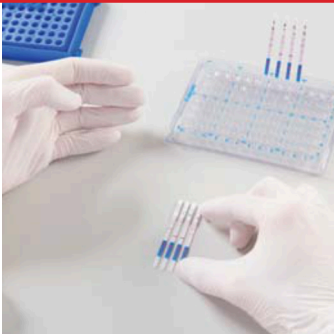




milenia biotec



# Detection of Beer Spoiling Germs

simple, fast, reliable

**Milenia GenLine**

## Table of Contents

<a href="#">About us</a>	3
<a href="#">Milenia GenLine</a>	4
<a href="#">Technology</a>	5
<a href="#">Laboratory Equipment Needed</a>	8
<a href="#">Sample Preparation</a>	9
<a href="#">Test Procedure</a>	10
<a href="#">Interpretation of the Test Results</a>	11
<a href="#">Milenia GenLine Lactobacillus / Pediococcus Screening</a>	12
<a href="#">Performance Data Milenia GenLine Lactobacillus / Pediococcus Screening</a>	15
<a href="#">Milenia GenLine Hop Resistance Gene Screening</a>	16
<a href="#">Performance Data Milenia GenLine Hop Resistance Gene Screening</a>	17
<a href="#">Available Products</a>	18
<a href="#">Features and Advantages of Milenia GenLine</a>	19
<a href="#">Contact Information</a>	20

## About us

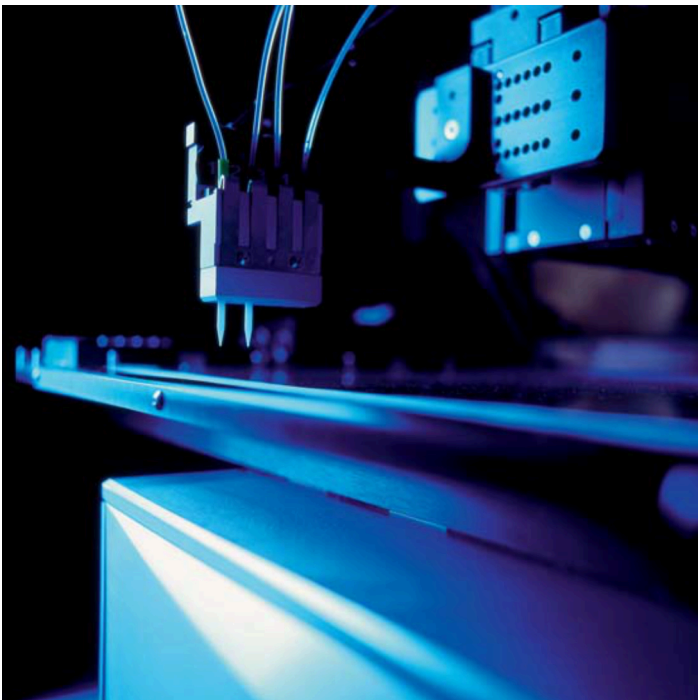
Milenia Biotec GmbH was founded in 2000 and is engaged in the development, production and distribution of rapid diagnostic tests based on the technology of lateral flow tests. The best-known example is the Corona rapid test.

The products are very easy to use and can be evaluated either visually or with simple devices. Results are available in a short time.

In order to provide products that meet our customers' expectations in terms of service and quality, Milenia Biotec GmbH has had a quality management system in place since 2003 that is certified by TÜV Rheinland in accordance with EN ISO 13485:2012.

Milenia GenLine is a product line of molecular biological detection systems with a modular structure. All GenLine tests are based on a universal test strip that can detect any molecular biological product. The strips are provided in a "[PCR Universal Module](#)". The molecular biology modules ([PCR Modules](#)) are developed in addition to the strips. This approach allows us to implement new developments quickly and also serve niche markets.

The first products in the Milenia GenLine product line are tests for the detection of beer spoiling bacteria.



