



sartorius

Microbiological Detection – The Easy Way

On the Use of Nutrient Pad Sets
for Quality Control in the Food and Beverage Industry

Elke Just



1
Prewetting Nutrient Pad Sets with sterile water (using the Sartorius Dosing Syringe, as pictured here, or a pipette)



2
Types of Nutrient Pad Sets



3
Placing a membrane filter on the frit of a filter holder

The nutrient pad method represents a valuable time- and labor-saving technique in microbiological testing of foods and beverages involving membrane filtration. The method not only makes it possible to economize work as opposed to conventional techniques, but it also guarantees reproducible results as well. Culture media for all the usual types of tests are available in the form of Nutrient Pad Sets. With them, the time-consuming work load involved in preparing, sterilizing and pouring agar culture media is eliminated.

Introduction

The consumer's ever increasing demands on the microbiological stability and the quality of foods and beverages entail certain problems for the producer. He cannot simply limit quality control to the final product, i.e. the bottled beverage or the prepared food product, if he wants to avoid ensuing losses and complaints. On the contrary, he must subject both the basic ingredients and the entire production process to continuous testing and control. Here, the microbiological and hygienic control plays a substantial role, because it is frequently the case that merely a few microbes are all it takes to spoil large quantities of a beverage.

Microbial detection can be considerably simplified by using Nutrient Pad Sets as opposed to agar or liquid media. Nutrient Pad Sets are sterile, dehydrated culture media, which are individually preinserted in petri dishes. After moistening them with sterile water, they are ready for immediate use. There is no time-consuming preparation, sterilization, or pouring, which are necessary when using agar culture media. With nutrient pads, the risk of infecting the uninoculated culture medium is also reduced.

Currently, there are more than 20 different types of Nutrient Pad Sets on the market. Their basic material is biologically inert cellulose. Microbial counts and growth on nutrient pads yield results comparable to those obtained using corresponding types of agar media.

Nutrient pads are used in combination with the membrane filtration method. The pore sizes of the filters for detection of bacteria are 0.45 μm and 0.65 μm ¹⁾ for the detection of yeasts and molds. If larger volumes of liquid are to be examined or if the colony count of a solution low in microbes is to be determined, filtration always takes precedence over the streak plate or pour plate method.

Solid foods can also be examined according to the membrane filtration method if they are suspended or homogenized in a suitable solvent (physiological saline solution, peptone water or emulsifier). In doing so, a prefilter attachment is used to trap and retain coarse particles while allowing bacteria to pass through. Emulsifiers and bacteriostats, if present, are removed by repeated rinsing with physiological saline solution (Figs. 1 to 5). It is also possible to place a membrane filter on a prewetted nutrient pad and streak it with a microbial suspension. This technique is recommended for detecting Salmonella, for instance, in an enrichment culture.

Types of Nutrient Pad Sets

1. For determining the colony count:

- a) "Standard" nutrient pads, non-selective media, contain no bacteriostats, and are formulated according to the "Standard Methods for the Examination of Water and Wastewater 1971."
- b) "Standard TTC" nutrient pads have the same formula as "Standard" nutrient pads, but additionally contain 2,3,5-triphenyltetrazolium chloride (TTC) which is reduced to red-colored formazan by most microorganisms, thereby facilitating enumeration of the colonies.
- c) "Caso" nutrient pads, formulated according to the United States Pharmacopeia (USP), constitute a non-selective medium, which does not contain any bacteriostats or dyes. These culture pads can be converted into selective media by mixing the wetting solution with additives before impregnating them.
- d) "Yeast extract" nutrient pads, non-selective media in accordance with the new European Drinking Water Directive, are formulated according to ISO 6222. With their rather low content in nutrients, they are especially suitable for detecting the total count of viable water bacteria.
- e) "R2A" nutrient pads, non-selective media for determining the total microbe count in water; these pads have an especially low nutrient content, such as RO water and highly purified water. Formulated according to the European Pharmacopoeia (EP), 4th edition.

¹⁾ These specifications refer to Sartorius membrane filters.



