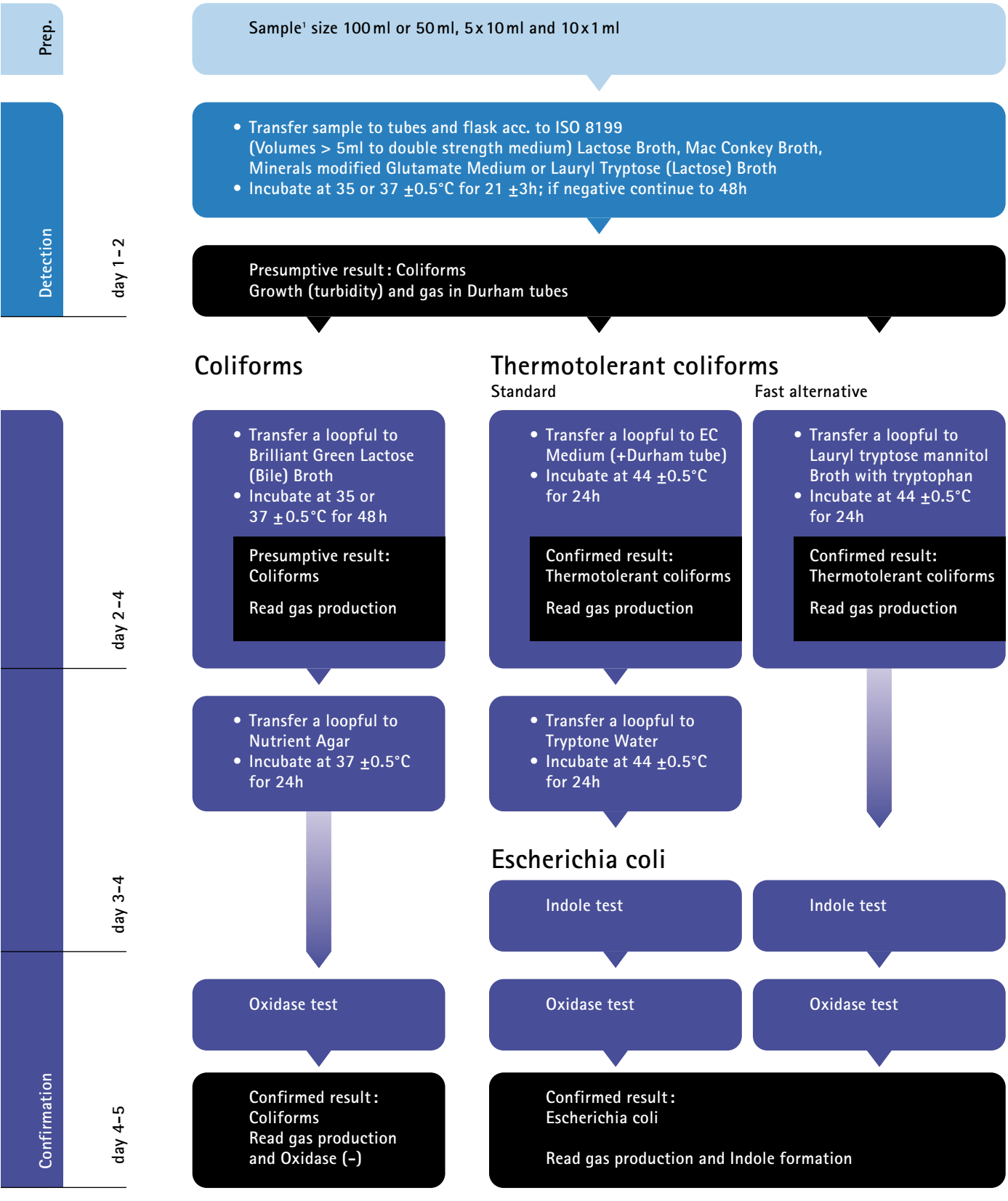
Product list

DIN EN 12780 2002 product description	MERCK product description	Merck Cat.No.	Stage
Pseudomonas selective agar (CN agar)	Pseudomonas Selektivagar (Basis)	1.07620.0500	**Detection**
	Pseudomonas CN Selektiv Supplement	1.07624.0001	
Kings B medium	KING agar B base (Dansk Standard)	1.10991.0500	**Confirmation**
Acetamide nutrient solution	-	-	
Nutrient agar	Standard II nutrient agar	1.07883.0500	
Oxidase reagent	Bactident® Oxidase	1.13300.0001	
Nessler's reagent	Nessler's reagent	1.09028.0100/0500	

[1] Sampling acc. to ISO 5667-1,2 and 3; Sample preparation, filter, dilution, and inoculation acc. to ISO 8199, 5667-3 and 6887-1

ISO 9308 - 2 1990 P-A/MPN

Detection and enumeration of coliform organisms, thermotolerant coliform organisms and Escherichia coli

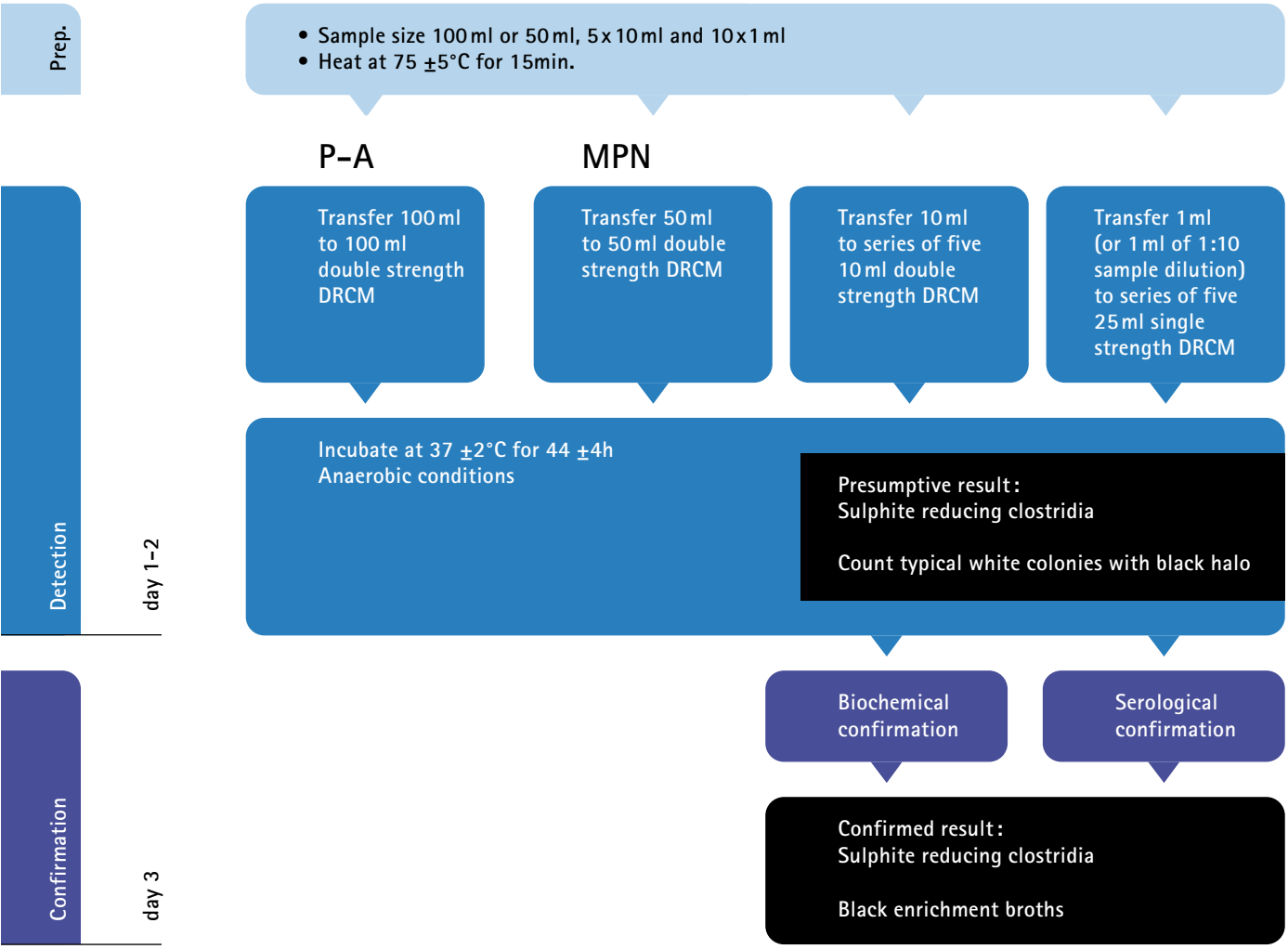


Product list

ISO 9308-2 1990 product description	MERCK product description	Merck Cat.No.	Stage
Lactose broth	Lactose broth	1.07661.0500	**Detection**
MacConkey broth	MacCONKEY broth	1.05396.0500	
Lauryl tryptose (lactose) broth	Lauryl sulfate broth	1.10266.0500	
Improved formate lactose glutamate medium	DEV glutamate broth	1.10687.0500	
EC medium	EC broth	1.10765.0500	**Confirmation**
Brilliant green lactose (bile) broth	Brilliant green-bile-lactose broth	1.05454.0500/5000	
Lauryl tryptose mannitol broth with tryptophan	-	-	
Nutrient agar	Standard II nutrient agar	1.07883.0500	
Tryptone water	Tryptone water	1.10859.0500	
Kovacs' reagent for indole	KOVACS' indole reagent	1.09293.0100	
	Bactident® Indole	1.11350.0001	
Oxidase reagent	Bactident® Oxidase	1.13300.0001	
Ringer's solution quarter strength	RINGER tablets	1.15525.0001	**Dilution**
Peptone diluent 0.1%	Peptone from casein, pancreatically digested, granulated	1.07213.1000/2500	
Peptone saline solution	Maximum recovery diluent	1.12535.0500	
Phosphate buffer solution	Potassium dihydrogen phosphate (ISO,Reag. Ph Eur)	1.04873.0250/1000/5000	
	Magnesium chloride	8.14733.0100/0500	

[1] Sampling acc. to ISO 5667-1, 2 and 3; Sample preparation, filter, dilution and inoculation acc. to ISO 8199, 5667-3 and 6887-1

Detection and enumeration of the spores of sulphite reducing anaerobes (clostridia)



