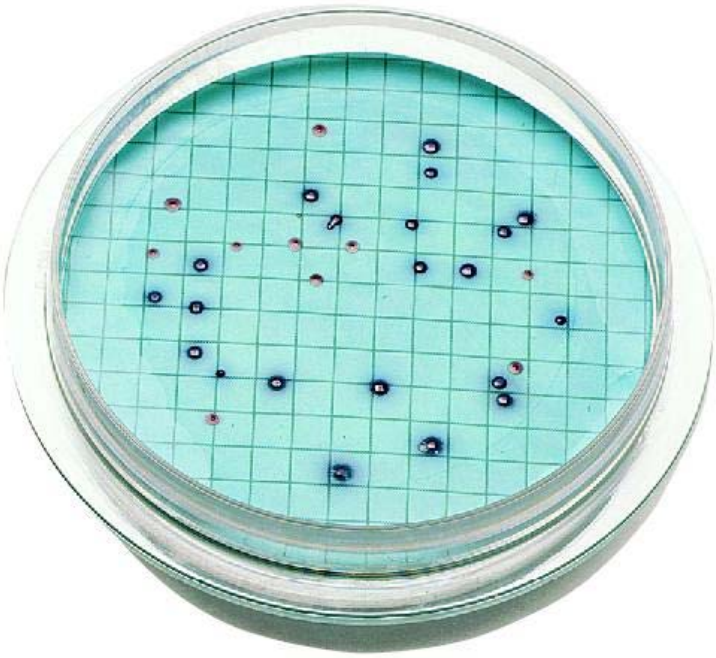
PRACTICE REPORT
MICROBIOLOGY
Author: Dr. Elmar Grabert, HACH LANGE GmbH, Germany



Tests microbiologiques : Pas seulement pour l'eau potable !

Summary: L'analyse des micro-organismes est un élément indispensable pour l'hygiène moderne et les mesures de désinfection. Le succès de ces mesures ne peut être atteint qu'à l'aide de tests microbiologiques ciblés. Pour intervenir le plus précocement possible, il est important que les résultats du dépistage soient rapides, car les analyses standard sont généralement coûteuses et fastidieuses. Vous trouverez ci-après une presentation succincte des tests microbiologiques et de leurs utilisations.

Bactéries aérobies ou moisissures ?
Les testeurs à palettes déterminent en 24 heures à peine si les surfaces ou les liquides ont été correctement désinfectés. Ils s'appliquent aussi pour les eaux de process industrielles, les papeteries, colorants, peintures ainsi que les huiles de coupe.

Contamination fécale ou non ?
La réponse est fournie par des tests P/A (présence/absence).

Les micro-organismes sont omniprésents dans l'eau. Ils peuvent presenter un risque pour la santé et forment souvent des biofilms en surface. Des dommages peuvent donc être engenders par une corrosion d'origine bactérienne ou des défaillances mécaniques dans les tuyaux. Pour réagir convenablement aux effets de ces invités indésirables, ceux-ci doivent être identifies à l'aide d'un système approprié. Les tests HACH LANGE ont déjà fait leurs preuves dans de nombreuses applications : ils sont faciles à réaliser et donnent des résultats fiables

Les bactéries E. coli servent ici d'indicateur de présence d'organismes fécaux réellement dangereux, tels que le choléra pathogène. Le test P/A mesure la concentration d'E. coli avec une précision d'un organisme dans un échantillon de 100 ml.

E. coli ou coliformes ?

En seulement 24 heures, le test M COLI BLUE24 , filtrage sur membrane, determine de manière sûre et fiable si l'eau potable contient ou non de telles bactéries et en quelle quantité. Cette information s'avère particulièrement essentielle
quand le nombre d'organismes revêt une importance cruciale, tel que dans les secteurs pharmaceutiques et cosmétiques ainsi que dans les eaux de surface et de baignade.

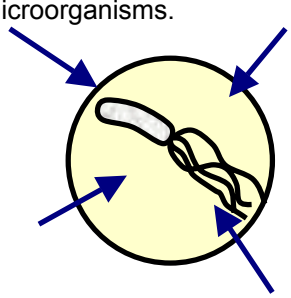
Identifier la source du problème ?

Filtre colmaté, mauvais fonctionnement d'un puits, odeurs nauséabondes, dans tous les cas les tests BART identifient rapidement et sans ambiguïté les bactéries coupables.

Complete Report: Microorganism-free drinking and process water? Fast microbiological tests give the answer

Introduction

Microorganisms, also referred to as microbes, are the smallest living organisms that are visible through a microscope. Bacteria, unicellular algae and moulds, yeasts and protozoa are all microorganisms.