Pouring the liquid to be examined into the funnel



Placing the membrane filter on a nutrient pad after filtration

2. For selective detection of problem microorganisms:

- a) "Endo" nutrient pads, culture media containing sodium sulfite and fuchsin, are designed for the detection of *E. coli* and coliform bacteria.
- b) "Tergitol" nutrient pads, formulated according to ISO 9308-1, comply with the requirements in the new European Drinking Water Directive for the detection of *E. coli* and coliform bacteria.
- c) "Teepol" nutrient pads correspond to the lauryl sulphate medium in the former ISO 9308-1 and are suitable for detecting lactose-positive *E. coli* and coliform bacteria.
- d) "MFC" nutrient pads are formulated according to the former ISO 9308-1 and clearly indicate the presence of lactose-positive *E. coli* and coliform bacteria.
- e) "Chromocult" nutrient pads are media that contain chromogenic substances, which make it easier to distinguish among *E. coli*, coliform bacteria and others.
- f) "ECD" nutrient pads are selective media for *E. coli* based on the MUG reaction.
- g) "MacConkey" nutrient pads, formulated according to the USP, are formulated for the detection of enterobacteria and are mainly used in the pharmaceutical industry.
- h) "Azide" nutrient pads according to ISO 7899-2 are suitable for detecting fecal enterococci as described in the European Drinking Water Directive.
- i) "Bismuth Sulfite" according to Wilson and Blair are used to detect *Salmonella*.
- j) "Chapman" nutrient pads are ideal for the detection of staphylococci.
- k) "Cetrimide" nutrient pads are formulated according to ISO 12780 for the detection of *Pseudomonas aeruginosa* as described in the European Drinking Water Directive.

3. For the detection of yeasts and molds:

- a) "Wort" nutrient pads contain the natural constituents of beer and are especially convenient for detecting yeasts and molds in the brewing industry.
- b) "Sabouraud" nutrient pads, formulated according to the USP and the EP, are the media of choice for the pharmaceutical industry.
- c) "Lysine" nutrient pads are used for the detection of "wild yeasts" in breweries according to Morris and Eddy.

d) "Schaufus-Pottinger" nutrient pads contain special ingredients specifically formulated for the soft drink industry; these ingredients combined with the pads' low pH effectively suppress bacterial growth.

e) "Malt extract" nutrient pads featuring a low pH of 3.5 have an especially high selectivity with respect to soft drink spoiling yeasts and molds, and largely prevent the growth of bacteria.

4. Culture media for acid formers, acid-tolerant microorganisms, and thermophiles:

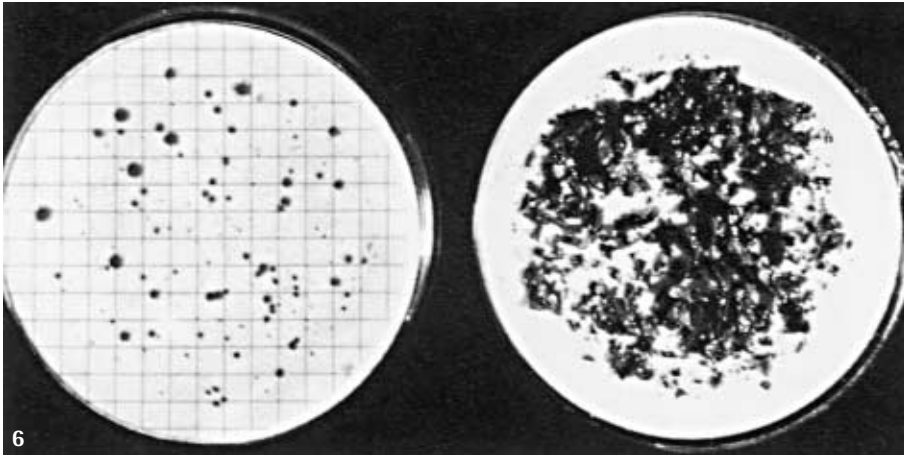
- a) "VLB-S7-S" nutrient pads are formulated for the detection of lactobacilli and pediococci in beverages and pickled or fermented foods according to Emeis, modified.
- b) "Orange Serum" nutrient pads are predominantly used for the detection of acid-tolerant microorganisms in the beverage industry. Formulated according to the "Recommended Methods for the Microbiological Examination of Foods (1966)" of the APHA.