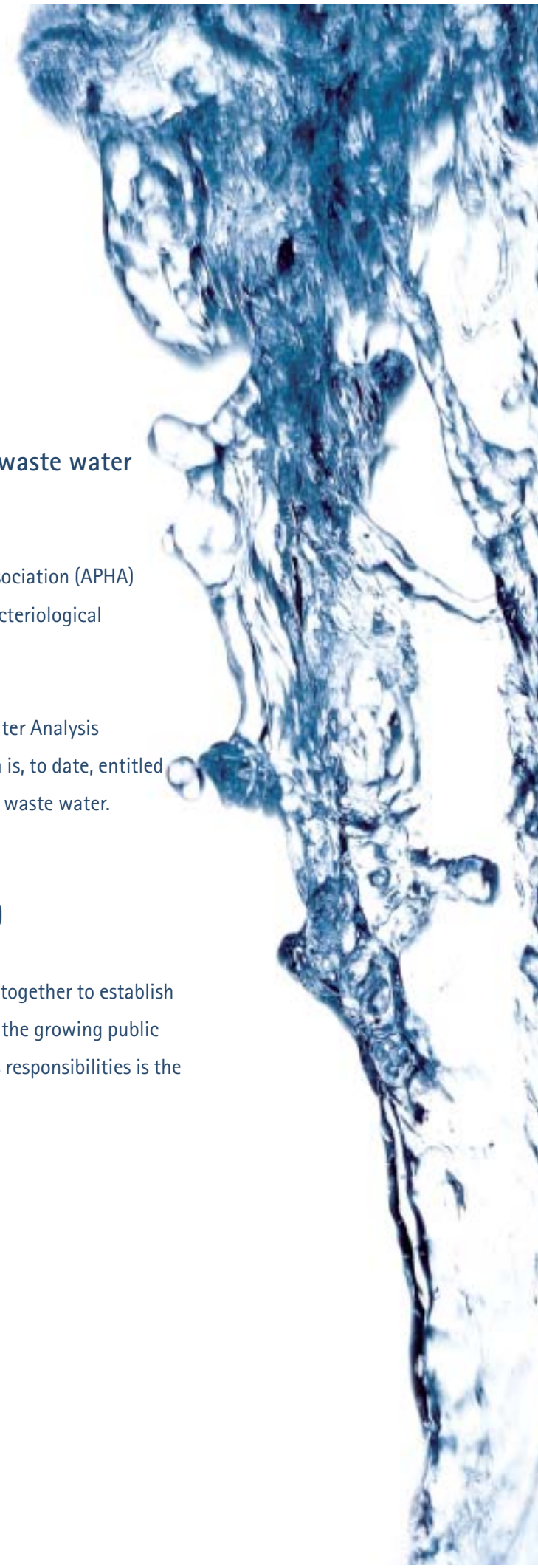
Product list

Stage	ISO 6461-1 product description	MERCK product description	Merck Cat.No.
Detection	DRCM medium	Differential reinforced clostridial broth (DRCM)	1.11699.0500
	Anaerobic conditions	Anaerobic jar 2.5 l-volume	1.16387.0001
Dilution	Ringer's solution	RINGER tablets	1.15525.0001
	Peptone diluent	Peptone from casein, pancreatically digested, granulated	1.07213.1000/2500
	Peptone saline solution	Maximum recovery diluent	1.12535.0500
	Phosphate buffer solution	Potassium dihydrogen phosphate (ISO,Reag. Ph Eur)	1.04873.0250/1000/5000
		Magnesium chloride	8.14733.0100 / .0500

Other products for anaerobic incubation

ISO 6461-1 product description	MERCK product description	Merck Cat.No.	Stage
Anaerobiosis	Anaerocult® A for microbiology (Reagent for the generation of ananaerobic medium in anaerobic jars)	1.13829.0001	Detection
	Anaerocult® A mini Gas generating system for the incubation of one to four petri dishes in an anaerobic atmosphere	1.01611.0001	
	Anaerocult® P for microbiology for generating an anaerobic atmosphere in the single Petri dish	1.13807.0001	
	Petri-dish rack for up to 12 petri dishes	1.07040.0001	
	Anaeroclip®	1.14226.0001	
	Anaerotest®	1.15112.0001	

SMWW - APHA/EPA



Standard Methods (SM) for water and waste water analysis (SMWW – APHA)

In 1895, members of the American Public Health Association (APHA) recognized the need for Standard Methods in the bacteriological examination of water.

In 1905, the first edition of Standard Methods of Water Analysis was published. The reference book in its 20th edition is, to date, entitled Standard Methods for the examination of water and waste water.

Environmental Protection Agency (EPA)

In July 1970, the White House and Congress worked together to establish the Environmental Protection Agency in response to the growing public demand for cleaner water, air and land. One of EPA´s responsibilities is the evaluation of analytical methods for drinking water.

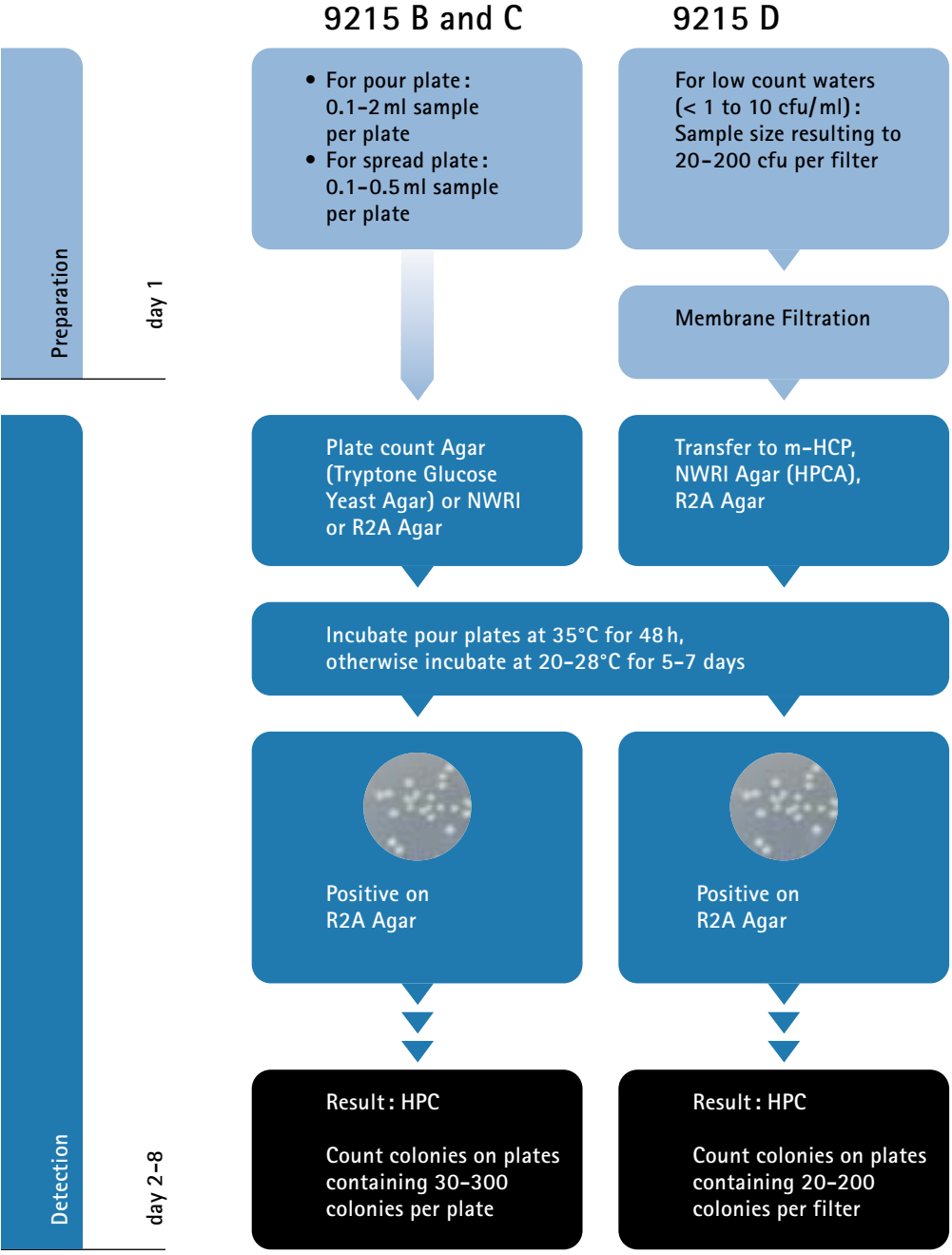
Plate Count (HPC)

Membrane Filtration (MF)

Presence-Absence / Multiple tube test (P-A / MPN)

= Verification

Heterotrophic Plate Count



Product list

Stage	Standard Method 9215 product description	MERCK product description	Merck Cat.No.
Detection	Plate count agar	Plate count agar	1.05463.0500/5000
		Merckoplate® Plate count agar	1.13108.0001
	m-HPC agar	-	-
	R2A agar	R2A Agar	1.00416.0500
		Merckoplate® R2A Agar	1.00073.0020
	NRWI agar	-	-

Products for dilution

See page 56 below

Standard total coliform Membrane Filtration procedure (Single step direct technique)

Sample 100 ml[1,2] for Membrane Filtration

Membrane Filtration

Delayed-Incubation

- Transfer filter to Petri dish with m-Endo preservative medium or M-ST holding medium
- Holding time up to 72h

day 1-3 Preparation

- Transfer filter to LES Endo Agar or m-Endo Agar
- Incubate at 35 ±0.5°C for 22-24h

Stop testing, if non-coliform colonies: Pink, red, white or colorless without metallic sheen

Presumptive result: Typical coliform colonies

Count pink to dark red colonies with metallic sheen

Presumptive result: Atypical coliform colonies

Count dark red or nucleated without metallic sheen

day 2-4 Detection

Go for verification of typical and atypical colonies to (page 46)