### Background

Beer is a medium in which germs find poor growth conditions due to the alcohol content and low pH value. Furthermore, the carbon dioxide and the bitter substances contained in hops represent additional growth barriers for microorganisms.

Nevertheless, some germs have managed to adapt to the beer environment and grow in it. These have beer-damaging potential, as their growth can lead to turbidity and changes in taste.

This is why testing for beer spoiling bacteria is an increasing challenge in breweries. The **Milenia GenLine** product line provides a test system for the molecular biological detection of beer spoiling organisms that is **fast, simple and reliable**.



## Technology

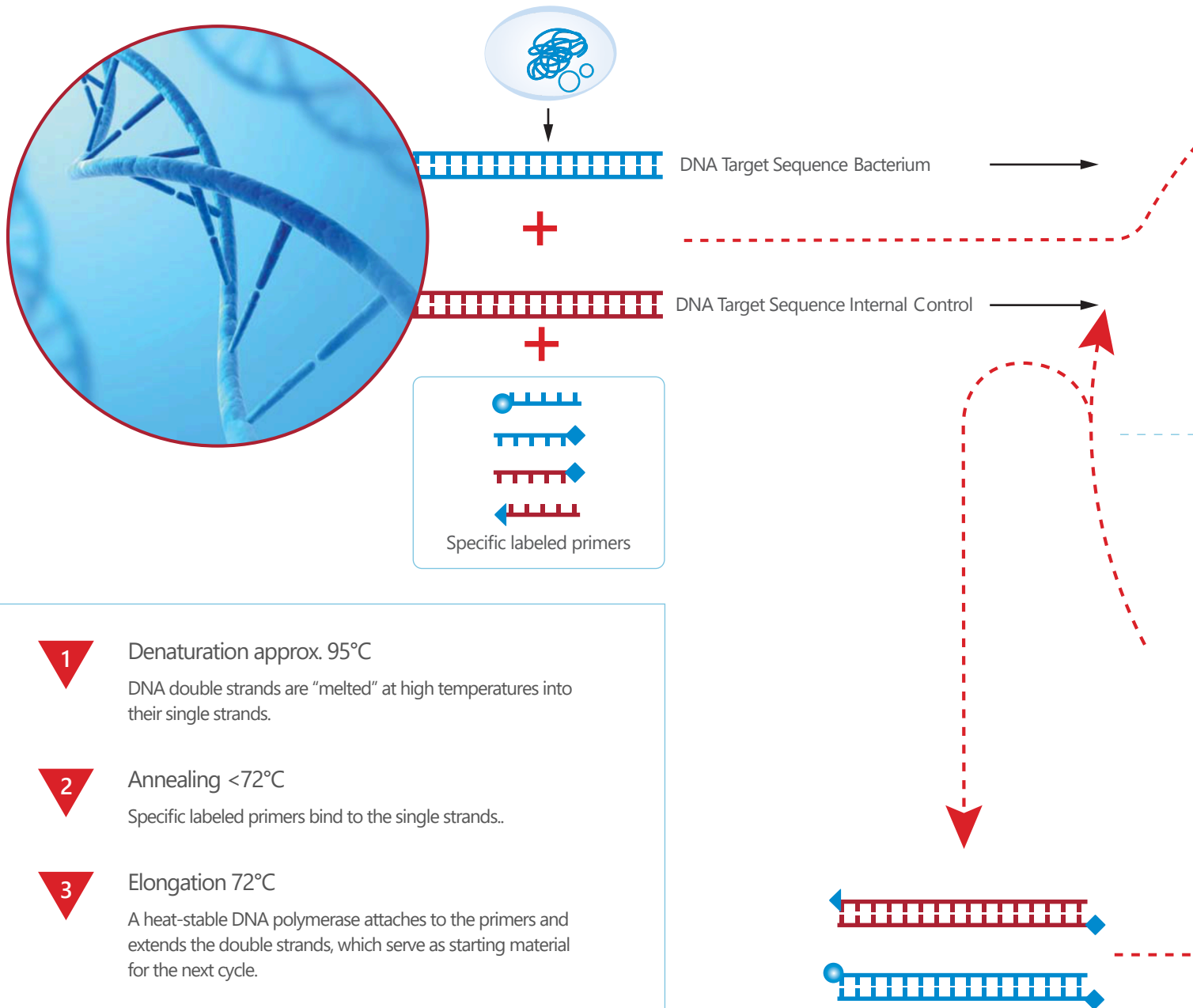
PCR (polymerase chain reaction) is used as the technological basis for the Milenia GenLine tests.

