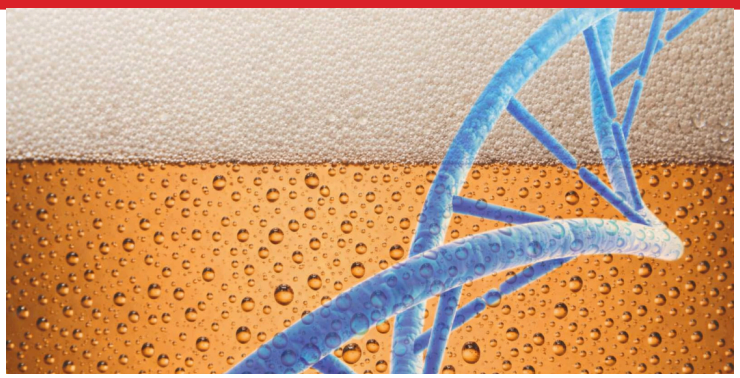
milenia biotec

Lateral Flow Rapid Tests *and more*



Milenia GenLine – Direct Detection of Beer Spoilage Bacteria from Swabs

Background

From a microbiological perspective, breweries have multiple critical areas where product spoilage microorganisms can enter beer. These areas are frequently cleaned and inspected via routine hygiene control procedures.

To assess hygiene-swabs, breweries usually apply classic culturing methods. The detection of anaerobic growing organisms in particular, for example *Megasphaera* and *Pectinatus* species, can take weeks in culture.

Therefore a significant period of time passes before a microbiological safety issue is identified.

The Right Solution …

PCR tests supplied by Milenia Biotec allow the detection of beer spoilage bacteria **directly from swabs**, enabling brewers to detect weak areas and to start counter actions earlier.

Direct Detection of Beer Spoilers from Swabs

About Workers and Secret Roommates

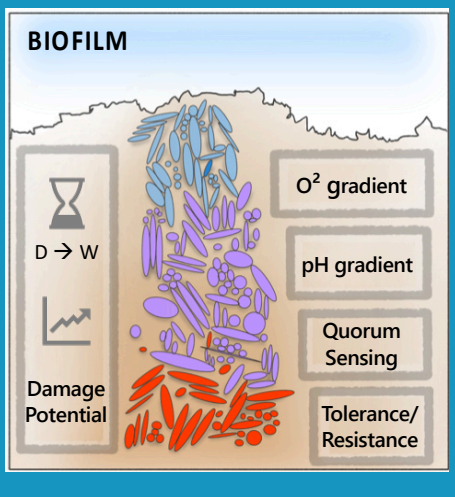
A brewery is a place where not only humans are working hard to make a high quality product. Brewing is a biotechnological process where **microorganisms** play an important role. One of the most diligent co-workers in the brewery is the brewing yeast. For microorganisms the brewery is not only a working place but it is also an **ecological niche**. In this environment, microorganisms may be present which are capable of creating off-flavours and consequently a large headache for brewers.

If the Beer Comes to Life

In the **filler areas**, the open bottle and product are in close proximity to each other and the open environment. The high level of moisture, frequent agitation and continuous overflow of beer creates the perfect situation for microbes to attach and flourish in such areas. In the warmer months of the year, **biofilms** can grow quickly and create an environment where beer spoiling organisms can grow. For this reason the **"secondary contamination"** is an issue brewers frequently deal with; close microbial monitoring of these areas is of utmost importance.

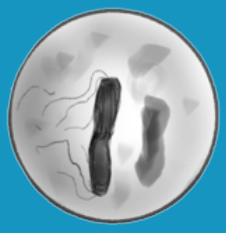
Presence of microorganisms, germ pressure, presence of potential and obligate beer spoilers:

The method of choice for the identification of these contaminants is the hygiene swab.



Biofilms are an exciting microenvironment; a **complex** and variable community of many different species, including bacteria, fungi, algae and protozoans.