▶ Method 9222 B-5f

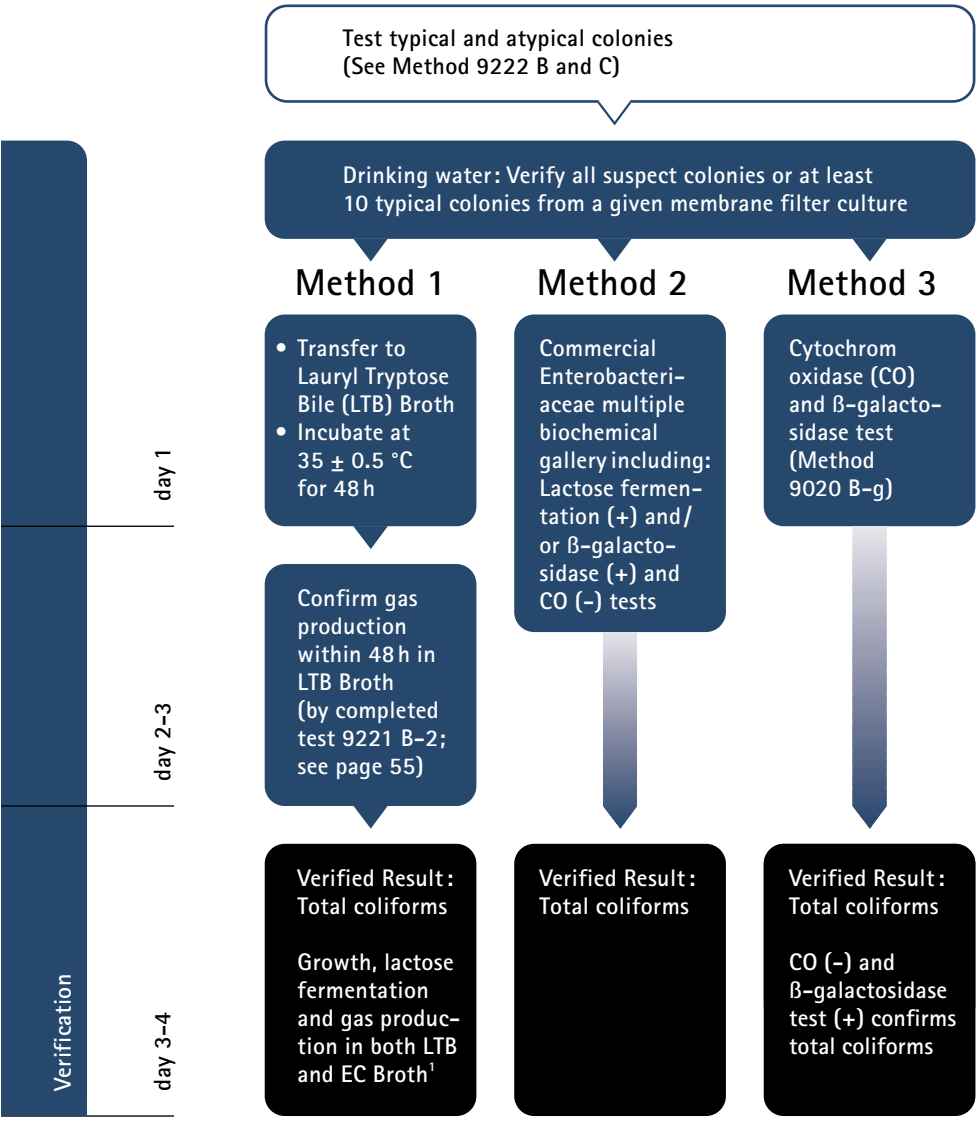
▶ Product list

Next page 46

[1] For regulation purpose 100 ml otherwise see note 2

[2] Ideal sample size results in 20 - 80 colonies but no more than 200

Verification of total coliforms



Product list

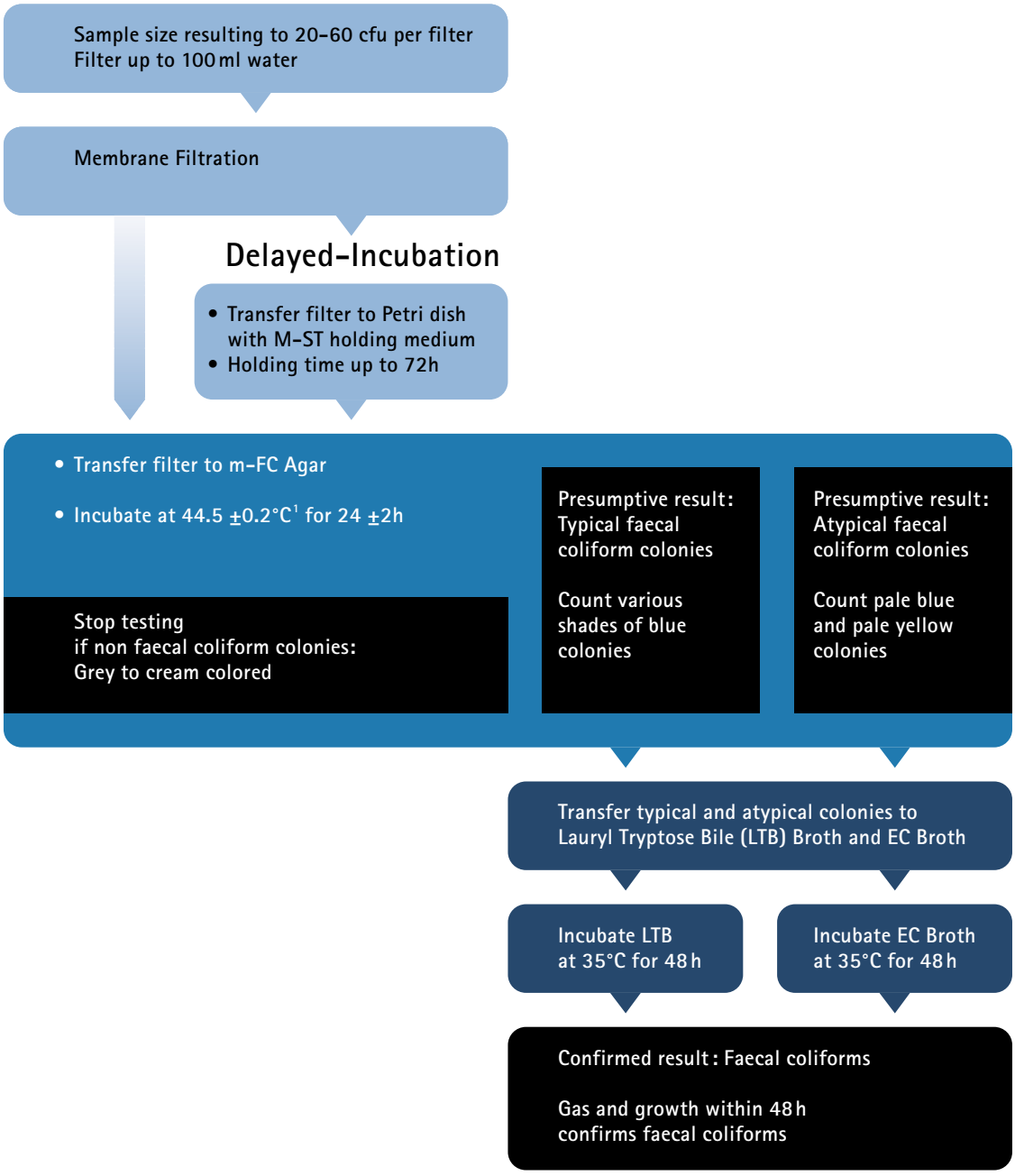
Stage	Standard Method 9222 B and C, 9221 B product description	MERCK product description	Merck Cat.No.
Preparation	M-Endo preservative medium	-	-
	M-ST holding medium	-	-
Detection	m-ENDO agar LES	m-ENDO agar LES	1.11277.0500
	M-Endo medium	-	-
Verification	Brilliant green-lactose-bile broth	Brilliant green-bile-lactose broth	1.05454.0500/5000
	Lauryl tryptose bile broth ß-galactosidase	-	-
	Cytochrom oxidase reagent	Bactident® Oxidase	1.13300.0001

[1] Inclusion of EC Broth at 44.5°C for 48 h confirms feacal coliforms; Inclusion of EC-MUG Broth at 35°C for 24 h confirms E. coli

Products for dilution

See page 56 below

Faecal coliform procedure (Single step direct technique)



Product list

Standard Method 9222 D and E product description	MERCK product description	Merck Cat.No.	Stage
M-ST holding medium	-	-	Preparation
M-FC medium	M FC Agar	1.11278.0500	Detection
EC broth	EC broth	1.10765.0500	Confirmation
Lauryl tryptose bile broth ß-galactosidase	-	-	

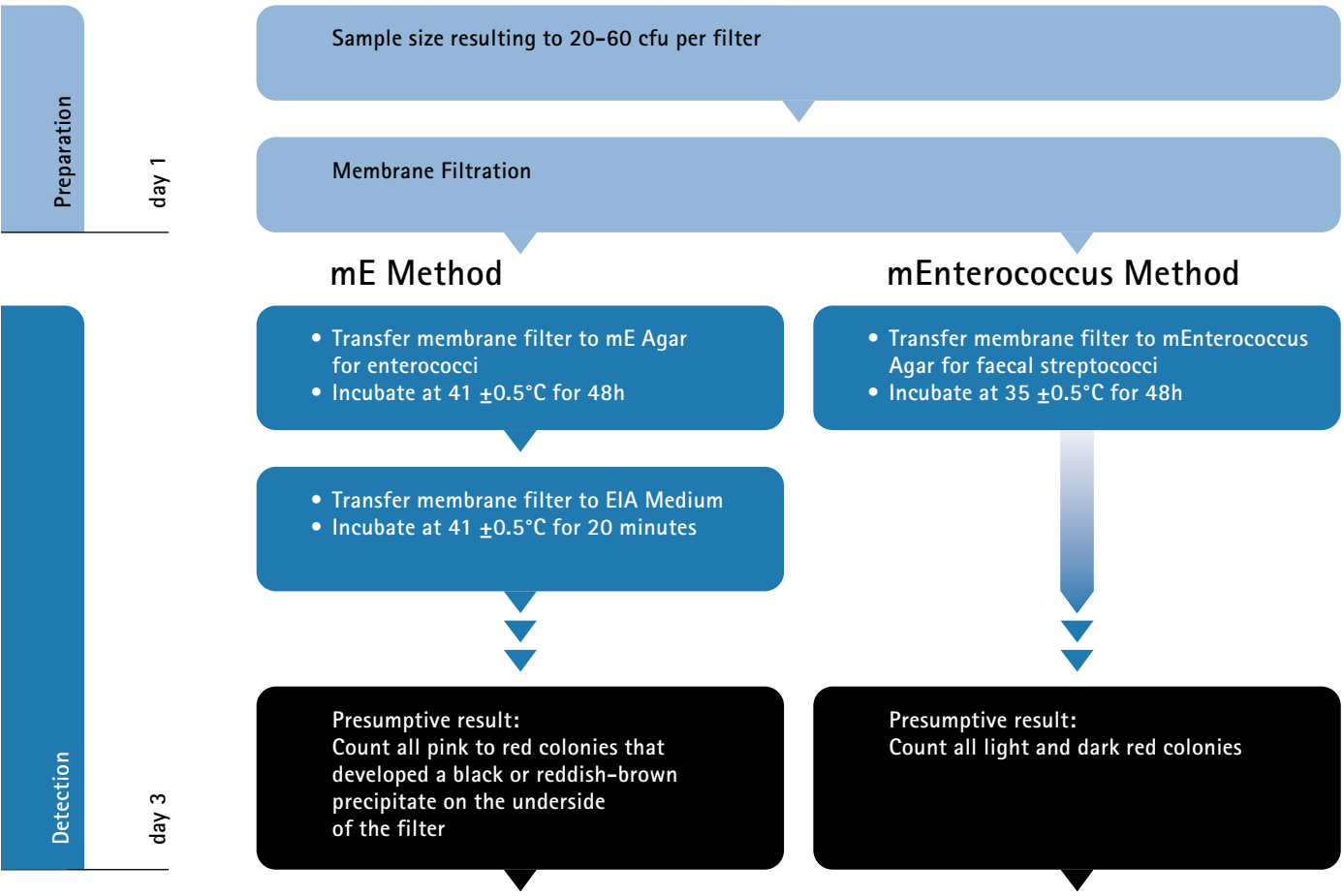
[1] Incubation at 45 ±0.2°C eliminates environmental *Klebsiella spp.*;