Bacterium in focus

Viruses are not microorganisms; they cannot reproduce independently but are dependent on the metabolic machinery of their host cells, and are therefore not regarded as "living".

All microorganisms play an important role in maintaining nature's metabolic balance, through their ability to convert (mineralise) biomass. They do this by using large, complex organic molecules and small inorganic molecules as sources of nutrients and breaking down the chemical bonds to create smaller, simpler structures.

Bacteria form the largest group of microorganisms. They have an amazing ability to survive under almost any conditions – with or without air, in darkness or in light, in water, oil or absolutely dry conditions, in or on other living organisms. It is therefore no surprise that bacteria can be found almost everywhere.

Depending on their type and mode of life, bacteria may be beneficial or they may harm human health, food, consumables or technical equipment. Legally binding regulations therefore exist, whose purpose is to prevent undesirable contact with pathogenic microorganism.

Strict hygienic regulations

These regulations stipulate which microorganisms must not be present, or may only be present up to a given limit. Hygiene regulations also define the individual measures that must be taken to prevent microbial contamination.

Examples are the Drinking Water Act, Mineral and Table Water Act, Milk Act, European Bathing Water Directive and the HACCP (Hazard Analysis of Critical Control Point) system.

Outside the scope of such legal regulations, measures are also needed to prevent the harmful effects of microbial action.

Prevent harms

Examples of such harmful effects are the fouling of process water and cooling water circuits, bacterial corrosion of pipes or coated cans of preserved food, and the impairment of the stability of paper, dyes and paint products.

Today an immense number of widely varied methods exist for detecting microorganisms. The most important microbiological tests are listed by application and described below.

Classification of test methods

➔ **General determination of aerobic bacteria and moulds:**

A) PADDLE testers

➔ **Determination of indicator organisms (organisms that indicate the possible presence of pathogens):**

B1) presence/absence (P/A) test

B2) membrane filtration test with M COLI BLUE24

➔ **Determination of bacteria that can harm technical equipment:**

C) BART tester

General determination of aerobic bacteria and moulds:

A) PADDLE testers

PADDLE testers are a simple way of determining within 24 hours whether large or small numbers of bacteria, fungi or moulds are present in a sample or on a surface.

One of the paddle's two sides is red, and indicates the presence of bacteria, while the other (yellow) side detects yeasts and moulds. On the one side is a trypton glucose extract agar (yellow, for the total organism count) and the other has a coating of a selective agar, which depends on the type of tester.

The paddle is either dipped in the sample or pressed against the bacteriologically contaminated surface. It is then returned to the vial in which it was supplied and then incubated for 24 hours at 35-37°C (see Fig. 1).

Fig. 1: Incubator for 24h incubation, and optical evaluation of a paddle tester.

The paddle is then evaluated optically. Red spots indicate the presence of colonies of bacteria. If the red spots are densely packed, there are correspondingly large numbers of bacteria in the sample, and if there are only a few or no spots, the level of bacterial contamination is low. The measuring and detection range of the paddle tester is approximately 10^2 to 10^7 organisms in 1 ml sample.

Reliable checking

The paddle tester is eminently suitable for checking surfaces or liquids that have been disinfected. If the findings are positive, this indicates a less than optimal disinfection result. If the paddle remains free of red spots, the disinfection was carried out correctly.

The paddle tester can also be used to good effect in other areas where bacteria can theoretically multiply but are basically undesirable, such as cooling water circuits, process water in the paper, dye and paint industries or in cutting fluids in the metal processing sector. PADDLE testers are of limited efficacy, however, for use with viscous liquids.

Determination of indicator organisms (organisms that indicate the possible presence of pathogens):

E. coli

Worldwide, the most widely tested-for bacteria are E. coli (Escherichia coli) and coliforms. Escherichia coli is a rod-shaped bacterium with a length of 2-4 µm and a diameter of 1 µm. It occurs naturally as a harmless inhabitant of the human intestine. Since E. coli can also survive for a certain length of time outside the intestine, however, and can also be easily detected, it serves as an indicator of faecal contamination in water, and especially in drinking water.

A positive identification of these bacteria in water always indicates contamination of the sample with intestinal bacteria. In principle, there is always a possibility that these relatively harmless bacteria are accompanied by really dangerous pathogens such as Salmonella, Cholera, or intestinal viruses, representing a risk to human health.