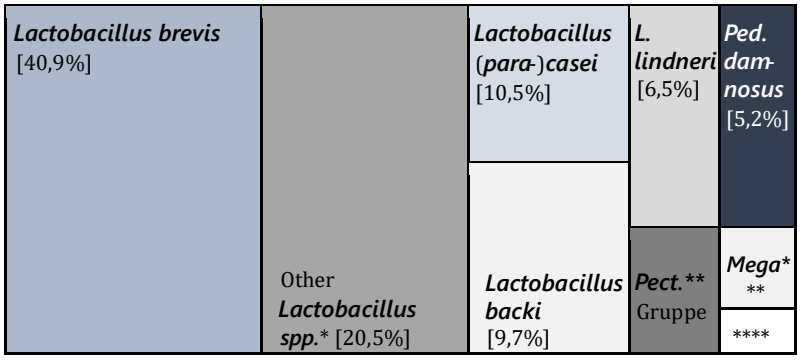
In breweries, biofilms with **spoilage potential** can develop rapidly. If such a biofilm is located in the area of the fillers, and enters a status where anaerobic growth is possible, **product spoilage lactic bacteria** and other potential obligate spoilers can grow. In practice the bacterial groups of ***Megasphaera*** and ***Pectinatus*** play an important role.



These obligate anaerobic bacteria show high spoilage potential, especially in non-pasteurized beers, and have a dramatic negative impact on product quality.

Beer spoilage is characterised by cloudiness and an off-flavour (similar to the smell of drains). Detection by culture is often difficult and time consuming. However, the results are of high importance to maintain a good level of brewery hygiene.

Statistical Frequency of Beer Spoilage Bacteria



Schneiderbanger et al. 2017 TUM BLQ Weihenstephan: „Statistical evaluation of beer spoilage bacteria by real-time PCR analyses from 2010-2016"

A frequent phenomenon seen in breweries is the accumulation of highly specialized bacteria, especially in biofilms. However, these bacteria cannot be grown in cultures. Known as, **'Viable But Non-Culturable'**, Lactobacillus acetotolerans is a good example of a potential spoiler that grows poorly in standard-enrichment media. **Yet, with a PCR-based direct test, such organisms become detectable.**

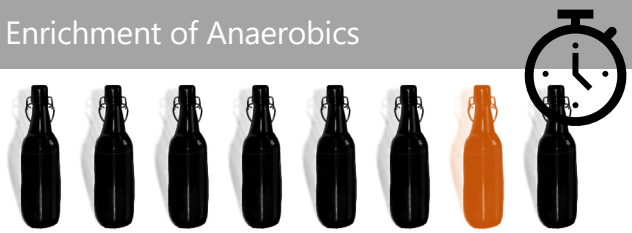
swabPCR - Detection from a Swab

The Idea Behind the Product

In close cooperation with a partner brewery, our goal was to detect obligate anaerobic beer-spoilers directly from swabs. Culture-based detection is too time consuming and does not allow timely countermeasures in daily practice.
Particularly in the warmer months over the summer, when large volumes of beer are produced and bottled to meet higher demands, bacteria find an ideal environment to grow. In this event, it is difficult to evaluate the hygiene status if detection procedures take weeks to yield results. **For this reason, methods which allow same-day results are invaluable**.

Conventional Method-

Enrichment of Anaerobics



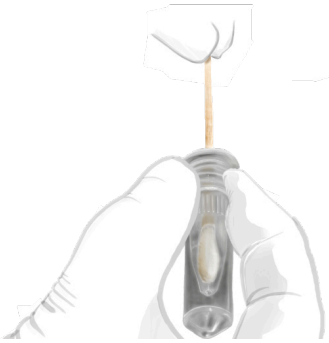
Time to Result: Days to Weeks

Cultural enrichment takes place under selective conditions, using for example , non-alcoholic beer. The volumes required are often large (0.2 – 0.5 L). Clouding due to contamination becomes visible after a couple of days or **several weeks**.

Method swabPCR - 3 Steps to the Result

Step 1: **Extraction fom the Swab**

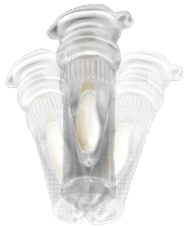
Mechanical breakup of the biofilm by vigorous twisting of the swab.