Products for dilution

See page 56 below

Standard Method 9230 C MF

Faecal *Streptococcus* and *Enterococcus* groups

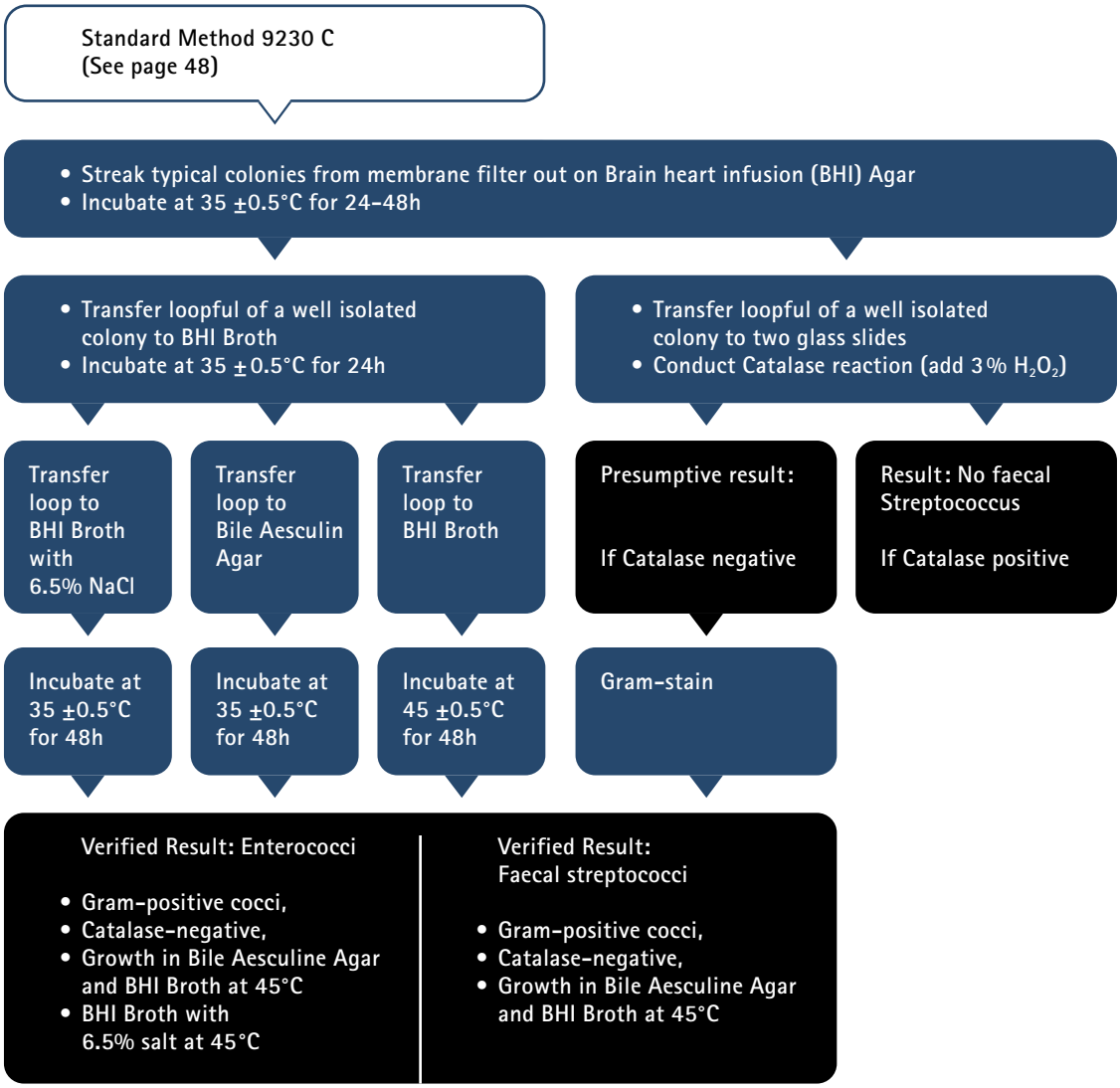


Go for verification of faecal Streptococcus and enterococci to (next page 49)

▶ Method 9230 C

▶ Product list
Next page 49

Faecal *Streptococcus* and *Enterococcus* groups
Verification



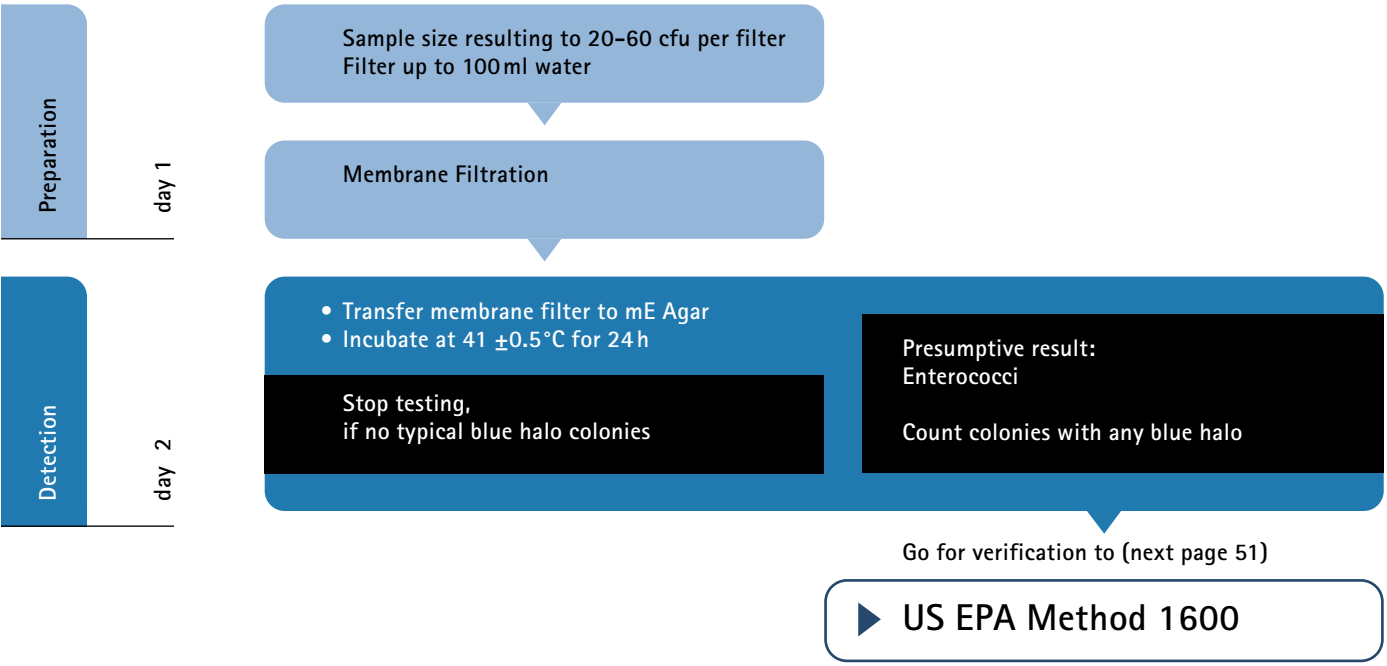
Product list

Standard Method 9230 C product description	MERCK product description	Merck Cat.No.	Stage
mE agar for enterococci	-	-	Detection
EIA medium	-	-	
mEnterococcus agar for faecal streptococcci	Membrane-filter enterococcus selective agar acc. to SLANETZ and BARTLEY	1.05262.0500	
Brain-heart infusion broth	Brain heart broth	1.10493.0500	Verification
Bile esculin agar	Bile Aesculin Azide Agar, acc. to ISO 7899-2	1.00072.0500	
	Merckoplate® Bile Aesculin Azide Agar	1.00077.0020	
Brain-heart infusion broth with 6.5% NaCl	Brain heart broth[1]	1.10493.0500	
	Sodium chloride (ACS, ISO, Reag. Ph Eur)[1]	1.06404.0500/1000/5000	
Gram stain	Gram-color stain set	1.11885.0001	
Catalase reagent	Bactident® Catalase	1.11351.0001	

[1] For preparation of Brain heart infusion broth – 6.5% NaCl

US EPA Method 1600 MF

The enumeration of *Enterococcus spp.*



Go for verification to (next page 51)

▶ US EPA Method 1600

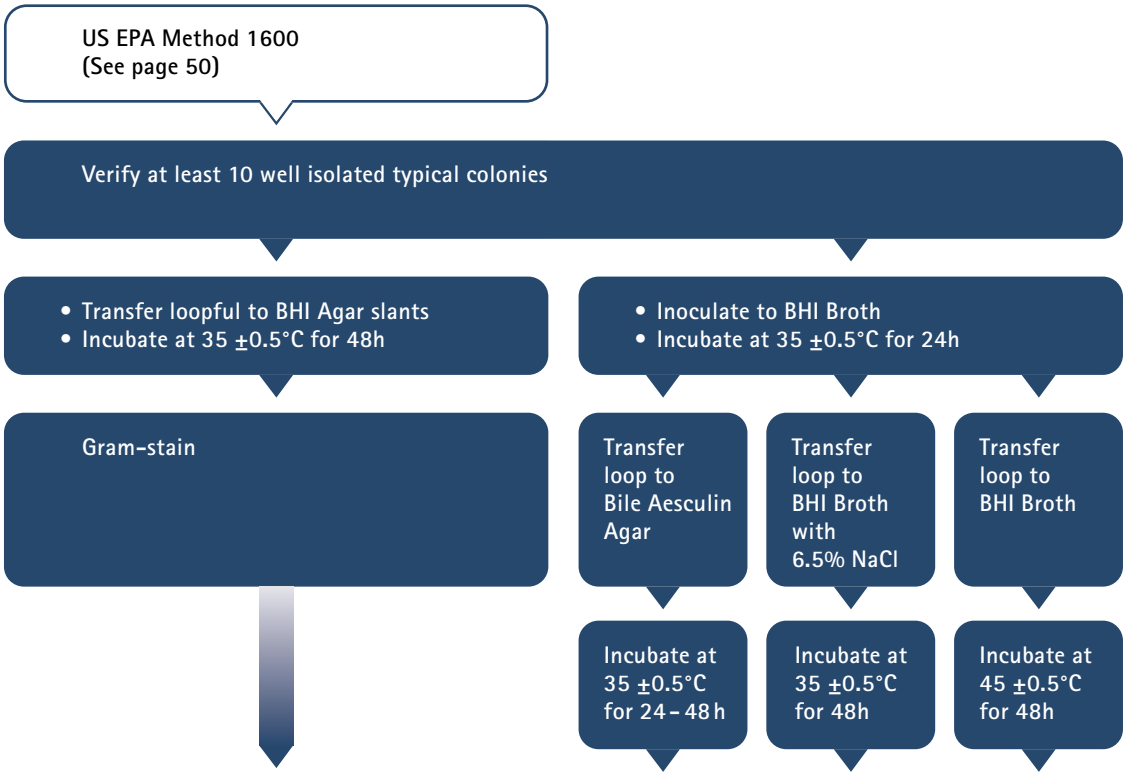
Product list

Stage	US EPA Method 1600 product description	MERCK product description	Merck Cat.No.
Detection	mEI agar	-	-
Dilution	Buffered dilution water	Sodium dihydrogen phosphate[1]	1.06346.0500/1000
		di-Sodium hydrogen phoshate[1]	1.06579.0500/1000/5000
		Sodium chloride (ACS, ISO, Reag. Ph Eur)[1]	1.06404.0500/1000/5000
	Phosphate buffered dilution water	Potassium dihydrogen phosphate (ISO,Reag. Ph Eur)[2]	1.04873.0250/1000/5000
		Magnesium chloride[2]	8.14733.0100/0500