For this reason a number of regulations have been enacted, prohibiting the presence of these organisms in drinking water. For instance, 100 ml tap water should not contain detectable levels of E. coli or coliforms.

Highly sensitive methods

Methods of analysing E. coli must therefore be highly sensitive. It must be absolutely certain that a negative test result really indicates the absence of E. coli in 100 ml original sample.

As an indication of the level of sensitivity required, detecting a bacterium in 100 ml water is equivalent to finding a grain of rye in a 200 km long goods train full of wheat. Two different methods can be used to detect coliforms and E. coli.

B1) Presence/absence tests (P/A tests)

As the name suggests, the presence/absence (P/A) test can be used to check for the presence or absence of coliforms in a water sample. It is simple to use and the results are very reliable.

The test reagent – a presence-absence broth – is introduced into a 120 ml sample bottle. 100 ml of the sample are added and the bottle is incubated for 24 hours (or not more than 48 hours) at 35-37°C (see Fig.2).

Fig. 2: P/A test in incubator

If the original red colour of the solution does not change, no coliforms and therefore no E. coli are present in the sample. A change of colour to yellow indicates the presence of coliforms or E. coli.

Reliable Detection

This fast test is a reliable detection method for use in water analysis. It gives the operator of a drinking water plant the option of quickly obtaining an overview of the hygiene situation in general after repairs or direct interventions have been carried out in the drinking water network. The sensitivity of the P/A test is1 organism per 100 ml sample.

B2) MEMBRANE FILTRATION method

A far more exact method for determining the type and number of specific indicator organisms such as E. coli, total coliforms, faecal coliforms, total aerobic organisms or pseudomonads is membrane filtration. For this reason, it is also mainly used in routine analysis. The ready-to-use test contains everything that is needed, including membrane filters, funnels, petri dishes, absorbent pads, and the indicator medium M COLI BLUE24.

The user only needs to provide a vacuum pump for the membrane filtration.

The sample is filtered through the membrane filter (nitrocellulose, 0.45 µm average pore diameter). All the bacteria in the sample remain on the surface of the filter. The filter is then placed in a petri dish containing an absorbent pad which has been soaked with the indicator medium.

A variety of indicator media are available to detect different bacteria (e.g.: mColiBlue24 for total coliforms and E. coli; m-Endo for total coliforms, m-FC for faecal coliforms, m-Pseudomonas broth for pseudomonads and m-HPC for the total organism count). The absorbent pad is soaked with the relevant indicator medium immediately before the test is performed.

The dish is then incubated for 24 hours at 35-37°C. Thanks to this preselection only the target bacteria can multiply on the membrane filter. Their presence is betrayed by variously coloured spots on the white filter. Figure 3 shows the detection of an E. coli organism in tap water.

The indicator medium M COLI BLUE24® can therefore be used to determine, within 24 hours, either the absence of E. coli or the exact number of E. coli (blue spots) and total coliforms (red spots).

The membrane filtration method requires relatively little effort and yields very reliable results. It is the ideal method for analysing process water in the pharmaceutical and cosmetic sectors as well as drinking water and bottled water, and for monitoring surfaces and bathing water, where the exact organism count is crucial.

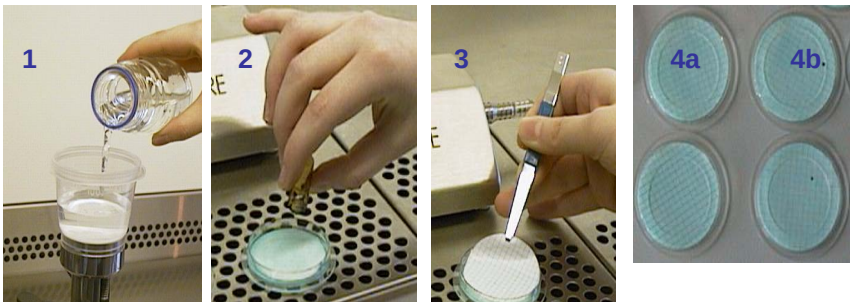


Fig. 3: Membrane filtration. The sample is filtered (1); indicator medium m-ColiBlue24 is poured onto the absorbent pad (2); the membrane filter is placed on the absorbent pad and incubation is carried out (3); the result is read (4a): tap water without E. coli, (4b): tap water with E. coli (1 organism/100 ml).

Determination of bacteria that can cause damage to technical equipment:

Wherever technical equipment comes into contact with liquids, as in pumps, pipelines, tank farms and storage facilities, but also in drinking water wells, bacteria cause undesirable effects such as corrosion, discolouration, block

of suspended solids, flocking, and changes of taste and odour.