A DNA polymerase and short single-stranded DNA pieces, called primers, are used to amplify defined DNA sequence regions of a sample in vitro. PCR is based on a temperature protocol consisting of several cycles that are run in succession. In this way, a doubling of the genetic target sequence is achieved per cycle. According to this principle, a single copy of the target DNA segment will result in over 1 billion copies after 30 cycles.

The primers used in the Milenia GenLine tests are provided with specific markers that are recognized by the test strips. The Milenia GenLine test enables the detection of two different gene products on one strip. By adding a control gene to the reaction preparations, a statement can be made as to whether the PCR has been carried out correctly in the specific preparation.



# Technology



1

Denaturation approx. 95°C

DNA double strands are "melted" at high temperatures into their single strands.

2

Annealing <72°C

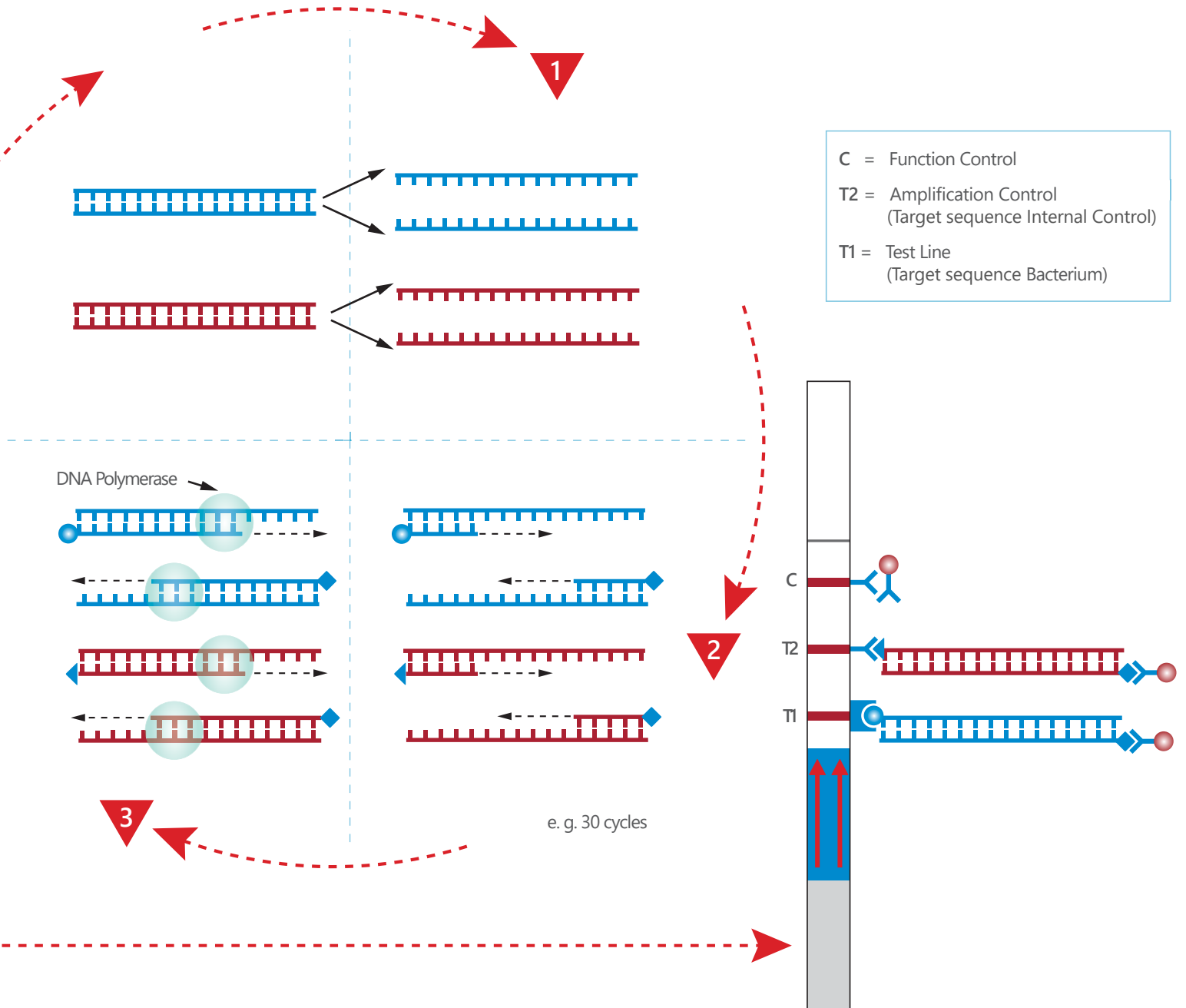
Specific labeled primers bind to the single strands.