[1] For preparation of Buffered dilution water

[2] For preparation of Phosphate buffered dilution water

The enumeration of *Enterococcus spp.*
Verification



day 1 - 3

Verification

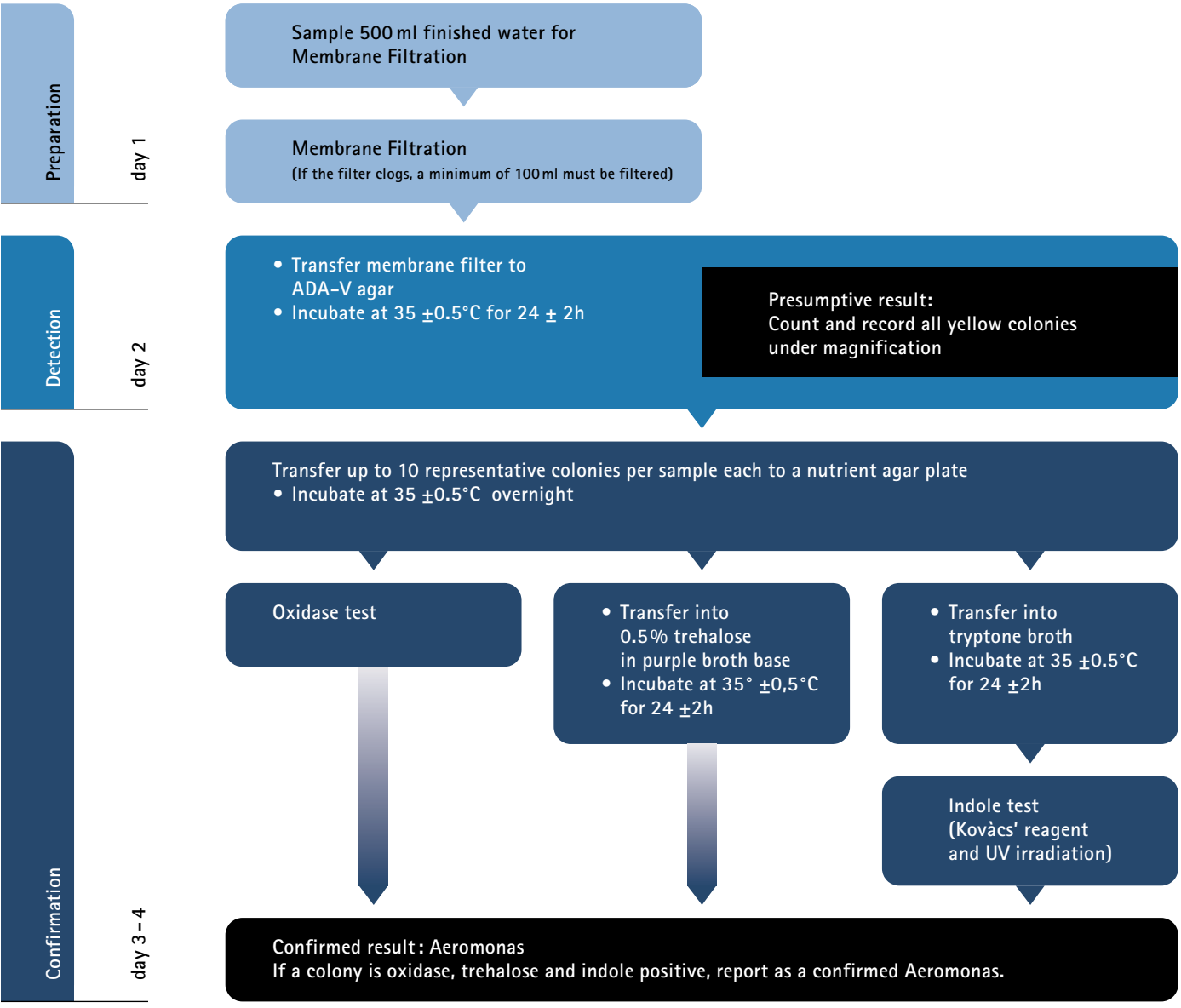
Product list

US EPA 1600 product description	MERCK product description	Merck Cat.No.	Stage
Brain heart infusion agar	Brain heart agar	1.13825.0500	Verification
Brain heart infusion broth	Brain heart broth	1.10493.0500	
Bile Aesculin Agar	Bile Aesculin Azide Agar, acc. to ISO 7899-2	1.00072.0500	
	Merckoplate® Bile Aesculin Azide Agar	1.00077.0020	
	Sodium chloride (ACS,ISO,Reag. Ph Eur)[1]	1.06404.0500/1000/5000	
Catalase reagent	Bactident® Catalase	1.11351.0001	
Gram stain	Gram-color stain set	1.11885.0001	

[1] For preparation of Brain heart infusion broth – 6.5% NaCl

US EPA Method 1605 MF

Aeromonas in finished Water by Membrane Filtration using Ampicillin-Dextrin Agar with Vancomycin

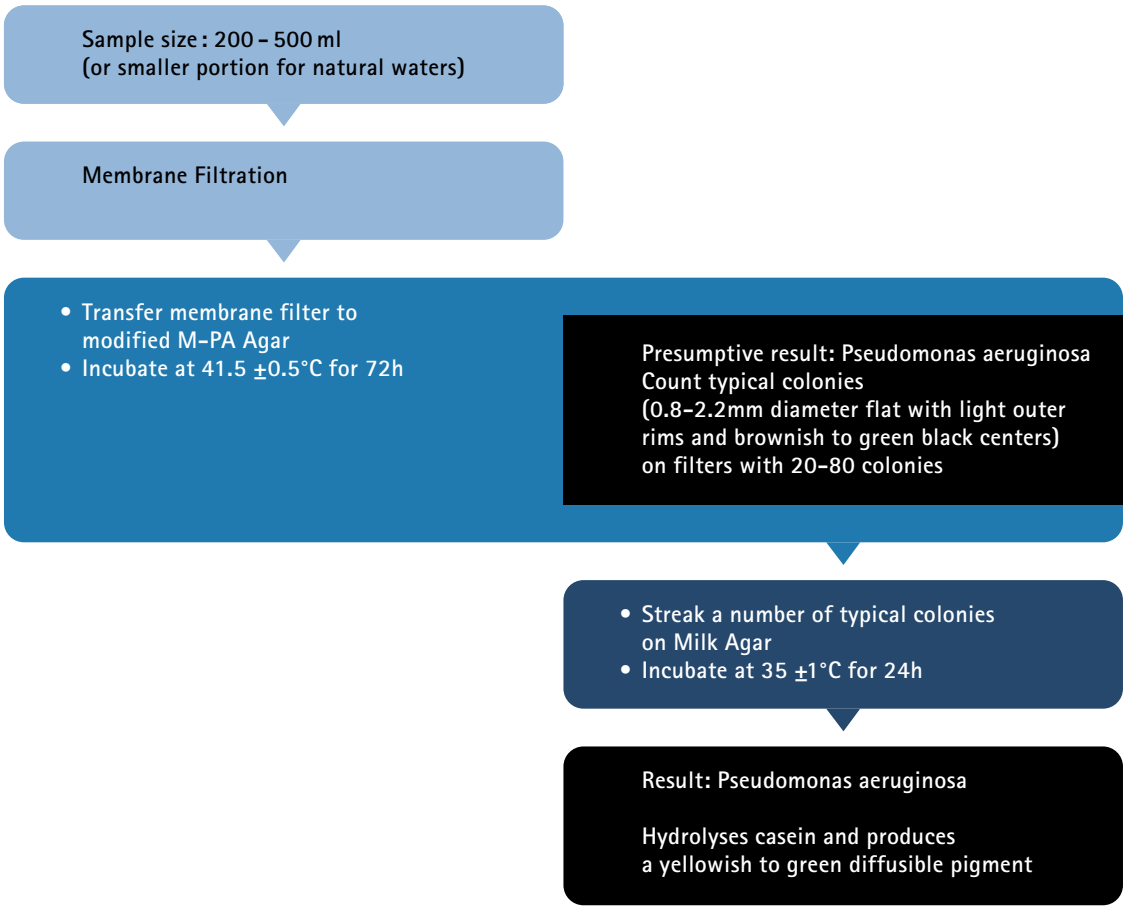


Product list

Stage	US EPA Method 1605 product description	MERCK product description	Merck Cat.No.
Detection	Ampicillin-dextrin agar with vancomycin (ADA-V)	m-Aeromonas Selective Agar (Base) (HAVELAAR)	1.07621.0500
		m-Aeromonas Selective Supplement	1.07625.0001
Confirmation	Nutrient agar	Nutrien agar	1.05450.0500
	Oxidase reagent	Bactident® Oxidase	1.13300.0001
	0.5% Trehalose in purple broth base	-	-
	Tryptone broth	Tryptone water	1.10859.0500
	Indole reagent	KOVACS' indole reagent	1.09293.0100
		Bactident® Indole	1.11350.0001
Dilution	Phosphate buffered dilution water	Potassium dihydrogen phosphate (ISO,Reag. Ph Eur)[1]	1.04873.0250/1000/5000
		Magnesium chloride[1]	8.14733.0100/0500
		Sodium hydroxide, pro analysi, ISO[1]	1.06498.0500/1000/5000