Under aerobic incubation conditions, this type of medium allows the growth of a few acid-tolerant bacteria and fungi, whereas under anaerobic conditions, it promotes the growth of lactic acid bacteria while inhibiting fungi.

- c) "Weman" nutrient pads are suitable for the detection of slime-forming mesophilic bacteria (especially *Leuconostoc mesenteroides*) in beverages, sugar, and sugar products.
- d) "Glucose-Tryptone" nutrient pads, formulated according to Williams, are used for the determination of thermophilic spore formers, particularly of "flat-sour" microorganisms in foodstuffs.

Test Objectives

On the one hand, microbiological testing of foods and beverages focuses on determining the colony count and, on the other hand, demonstrating the absence of specific pathogenic or toxigenic microbes or, by way of substitution, detecting the microorganisms which indicate the possible presence of pathogenic organisms, known as indicator microbes. Even in a perfectly edible state, many foods have a high microbial content, e.g. milk, cheese, or special types of uncooked sausage (salami). These foods require bacteria in order to ripen. In milk and milk products, 10^5 cfu/ml is a long way from indicating poor quality, and in vegetables, either fresh or frozen, colony counts between 10^4 and 10^8 cfu/g are found.



Filtration of parsley; left: prefilter, right: bacteriological filter on a Standard TTC nutrient pad after incubating for 48 hours

Generally speaking, the microbiological and hygienic quality of a foodstuff can be judged by its colony count. High counts always indicate unsanitary processing, too long a storage period, or packaging which is of a hygienically poor quality. On the other hand, a low colony count does not exclude the presence of pathogens or of toxic bacterial metabolites. Products rich in proteins begin to spoil at a count of 10^7 to 10^8 cfu/ml; for foods low in proteins, the limit is higher. At the top of the list of specific pathogens are salmonellae, the microbes that cause typhus, paratyphus or enteritis. Just a few bacteria are all it takes to initiate the outbreak of one of these diseases. Salmonella infections usually result from eating meat, eggs, shellfish, or drinking potable water that is fecally contaminated.

Disease can then be spread through contagion by a "carrier," i.e. by the person who has contracted the disease. Therefore, even microbial detection itself is not without risk and must be carried out with great care and caution so that salmonellae, which have been detected, do not cause further contagion. (Detection with "Bismuth Sulfite" Nutrient Pad Sets followed by biochemical differentiation according to the IMVIC test.) Within the scope of in-process control (for internal use by the plant only), the results of such tests are negative. If it is suspected that a test will yield positive results, the competent authorities should be immediately notified. For the industrial sector, official permission is always required for working with pathogens.

Clostridium botulinum causes acute food poisoning by endotoxins. Clostridia are found in sterile, canned foods which are slightly acidic to neutral. Clostridia grow only under anaerobic conditions. Exact detection of the toxins they produce is only possible in animal experiments. In cafeterias, large kitchens and similar food service facilities, contamination by *Clostridium perfringens* occurs more frequently. These bacteria multiply rapidly when cooked foods are kept above 10°C . Growth is detected on membrane filters incubated on nutrient pads (types: Standard, Standard TTC, and Caso) in an anaerobic system, e.g. in a container for anaerobic incubation. For these strictly anaerobic forms, the pyrogallol technique is usually insufficient.

Staphylococci are pyrogenic organisms that are transmitted to foods from infected wounds (usually harmless, minor hand cuts). However, they are also frequently transmitted from the nasopharyngeal area, e.g. by sneezing. When foods, which have been contaminated with staphylococci, are left in an uncooled place for a considerable length of time, these microorganisms proliferate at a high rate and release toxic metabolites in the process. If they are completely destroyed by heating, the thermostable toxins are nevertheless still potent. For this reason, negative test results for staphylococci (e.g. on "Caso" nutrient pads which have been prewetted with a 7.5% NaCl solution or on "Chapman" nutrient pads) are not a reliable indication that toxins produced by staphylococci are absent.

