SARTORIUS

Practical Guide to Easy Microbiological Testing for Craft | Micro Breweries

Brew with Confidence - Spoilage Detection by Sartorius

The art of brewing and the science of microbiology are an interesting and dynamic partnership which has been centuries in the making, one cannot exist without the other. Besides brewer's yeast there also lurk other micro-organisms whose effects can cause detrimental changes in aroma and flavor and ultimately in the quality of the product. Any brewery, whether a craft, regional or multinational, all share the same concerns that their product hits the shelf in prime condition and is safe for consumption.

This practical guide has been developed to help brewers improve their beer quality by finding appropriate solutions to detect the most important beer spoilage microorganisms, during the process, or in the final product.

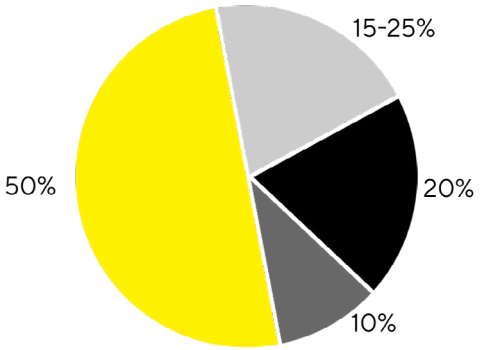
Quality Control

Introduction

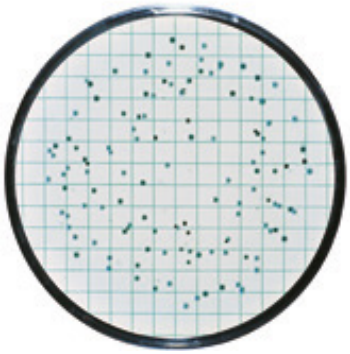
Despite beer's intrinsic anti-microbial properties, due to the presence of hop compounds (e.g. iso-α-acids) and alcohol, the product is not 100% protected and contamination can still occur at many stages of the brewing process.

Among all the potential microorganisms that can negatively affect beer taste and quality, the most important ones are *Lactobacillus brevis, Lactobacillus lindneri* and *Pediococcus damnosus*. To a lesser extent, other potential contaminants, such as molds, wild yeasts and *Saccharomyces* species, can survive hostile conditions and have an undesirable effect on beer quality as well.

Lactobacillus and *Pediococcus* species are hop resistant and can develop off-flavors like diacetyl, lactic acid, acetoin and be at the origin of ropiness and turbidity.



Absolute beer spoilage microorganisms classified according to their degree of importance in beer spoilage incidents (based on studies from Back et al. , 1988; Back, 1994; Hollerová and Kubizniaková, 2001)



Colonies of *Lactobacillus brevis* after filtration and consecutive growth in anaerobic conditions on the selective culture medium VLB-S7-S

The responsibility of *Lactobacillus brevis* is predominant in the apparition of beer-spoilage incidents.

Microbiological Control by Filtration of Large Samples Using Nutrient Pad Sets

How to proceed

1. Before starting, remove everything from the bench that is not essentially needed.
2. Carefully clean and disinfect your working area.
3. For simple microbiological tests a laminar flow hood is not always needed. A good protection against airborne contamination, is to work close to a Bunsen burner[1]. Instruments like forceps can be placed into a small glass recipient containing alcohol.

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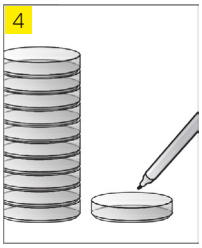

Label the needed amount of Nutrient Pads. Sample identification and date are needed for good traceability.

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Wet the Nutrient Pad with 3.5 ml of sterile, deionized or distilled water using the dosing syringe and a Minisart®. Open the lid of the dish only slightly to avoid airborne contamination.

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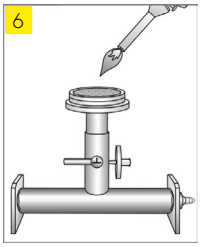

Start the vacuum source and open the Microsart® Manifold valve. Carefully flame[1] the filter support for ~5 sec. Close the valve again.

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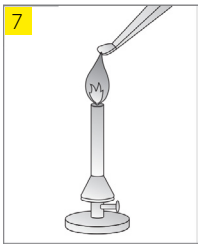

The forceps should always be stored in a small glass with alcohol. Take them out and flame[1] them. Let them it cool down for a few seconds before use.

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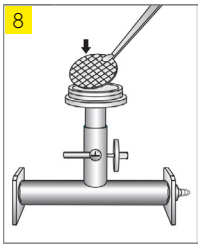

Open the membrane filter individual pack. Using the forceps remove the membrane filter and place it onto the filter support.

[1] in areas where the use of a flame is forbidden, use alcohol

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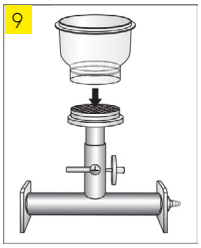

Adapt the Microsart® Funnel tightly on the filter support.