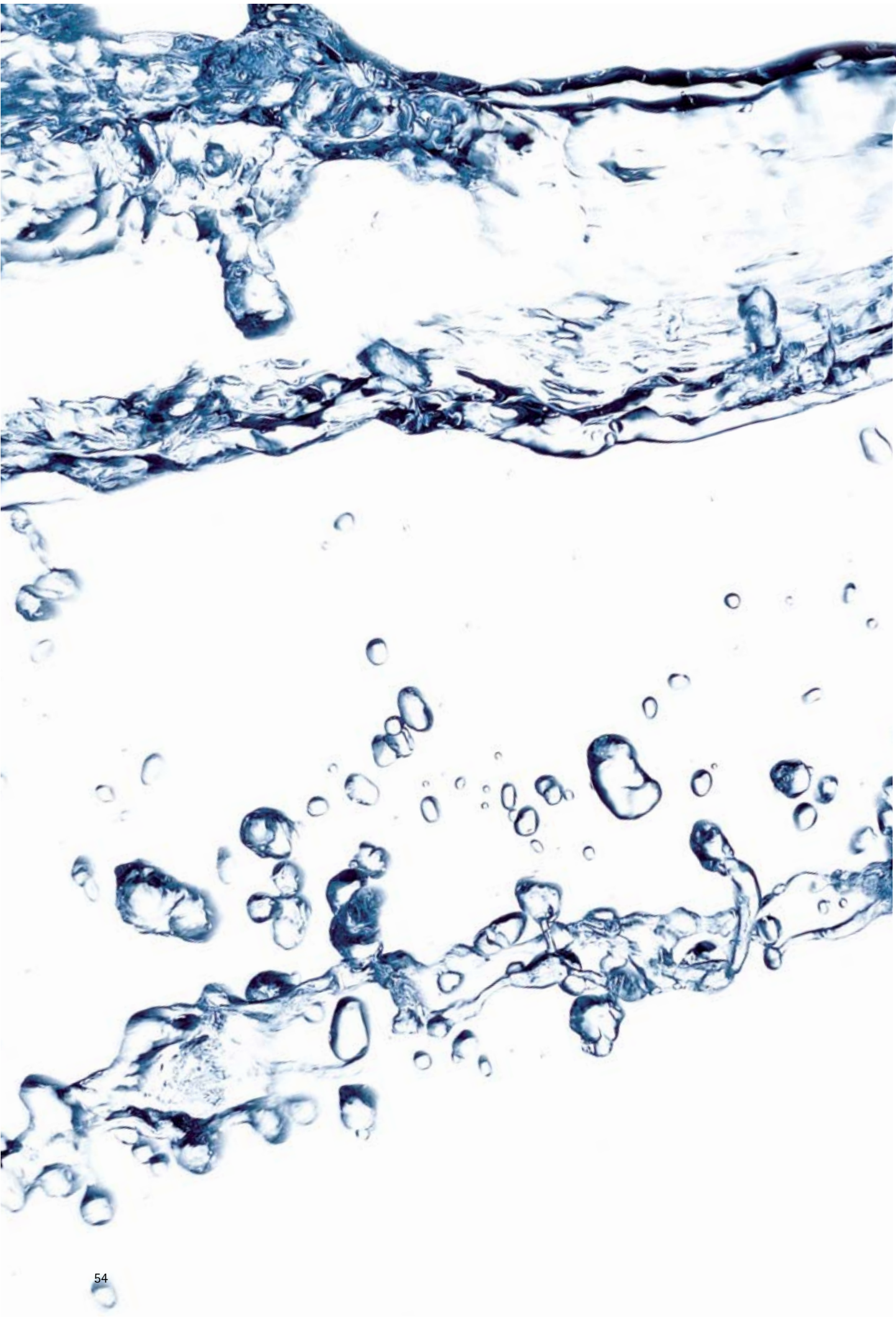
Standard Method 9213 MF

The enumeration of *Pseudomonas aeruginosa*



Product list

Standard Method 9213 E product description	MERCK product description	Merck Cat.No.	Stage
Modified M-PA-Agar	-	-	Detection
Milk Agar	-	-	Confirmation



Standard total coliform fermentation technique

day 1 — Preparation

Sample potable water: 1 x 100ml or 5 x 20ml or 10 x 10ml

Sample non potable water: 5 x 10ml, 1ml, 0.1 ml ... etc.

day 2-4 — Detection

- Add sample to equal volume of triple to double strength Lauryl Tryptose Bile (LTB) Broth (triple for 100ml; double for < 100 ml sample)
- Incubate at 35 ±0.5°C for 24 ±2h and reexamine after 48 ±3h

Presumptive result: Total coliforms

Growth, acid (yellow color) and gas production

day 5-6 — Confirmation

- Transfer from presumptive coliform tubes an aliquot to Brilliant Green Lactose Bile (BGLB) Broth
- Incubate at 35 ±0.5°C for 6, 24 and 48 h

Confirmed result: Total coliforms

Gas in BGLB Broth confirms coliforms present

Go for verification of total coliforms (page 58)

▶ Method 9221 B-3

Go for verification of faecal coliforms (page 59)

▶ Method 9221 E

Go for verification of E.coli (page 60)

▶ Method 9221 F

▶ Product list

Next page 56

Product list

Stage	Standard Method 9221 B and D product description	MERCK product description	Merck Cat.No.
Detection	Lauryl tryptose broth	Lauryl sulfate broth	1.10266.0500
Confirmation	Brilliant green lactose bile-broth	Brilliant green-bile-lactose broth	1.05454.0500/5000
Coliforms	LES Endo agar	m-ENDO agar LES	1.11277.0500
	Nutrient agar	Standard II nutrient agar	1.07883.0500
	Lugol's solution, Gram's modification	Gram-color stain set	1.11885.0001
Feacal coliforms	EC medium	EC broth	1.10765.0500
	A1 broth	A-1 Medium	1.00415.0500
E.coli		Fluorocult® DEV lactose peptone broth[1]	1.04037.0500
		Fluorocult® Lauryl sulfate broth[1]	1.12588.0500
Dilution	Peptone diluent (1%)	Peptone from casein, pancreatically digested, granulated	1.07213.1000/2500
	Phosphate buffer solution	Potassium dihydrogen phosphate (ISO,Reag. Ph Eur)[2]	1.04873.0250/1000/5000
		Magnesium chloride[2]	8.14733.0100/0500

[1] Merck's alternative product
[2] For preparation of Phosphate buffer solution

Products for dilution

Stage	Standard Method product description	MERCK product description	Merck Cat.No.
Dilution	Peptone water, buffered (1%)	Peptone water (buffered); acc. to ISO 6579	1.07228.0500/2500
	Peptone diluent (1%)	Peptone from casein, pancreatically digested, granulated	1.07213.1000/2500
	Peptone saline solution	Maximum recovery diluent	1.12535.0500
	Phosphate buffer solution	Potassium dihydrogen phosphate (ISO,Reag. Ph Eur)[2]	1.04873.0250/1000/5000
	Magnesium chloride[2]	8.14733.0100/0500	

[1] For preparation of Phosphate buffered dilution water
[2] For preparation of phosphate buffer solution

Presence-Absence (P-A) coliform test

day 1 — Prep.

Sample size 100 ml

day 2-3 — Detection

- Add to 100ml sample to 100ml triple strength P-A Broth or Lauryl Tryptose Broth (LTB)
- Incubate at 35 ±0.5°C for 24-48h

Presumptive result: Coliform bacteria
Any amount of gas and/or acid formation

day 3-5 — Confirmation

- Transfer from presumptive coliform bottles an aliquot to Brilliant Green Lactose Bile (BGLB) Broth
- Incubate at 35 ±0.5°C for 24-48h

Confirmed result: Coliform bacteria

Gas in BGLB Broth after 48 h confirms coliform present

Go for verification of total coliforms (page 58)

▶ Method 9221 B-3

Go for verification of faecal coliforms (page 59)

▶ Method 9221 E

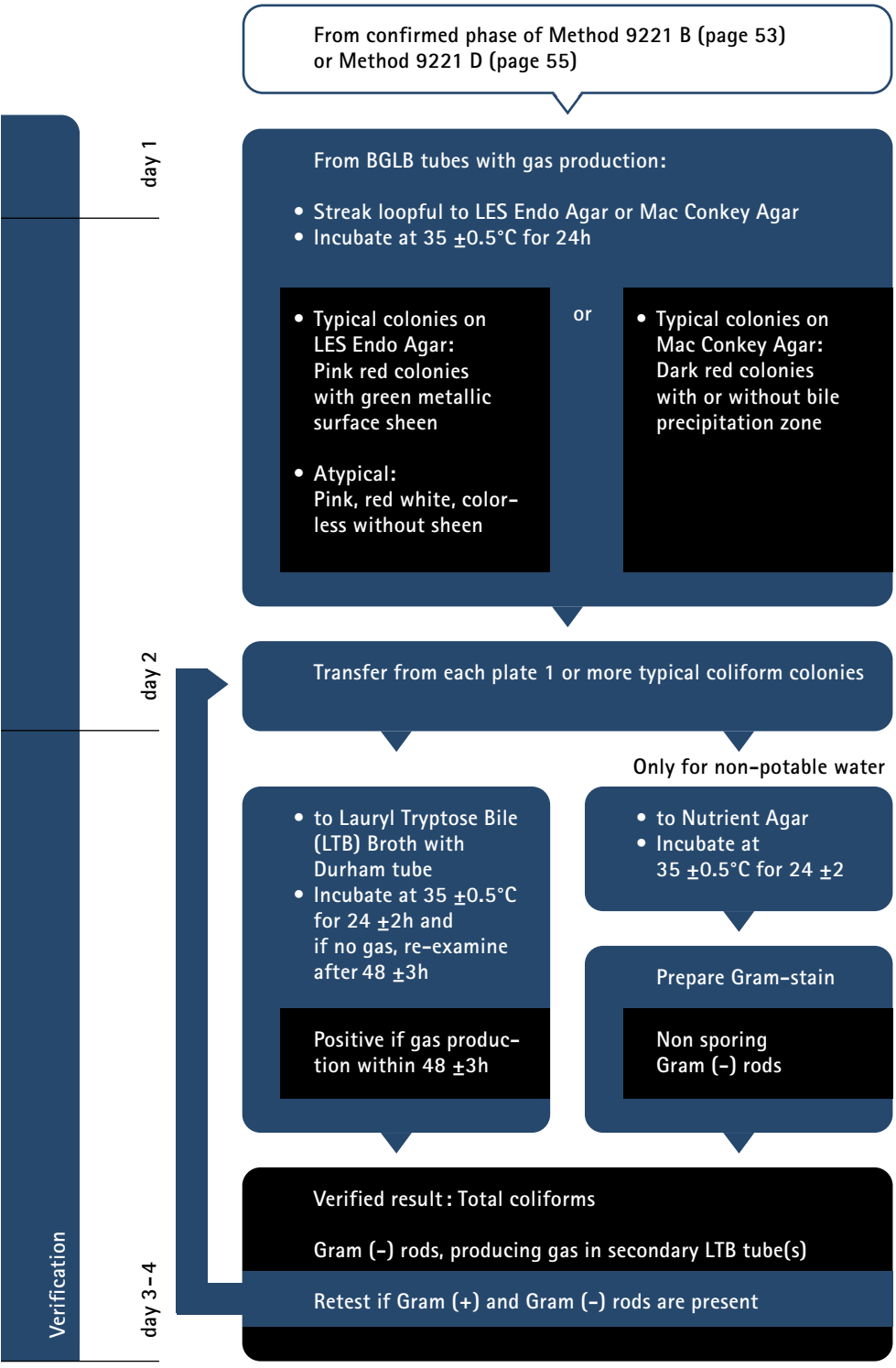
Go for verification of E.coli (page 60)

▶ Method 9221 F

◀ Product list

Previous page 56 above

Coliform test – Completed phase
Verification



Faecal coliform procedure Verification[1]

From Method 9221 B (page 53)
or Method 9221 D (page 55)
or Method 9222 D (page 47)

Transfer an aliquot of presumptive positive BGLB tubes or typical or atypical colonies from m-FC Agar to EC Broth

Incubate at 44.5 ±0.2°C (water bath) for 24 ±2h

Verified result: Faecal coliforms

Growth and gas production within 24 ±2h

day 1

day 2

Verification

Product list

Standard Method 9221 E product description	MERCK product description	Merck Cat.No.	Stage
Brilliant green lactose bile broth	Brilliant green-bile-lactose broth	1.05454.0500/5000	Verification
EC medium	EC broth	1.10765.0500	
A1 broth	A-1 Medium	1.00415.0500	
Peptone diluent (1%)	Peptone from casein, pancreatically digested, granulated	1.07213.1000/2500	Dilution
Phosphate buffer solution	Potassium dihydrogen phosphate (ISO,Reag. Ph Eur)[2]	1.04873.0250/1000/5000	
	Magnesium chloride[2]	8.14733.0100/0500	