Frequently, the detection of pathogenic or toxic microbes is too arduous or too time-consuming to be used in routine testing. This applies to the detection of toxins, for instance. Toxin detection is sidestepped by testing for the so-called indicator bacteria. Such bacteria are not necessarily pathogenic, but they often do occur in association with harmful microorganisms, e.g. *E. coli* and coliforms with salmonellae. *E. coli* is a colon bacillus, and its presence indicates fecal contamination of water, beverages, or foods. When large numbers of coliforms are present, we can obviously suspect that salmonellae are involved. Coliforms as well as *E. coli* are easily detectable (see nutrient pad types). However, positive verification on a culture medium must always be followed by biochemical analysis using the IMVIC test if final identification is required. This applies to *E. coli*, salmonellae, coliform bacteria, other enteric bacteria and, ultimately, to all types of bacteria.

Another indicator of fecal contamination is *Streptococcus faecalis* (detection on "Azide" nutrient pads) which belongs to the enterococcus group. Resistant enterococci can be detected in contaminated media over longer periods of time than can fastidious coliforms. This is the reason that they are used for testing foods that are deep-frozen, dried or processed in any manner, or which contain preservatives.

Psychrophilic microorganisms (optimum growth temperature around 10°C) are gaining importance since the refrigeration of meat, milk products, and vegetables largely inhibits the growth of mesophiles and thermophiles. To be sure, some enteric bacteria do grow at temperatures just above the freezing point (*E. coli* at 3.1 to 5°C , enterococci at 0°C). Even so, their rate of proliferation is so minimal that the importance of these food-spoilage bacteria has diminished. At low temperatures, the protein-decomposing bacteria that are taken into account include pseudomonads, certain *Lactobacillus* and *Leuconostoc* species, a few types of streptococci and enterococci, *Achromobacter*, etc. To detect these bacteria, "China Blue" nutrient pads may be used. Non-acid-forming bacteria that decompose proteins have a white to yellow color on this culture medium, while the typical acid-formers display blue colonies. *E. coli* and staphylococci develop grayish-blue to greenish-blue colonies.

Areas of Application

1. Water

In the food and beverage industries, excellent water quality is an absolute prerequisite since water is used as an additive in many foods and beverages and also needed to wash foods and clean containers and equipment utilized in production. Drinking water and industrial water are examined for their colony counts (usually "Standard TTC" nutrient pads) and for the presence of *E. coli* (with "Endo" or "Tergitol" nutrient pads, among others).

2. Beverages

For testing milk and milk products, the following culture media are recommended: "Tergitol" nutrient pads, "Endo" nutrient pads formulated for milk testing, "Standard TTC" or "China Blue" nutrient pads.

Since only small amounts of milk and milk products may be filtered, it is recommended that an emulsifier (nonionic wetting agent, e.g. Tween 80) be added. However, due to its potential bacteriostatic effect, this emulsifying agent must be washed out as quickly and thoroughly as possible by rinsing with a physiological saline solution.

Soft drinks and fruit juices are examined for their colony counts ("Standard" or "Standard TTC" nutrient pads). Detecting yeasts and molds ("Wort" and "Schaufus-Pottinger" nutrient pads) is even more important because these microbes represent a particular risk for fruit drinks and sweetened beverages. Other spoilage bacteria, lactobacilli ("VLB S7-S" nutrient pads) and acid-tolerant bacteria ("Orange Serum" or "Weman" nutrient pads), are also frequently detected.

In breweries, tests are performed to detect yeasts, molds, lactobacilli, pediococci ("VLB S7-SS" nutrient pads for the last two) and "wild yeasts" that may be present ("Lysine" nutrient pads for "wild yeasts" not belonging to the genus *Saccharomyces*).

Microbiological testing of wine is predominantly geared towards detecting yeasts and, in addition, lactobacilli and acetic acid bacteria. "Orange Serum" or "Wort" nutrient pads, which have been prewetted with a 5 to 8% alcohol solution, are used to detect acetic acid bacteria.