C) BART tester

A reliable aid to the prompt identification of these bacteria is the BART™ tester (see Fig.4).

Fig. 4: Three BART tests

Universal use

The Biological Activity Reaction Test (BART™) is a water test for a variety of bacteria whose presence in water is undesirable. It is available as a number of test kits.

These determine the activity of the bacteria in the water by the time that is needed for an easily visible reaction in the test system. The longer the time before the reaction can be observed, the lower the level of biological activity. The evaluation is carried out in two steps.

First of all the biological activity is determined by the time that elapses before a certain reaction is observed, and secondly the bacterial population that is present can be deduced from the reaction pattern.

The monitoring of drinking water wells is one application for which the BART tests are used. During the use of groundwater wells, symptoms of ageing appear, paired with loss of performance. The diminished efficiency of the well performance is attributable to physical effects such as sanding, fusion and ferric incrustation; of these, ferric incrustation is often caused by biological factors.

In order to be able to determine the right time to regenerate a well, extensive testing must be carried out.

Easy to perform

The BART™ test for determining biological activity in wells and groundwater is an important aid here. The BART tests are very easy to perform.

The tube is filled up to the mark with the sample. No further mixing occurs and the tube is incubated for 7–9 days at room temperature.

The tubes are checked each day for the appearance of certain reactions described in the instruction leaflet (e.g. colouration, turbidity, slime formation).

The earlier and stronger the reaction, the larger and more aggressive the bacterial population that causes it. The reaction is not analysed on a nutrient plate (solid medium) but in the natural habitat (sample water).

Seven different test kits

There are seven different test kits. These tests are specific for iron related bacteria (*RB*), sulphate reducing bacteria (*SRB*), heterotrophic aerobic bacteria (*HAB*), slime forming bacteria (*SLYM*), nitrifying bacteria (*N*), denitrifying bacteria (*DN*) and fluorescent Pseudomonads (*FLOR*). As each group of bacteria causes different problems, a combination of tests is advisable.

Berliner Wasserbetriebe

A BART test was used for a trial period to monitor a drinking water well of the Berliner Wasserbetriebe.

Samples were taken from a freshly regenerated well over a period of weeks and the water, or rather the bacteria in the water, were tested using the BART™ test.

Result: faster, more intensive!

As a comparison, parallel controls were set up with tap water and sterile tap water. It was observed that as the period of operation of the well increased, the reaction to the BART™ test occurred faster and became more intensive.

The observed reaction in the tap water control was very slight and occurred after some delay. No reaction was observed in the sterile tap water control.

The results indicated an increasing number of bacteria and increasing aggressiveness of the bacterial population as the well operation time increased. In parallel to this, the organism count was determined with a conventional dilution series on nutrient plates.

The evaluation showed that reproducible and comparable results were obtained for the organism number on nutrient plates and with the BART™ test (see Tab.2)

The other studies using BART™ tests and parallel pump tests also showed that the causes of the drop in performance of the studied wells were purely physical and chemical as well as biological

Summary

Analysis of microorganisms is now an indispensable part of a modern and comprehensive system of hygiene monitoring. .The results obtained reveal the success of failure of any hygiene measures that have been carried out.

Fast biological tests that can be easily carried out in the field are playing an increasingly important role and quickly provide the user with the results he needs to respond promptly to events. Costly and time-consuming routine analysis often cannot offer this option.

Sample	Plate test "R2A – Agar" [organisms/ml]	BART™ test "HAB" [organisms/ml]
Bacterial solution	5.1×10^5	5×10^5
Groundwater	1×10^1	1×10^1

Tab. 2: Comparison of the determination of the organism count of aerobic bacteria using the dilution method on nutrient plates and the BART™ test.
Source: Umwelttechnik Dr. Bartetzko GmbH

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HACH LANGE
HACH LANGE SAS
33, Rue du Ballon
F-93165 Noisy Le Grand
Tél. +33(0)1 48 15 80 80
Fax +33(0)1 48 15 80 00
info@hach-lange.fr
www.hach-lange.fr

HACH LANGE SA
Mostraat 54
B-2800 Mechelen
Tél. +32 (0)15 42 35 00
Fax +32 (0)15 41 61 20
info@hach-lange.be
www.hach-lange.be

DR. BRUNO LANGE AG
Juchstrasse 1
CH-8604 Hengnau
Tél. +41(0)1 9 45 56 10
Fax +41(0)1 9 45 56 76
info@hach-lange.ch
www.hach-lange.ch