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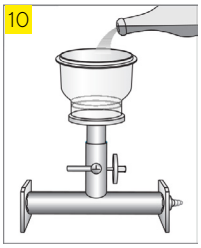

Pour the sample, open the valve and start filtration. Close the valve when there is no sample left in the funnel.

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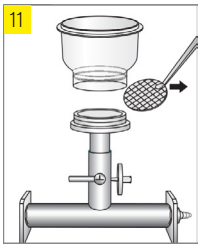

Lift the funnel up and remove the membrane filter disc using the sterile forceps. (see step 7)

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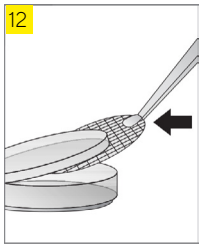

Place the filter on the Nutrient Pad, avoid entrapping air bubbles below the filter. Open the lid only slightly to avoid airborne contamination.

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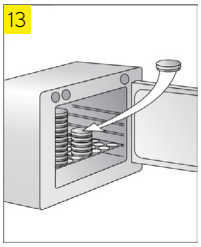


Place the Petri dish in the incubator, lid above. For generating anaerobic conditions, place several dishes in a bag containing a gas generator*. Incubate according to the recommendations. Evaluate immediately after the end of the incubation time.

*for generating anaerobic conditions, we recommend placing the plates in bags where a gas generator is added. Oxoid AnaeroGen™ W-Zip (part Number AN0010W) ready to use kits including pouches and reagents are available from Thermo Fisher Scientific™.

Culture Media VLB-S7-S for the Detection of *Lactobacillus* and *Pediococcus,* Quality Control by Sartorius and Evaluation of Results With the Membrane Filtration Method.

VLB-S7-S NPS

Part Number 14059-47--N
100 nutrient pads plus 100 membrane filters

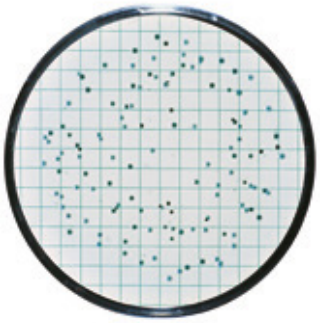
For the detection of *pediococci* and *lactobacilli* according to Emeis; modified acc. to Rinck and Wackerbauer. Dehydrated culture medium for cultivating microorganisms from beer and other products.

References:
EBC, MEBAC, VLB-BERLIN*

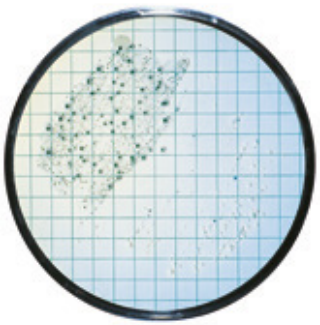
Incubation Conditions:
5-8 days at 24-28°C anaerobic
(microaerophilic)

Evaluation and Typical Results:
Pediococci ("Sarcina") develop round pale green colonies with smooth peripheries and approx. 1 mm in diameter. *Lactobacilli* grow as slightly rounded, irregularly lobed colonies with approx. 2 mm in diameter which are initially light green and later dark green.

Remarks: This medium must be incubated under anaerobic to microaerophilic conditions.



Lactobacillus brevis



Lactobacilli and pediococci from sediment, streak

Quality Control by Sartorius - Growth of Tested Microorganisms

Positive

Lactobacillus brevis
Lactobacillus lindneri
Pediococcus damnosus

Negative

Enterobacter aerogenes

*EBC (European Brewery Convention), MEBAC (Middle European Brewery Analysis Commission), VLB-BERLIN (Versuchs- und Lehranstalt für Brauerei in Berlin

Equipment and Consumables Needed

Description	Purpose	Number of units	Part Number
Equipment			
1 branch Microsart® Manifold for Microsart® Funnels[1] (includes 20 cm of vacuum hose for pump connection)	Filtration of one large sample at a time	1	168M1-MS
Microsart® e.jet transfer pump	Generates vacuum to filter the samples	1	166MP-4
Silicone tubing pressure-sided, 2 m	For sample drainage to waste	1	1ZAS--0019
Stainless steel forceps	For transferring membrane filters to culture media or filtration base	1	16625
Dosing syringe (0.5-5 ml)	To wet the culture media pads with 3,0-3,5 ml of sterile water	1	16685-----2
Benchtop Incubator 100-240V, 50/60Hz, 18 liters	To incubate the samples after filtration	1	18119
Consumables			
VLBS7-S - Nutrient Pad Sets [2][3] Petri dishes, Culture media pads and membrane filters	Detection of *Lactobacilli, Pediococci* and other beer spoiling microorganisms	100	14059--47------N
Minisart® High-Flow sterile (individually packed)	Sterile syringe filters for wetting the nutrient pads	50	16532----K
Microsart® Funnel 250 (sterile) – 6 bags containing 16 funnels each	Sterile 250 ml funnels for beer samples filtration	96	16A07--25------N

[1] 3 branch (168M3-MS) and 6 branch (168M3-MS) Microsart® manifolds are available in case higher throughput is needed. All manifolds can be connected together with rapid connectors.