3. Sugar, Syrup, and Sugar Products

Candy and sugar products are the prime targets for invading yeasts and molds; slime-forming bacteria are mainly responsible for bacterial invasions (detectable on "Weman" nutrient pads). For testing, sugar, syrup, candy, and other sugar products are dissolved in 10 to 100 times the amount of sterile water by shaking; they can be more easily dissolved by heating to approx. 40°C.

4. Solid Foods

Whereas water, beverages, and sugar products can normally be prepared without difficulty using the membrane filter method, some preparation is necessary to filter solid foods. Vegetables and spices are added to warm (40° to 45°C) physiological saline solution (1:10 or 1:100) and left to soak for about 20 minutes to allow them to expand and soften. Next, this material is broken down further by adding glass beads to the suspension and shaking, or it is completely dispersed in a homogenizer, thereby releasing as many individual microbes as possible into the liquid. A dilution series is prepared from the homogenisate, and different levels of dilution from this series are filtered. After blending, it is necessary to work quickly as the material should be immediately filtered and the membrane filter thoroughly rinsed. In testing foods with a high fat content, such as sausage, cheese, etc., an emulsifier must be added prior to homogenization (0.5% to 1% if Tween 80 is used). The best way to determine the type and concentration of an emulsifier for each particular case is to conduct preliminary experiments. Warming the solution, and, if necessary, the funnel of the filter holder, too, enhances the filterability of the test material.

If large amounts of muscle fibers, vegetable matter, fruit pulp, and similar substances remain on the surface of the filter, the coarse layers may strongly interfere with microbial growth. In this case, a prefilter on top of the bacteriological filter may be used in filtration. It has a pore size of 12 µm and traps all interfering substances on its surface, while allowing most microorganisms to pass through (Fig. 6). In a recovery rate test, less than 5% of the bacteria from a coli suspension was retained by the prefilter. Of course, it must be mentioned that a thick layer of fine-grained matter (flour, cocoa, and similar substances) creates a "deep filter effect," whereby the retention rate sharply increases. If yeasts are to be predominantly detected, we must consider the fact that larger yeast cells may be trapped by the prefilter when their diameters are greater than 10 µm.

Summary

Standardized work procedures and the implementation of in-plant guidelines substantially help the producer bacteriologically test the vast number of foods and beverages and their initial ingredients. In this context, I would like to mention the "Sammlung von Prüfvorschriften zur Mikrobiologischen Untersuchung von Lebensmitteln" (German Collection of Test Specifications for the Microbiological Testing of Foods) published at the beginning of 1980. Membrane filtration combined with Nutrient Pad Sets is a method that permits most alimentary substances to be tested, allows rapid and easy handling, and yields reproducible results.

Literature

[1] Nickersen, J., and Sinskey, A.: Microbiology of Foods and Food Processing. American Elsevier Publishing Company, Inc., 1974.

[2] Sartorius brochure "Microbiological Testing of Foods, Beverages and Pharmaceuticals", Publication No.: SM-4017-e97116.

[3] Schmidt-Lorenz, W.: Moderne Tendenzen bei der mikrobiologischen Untersuchung von Lebensmitteln: Standards – Grenzkeimzahlen – Spezifikationen – Standardisierung der Methoden. (Modern trends in the microbiological control of foods: standards – limiting number of microbes – specifications – standardizing – methods) Chem. Rundsch. 4/1975.

[4] Schmidt-Lorenz, W.: Sammlung von Prüfvorschriften zur mikrobiologischen Untersuchung von Lebensmitteln, 1980. (German Collection of Test Specifications for the Microbiological Testing of Foods).

[5] Scharf, J.M.: Recommended Methods for the Microbiological Examination of Foods. American Public Health Association, Inc. New York, 1966.

Please note that German titles have been translated into English for your convenience; however, no English translations of these publications are available.

Sartorius AG
Weender Landstrasse 94-108
37075 Goettingen, Germany

Phone +49.551.308.0
Fax +49.551.308.3289

www.sartorius.com

Specifications subject to change without notice.
Printed in Germany on paper that has been
bleached without any use of chlorine.
W4A000
Publication No.: SM-8012-e03072
Order No.: 85030-504-16