[1] This method can also be a rapid faecal coliform tests for drinking water stream pollution, raw water, waste water treatment systems, bathing waters, sea water and general water-quality monitoring

[2] For preparation of Phosphate buffer solution

Escherichia coli procedure
Verification

From confirmed tubes (growth, acid production, or gas within 48 ±3h) of Method 9221 B-3 (page 58) or Method 9221 D (page 57)

Verification

day 1

Submit an aliquot of all BGLB tubes or bottles allowing growth, gas or acidity within 48 ±3 h, to EC-MUG Broth

Incubate at 44.5 ±0.2°C (water bath) for 24 ±2h

day 2

Result: E.coli

Growth and fluorescence (365-366 nm)

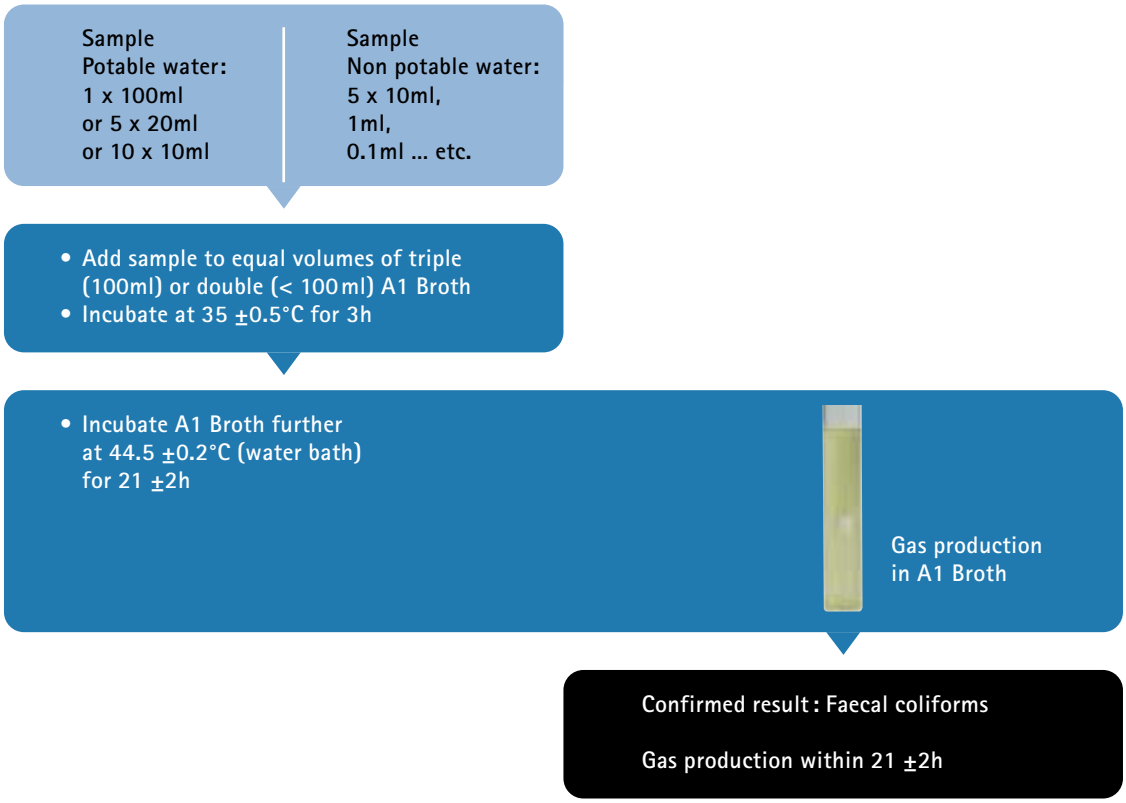
Product list

Stage	Standard Method 9221 F product description	MERCK product description	Merck Cat.No.
Verification	EC-MUG medium	Fluorocult® BRILA broth[1]	1.12587.0500
		Fluorocult® DEV lactose peptone broth[1]	1040370500
		Fluorocult® Lauryl sulfate broth[1]	1.12588.0500
Dilution	Peptone diluent (1%)	Peptone from casein, pancreatically digested, granulated	1.07213.1000/2500
	Phosphate buffer solution	Potassium dihydrogen phosphate (ISO,Reag. Ph Eur)[2]	1.04873.0250/1000/5000
		Magnesium chloride[2]	8.14733.0100/0500

[1] Merck's alternative product

[2] For preparation of Phosphate buffer solution

Faecal coliforms direct test



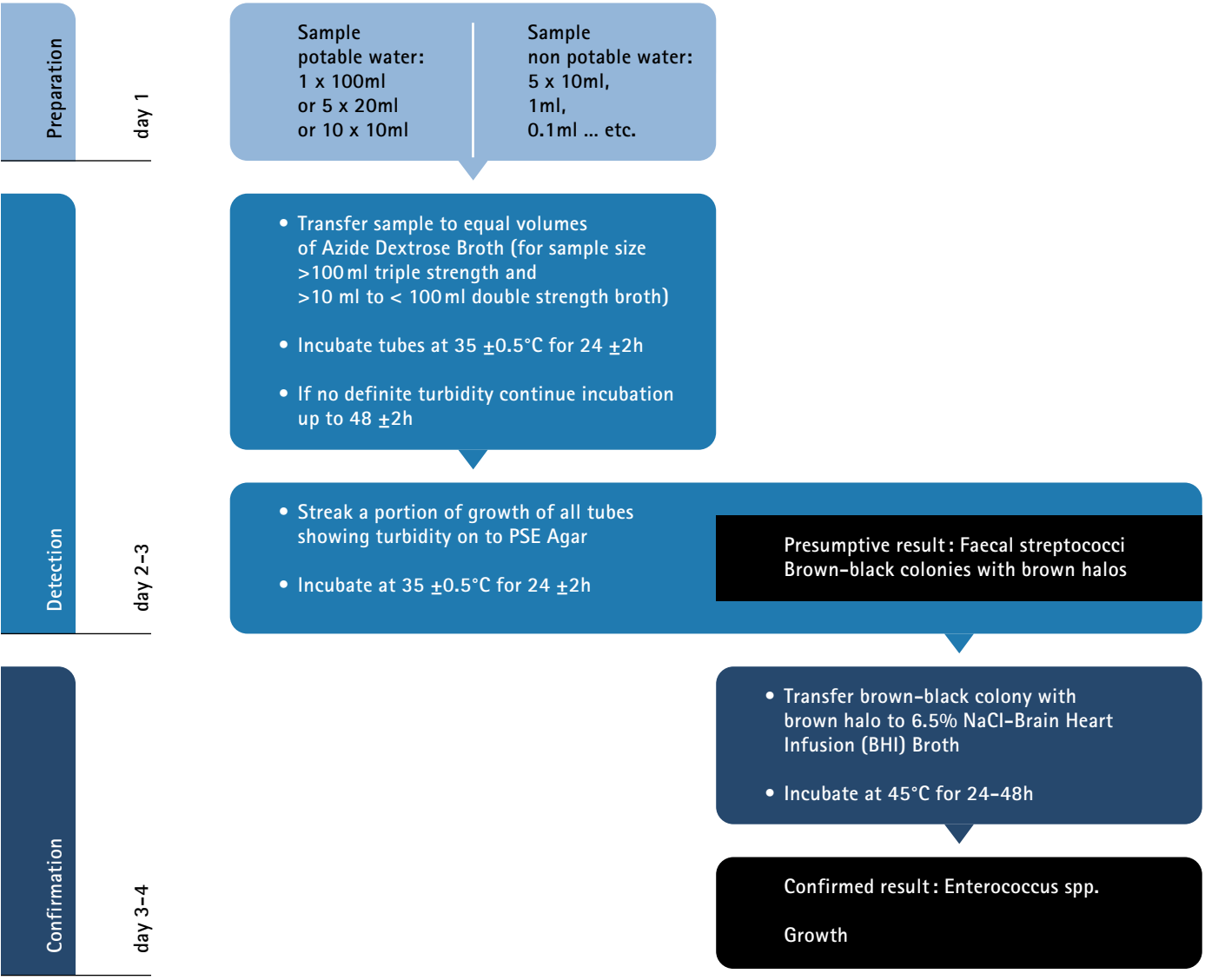
Product list

Standard Method 9221 E product description	MERCK product description	Merck Cat.No.	Stage
A1 broth	A-1 Medium	1.00415.0500	Detection
Peptone diluent (1%)	Peptone from casein, pancreatically digested, granulated	1.07213.1000/2500	Dilution
Phosphate buffer solution	Potassium dihydrogen phosphate (ISO,Reag. Ph Eur)[1]	1.04873.0250/1000/5000	
	Magnesium chloride1	8.14733.0100/0500	