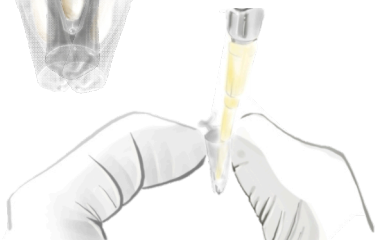


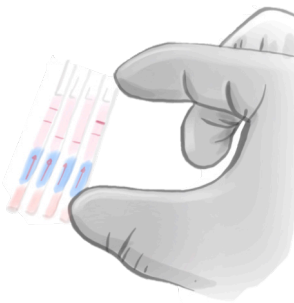
Break off the swab and pipette the Milenia Swab Detection Buffer directly onto the cotton pad.

In order to collect all components of the biofilm, centrifuge the tubes.

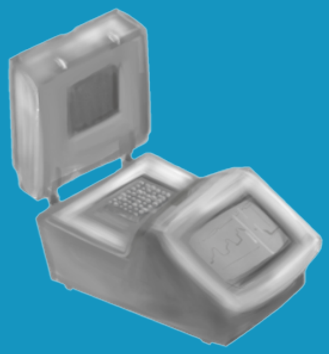
After extensive mixing, transfer a 2 µL sample from the tube to the PCR mix.







After completing the PCR and test strip run, results can be interpreted by the naked eye.



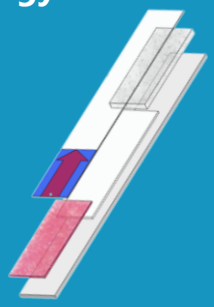
LabCycler 48 s - PCR Thermal Cycler

Step 2**: DNA Amplification**

Milenia GenLine Tests are based on PCR technology. PCR allows the amplification of very specific fragments of bacterial genes. The enzyme-based DNA amplification is done within a thermocycler, an instrument that is capable of quickly running defined temperature profiles. The PCR procedure in the thermocycler takes 50 minutes.

Step 3: **Lateral Flow Technology**

The resulting PCR products can be detected easily and quickly using the lateral flow test strip of the Universal Module (REF: MGUP 1).
2 µL of the PCR sample is pipetted directly onto the strip, which is then dipped into a buffer for 5 minutes. The strip is as easy to interpret as a pregnancy test.



Lateral Flow Test Strip

Time from Sampling to Result: 2 Hours

Conclusion:

The **Milenia swabPCR-System** allows the direct detection of obligate spoilers from a swab. Areas of poor hygiene and hotspots of biofilm formation can be quickly identified and monitored.
In practice the Milenia GenLine *Megasphaera/ Pectinatus* test showed excellent correlation with the reference culture.

Features and Benefits of Milenia GenLine

- Sensitive and Specific
- Internal PCR Control included in the test strip
- Positive Controls included in the Test Kit
- Direct Sample Application without DNA Purification
- Low Investment in equipment
- Minimal Influence of Yeast
- Simple workflow with fast turnaround from sample to result
- Robust Detection System
- Results in 2 h

Order Information

Milenia Swab Extraction

Product	REF	Content
Swab Detection Buffer	MGSDB	10 ml
Milenia GenLine Extraction System	MGES 1	1000 Reactions

PCR and Lateral Flow Evaluation

PCR Universal Module	MGUP 1	48 Tests

The Universal Module is always used in combination with the PCR Modules.

PCR Modules

Lactobacillus/Pediococcus Screen	MGScLP 1	48 Tests
Hop Resistance Gene Screen	MGScHOR 1	48 Tests
Megasphaera/Pectinatus Screen	MGScMP 1	48 Tests

Accessories

LabCycler 48s - PCR Thermal Cycler	MLCY	1 Device

Contact

Any questions, requests, or suggestions? Feel free to contact us at any time.

Milenia Biotec GmbH
Versailler Str. 1
35394 Gießen
Germany

www.milenia-biotec.com
info@milenia-biotec.de
+49 641 948883-0