[2] for generating anaerobic conditions, we recommend placing the plates in bags where a gas generator is added. Oxoid AnaeroGen™ W-Zip (part Number AN0010W) ready to use kits including pouches and reagents are available from Thermo Fisher Scientific™.

[3] Many other culture media are available for the detection of potential beer spoilage microorganisms such as wild yeasts (e.g. Lysine) or for total count determination of yeast and molds (e.g. Wort).

Microsart® one branch manifold

Dosing syringe with Minisart® High-Flow

Microsart® funnel 250ml

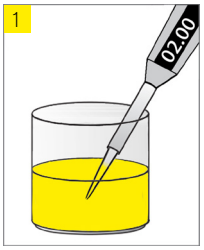
Microsart® e.jet transfer pump

Notrient Pad Sets (include dish, pad and membrane filter

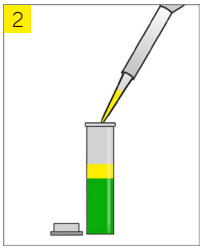
Microbiological Incubator 18 liters

Microbiological Control by Direct Inoculation of Small Samples (Approx. 2ml – Usually in-Process Samples) in Liquid Culture Media

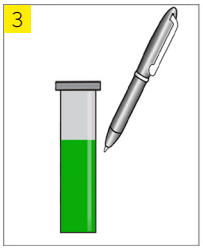
How to proceed



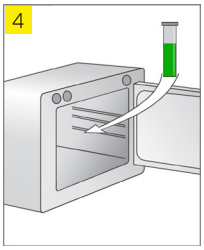
Pipette 2 ml of beer, wort or any other liquid sample to be analyzed.



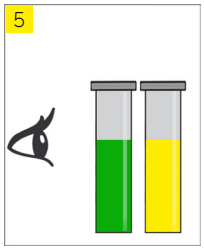
Open the VLB-S7-S broth tube, inoculate the sample into the tube.



Close the tube tightly and immediately and identify the sample by labelling the tube or writing on it.



Place the inoculated tube and a control tube containing no sample in the incubator at 26-28°C for 7 days maximum.



1. If clear and green: no contamination
2. If a yellow coloration and turbidity appear: growth of *Lactobacillus* and/or *Pediococcus*.

Culture Media for the Detection of *Lactobacillus* and *Pediococcus*, Quality Control by Sartorius and Evaluation of Results in the Case of Direct Inoculation of the Sample in the Culture Medium Broth.

VLB-S7-S

Part Number 14127———-K
50 tubes each containing 20 ml of broth

For the detection of *pediococci* and *lactobacilli* according to Emeis; modified acc.to Rinck and Wackerbauer. Liquid culture medium for cultivating microorganisms by direct inoculation of a small sample into the tube.

References:
EBC, MEBAC, VLB-BERLIN*

Incubation Conditions:
5-7 days at 26-28°C anaerobic (conditions are given when the tube is closed)

Evaluation and Typical Results:
When not inoculated, the culture medium broth is green. A pH indicator (bromocresol green) is present and will turn to yellow when acidity is produced. The change of color and the development of turbidity in the tube clearly indicate a microbial contamination, with a strong presumption of *Lactobacilli* or *Pediococci*.

Quality Control by Sartorius

The left tube (green color) shows a typical negative result. From left to right (yellow color and turbid), positive growth of : *Pediococcus damnosus, Lactobacillus brevis, Lactobacillus lindneri*

*EBC (European Brewery Convention), MEBAC (Middle European Brewery Analysis Commission), VLB (Versuchs-und Lehranstalt für Brauerei in Berlin)

Equipment and Consumables Needed

Description	Purpose	Number of units	Part Number
Equipment			
Proline® Plus Pipette 0,5 - 5ml	For inoculating 2ml of sample in the VLBS7-S Broth	1	728080
Incubator, 100-240V 50/60Hz, 18 liters	To incubate the samples after inoculation	1	18119
Consumables			
Safetyspace™ Filter Tips, 100-5000 µl, single tray (pre-sterilized)	For transferring 2ml of beer in the VLBS7-S Broth	50	LH-795001F
VLBS7-S Broth Media in 20 ml Tubes, 50 Tubes per Box	To be inoculated with 2 ml of sample for the detection of *Lactobacolli, Pediococci* and other beer spoiling microorganisms	50	14127----------K

Proline Plus pipette 0,5 – 5 ml

Safety Space Filter Tips

VLB-S7-S broth media 20 ml tubes

Microbiological Incubator 18 liters

https://www.youtube.com/watch?v=byexb5DA-o4

Nutrient Pad Sets - For Quality Control of Beverages and Water Samples

https://www.youtube.com/watch?v=d5gTxYrL2cU

Microsart® Manifolds - Adaptable to your needs

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