[1] For preparation of Phosphate buffer solution

Standard Method 9230 B P-A / MPN

Faecal *Streptococcus* and *Enterococcus* groups



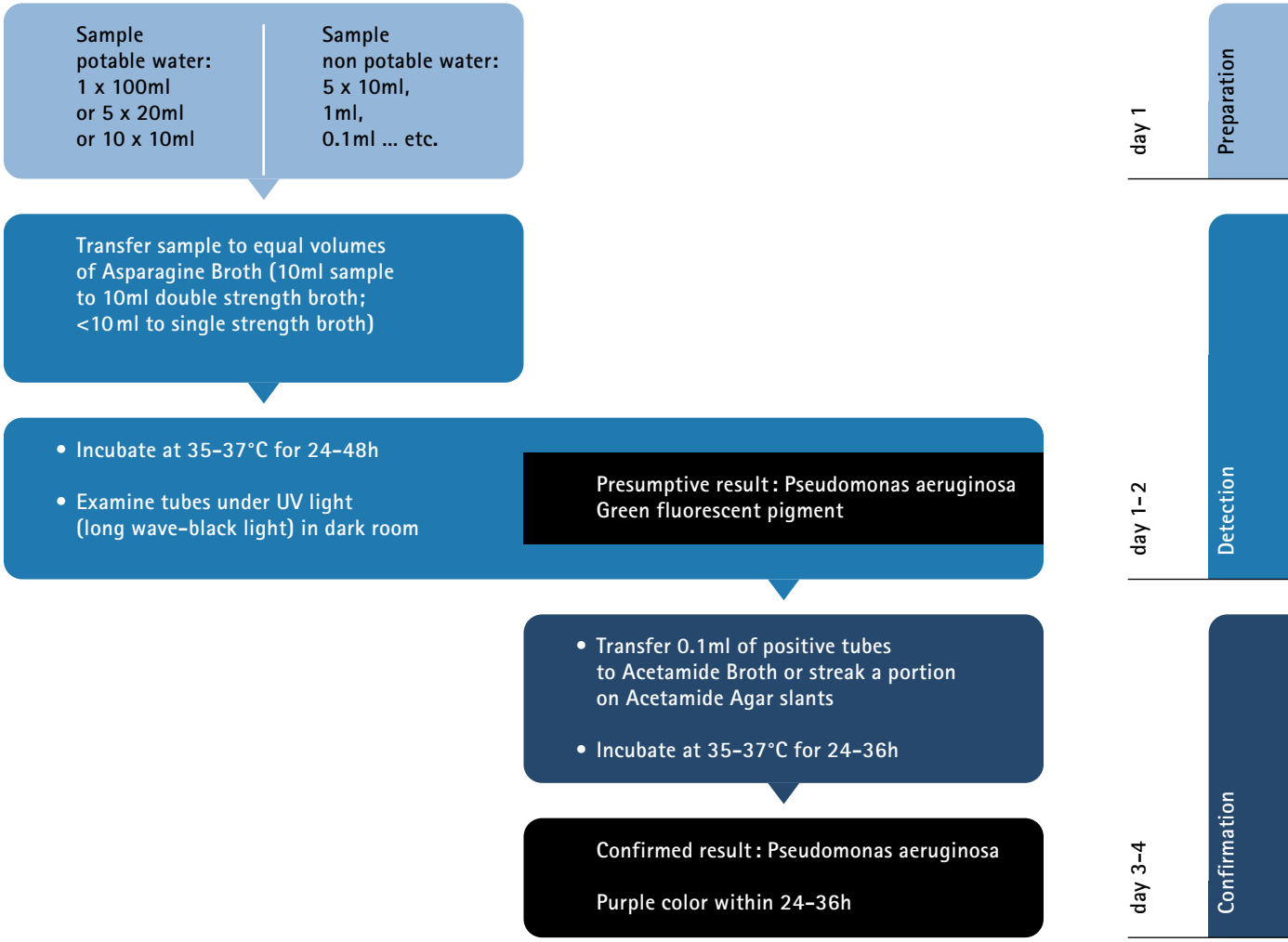
Product list

Stage	Standard Method 9230 B product description	MERCK product description	Merck Cat.No.
Detection	Azide dextrose broth	Azide dextrose broth	1.01590.0500
Confirmation	Pfizer selective enterococcus (PSE) agar	-	-
	Brain heart infusion broth – 6.5% NaCl	Brain heart broth[1]	1.10493.0500
		Sodium chloride (ACS,ISO,Reag. Ph Eur)[1]	1.06404.0500/1000/5000
Dilution	Peptone diluent (1%)	Peptone from casein, pancreatically digested, granulated	1.07213.1000/2500
	Phosphate buffer solution	Potassium dihydrogen phosphate (ISO,Reag. Ph Eur)[2]	1.04873.0250/1000/5000
		Magnesium chloride[2]	8.14733.0100/0500

[1] For preparation of Brain heart infusion broth – 6.5% NaCl

[2] For preparation of Phosphate buffer solution

The enumeration of *Pseudomonas aeruginosa*



Product list

Standard Method 9213 F product description	MERCK product description	Merck Cat.No.	Stage
Asparagine broth	-	-	Detection
Acetamide agar	-	-	Confirmation
Peptone diluent (1%)	Peptone from casein, pancreatically digested, granulated	1.07213.1000/2500	Dilution
Phosphate buffer solution	Potassium dihydrogen phosphate (ISO,Reag. Ph Eur)[1]	1.04873.0250/1000/5000	
	Magnesium chloride[1]	8.14733.0100/0500	

[1] For preparation of Phosphate buffer solution

European Pharmacopoeia (EP)

The European Pharmacopoeia promotes public health by providing recognized common standards for use by health-care professionals and others concerned with the quality of medicines. European Pharmacopoeia monographs and other texts are specifically designed to the needs of regulatory authorities, those engaged in the control of quality, manufacturers of starting materials and medicinal products.

The European Pharmacopoeia defines methods for the quality control and monitoring of water and details, in monographs, specifications for water e.g. like purified water (aqua purificata) and water for injection (aqua iniectabilia).

Plate Count (TVC)

EP-Method 2.6.12	Testing of water	Page 65
EP-Method 2.6.13		

Membrane Filtration (MF)

EP-Method 2.6.12	Testing of water	Page 66
EP-Method 2.6.13		

Testing of water for total viable aerobic count

Sample

- Mix min. 1ml of sample with 15-20ml R2A Agar
- Plate out
- Incubate at 30-35°C or 20-25°C for up to 5 days[1]

Positive on R2A Agar

If required, characterise colonies by colony morphology and Gram-stain

Result: TVC

Count colonies on plates containing 10-150 colonies

day 1 Prep.

day 2-5 Detection

Product list

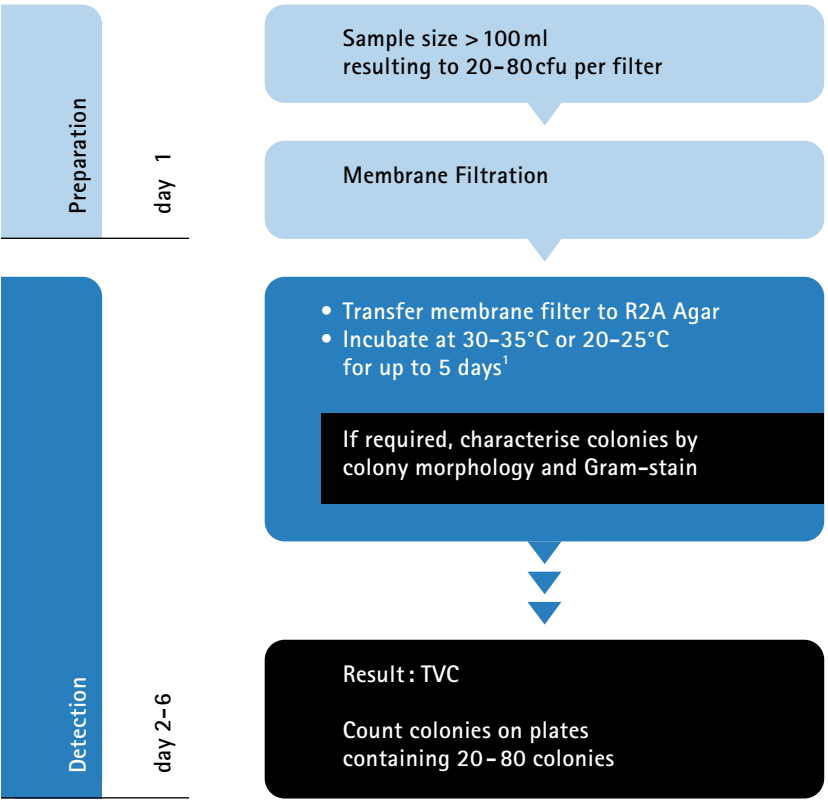
EP Method product description	MERCK product description	Merck Cat.No.	Stage
R2A agar	R2A Agar	1.00416.0500	Detection
	Merckoplate® R2A Agar	1.00073.0020	
Gram stain	Gram-color stain set	1.11885.0001	
Buffered Sodium chloride peptone solution pH 7.0	Sodium chloride peptone broth (buffered)[2]	1.10582.0500/5000	Dilution

Other products for microbiology

ISO product description	MERCK product description	Merck Cat.No.	Stage
Peptone water, buffered (1%)	Peptone water (buffered); acc. to ISO 6579	1.07228.0500/2500	Dilution
Peptone diluent (%)	Peptone from casein, pancreatically digested, granulated	1.07228.1000/2500	
Peptone saline solution	Maximum recovery diluent	1.12535.0500	
Ringer's solution	RINGER tablets	1.15525.0001	
Phosphate buffer solution	Potassium dihydrogen phosphate (ISO,Reag. Ph Eur)[3]	1.04873.0250/1000/5000	
	Magnesium chloride[3]	8.14733.0100/0500	

[1] Unless a reliable count is obtained in shorter time [2] Conforms with German Pharmacopeia DAB 10 (1991) [3] For preparation of Phosphate buffer solution

Testing of water for total viable aerobic count



Product list

Stage	EP Method product description	MERCK product description	Merck Cat.No.
Detection	R2A agar	R2A Agar	1.00416.0500
		Merckoplate® R2A Agar	1.00073.0020
	Gram stain	Gram-color stain set	1.11885.0001
Dilution	Buffered Sodium chloride peptone solution pH 7.0	Sodium chloride peptone broth (buffered)[2]	1.10582.0500/5000

[1] Unless a reliable count is obtained in shorter time

[2] Conforms with German Pharmacopeia DAB 10 (1991)

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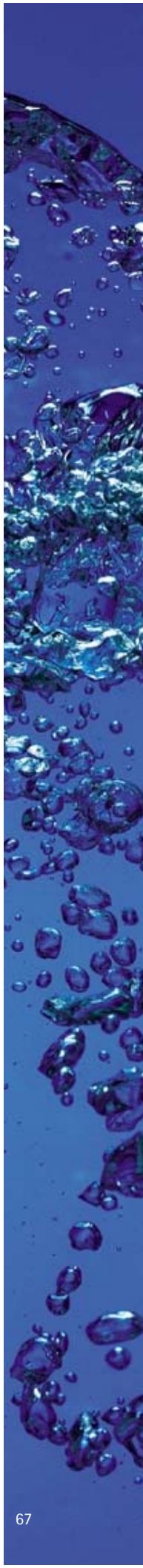

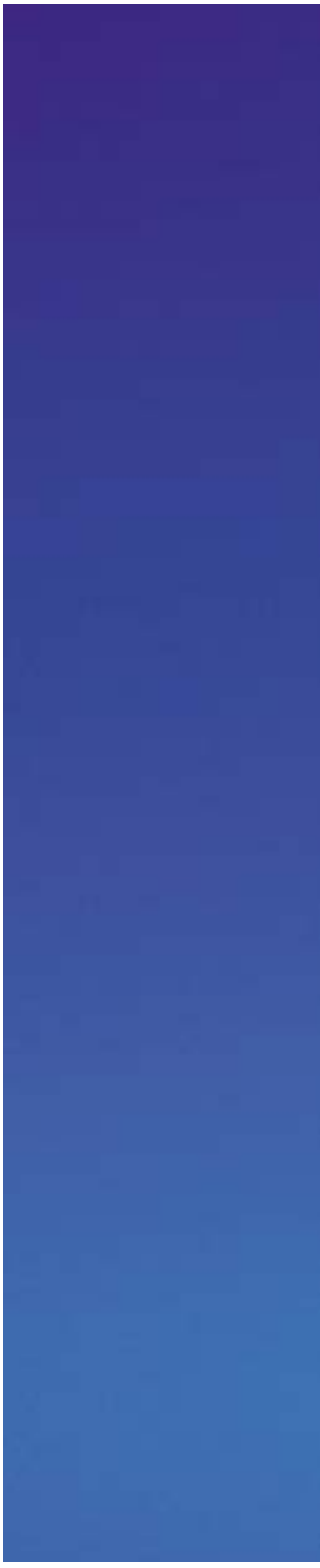

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