3

Elongation 72°C

A heat-stable DNA polymerase attaches to the primers and extends the double strands, which serve as starting material for the next cycle.

# Technology



## Laboratory Equipment Needed

- ▼ Simple Thermal Cycler
- ▼ Pipettes (2-20 $\mu$ l and 20-200 $\mu$ l)
- ▼ Pipette Tips
- ▼ PCR Reaction Tubes
- ▼ Eppendorf Tubes
- ▼ Stand for Reaction Tubes
- ▼ Mini Centrifuge
- ▼ Vortexer
- ▼ Freezer





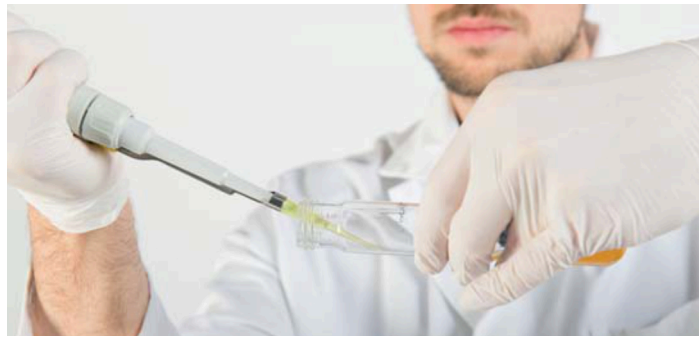
## Sample Preparation

The Milenia GenLine tests for the detection of beer spoiling bacteria can be carried out without DNA isolation from the sample. Most common liquid enrichment media are suitable as sample material. These include the media from Döhler (NBB-B, NBB-B-AM, NBB-C), other MRS-based media, wort and beer.

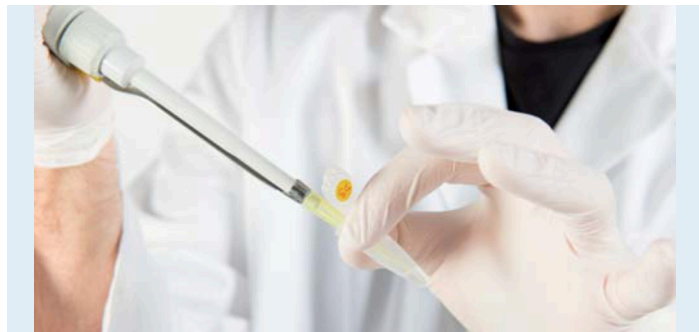
In-house media can be tested for compatibility very easily.  
Yeast-containing samples can also be analyzed directly.

Furthermore, the Milenia GenLine tests allow direct analysis of single colonies of solid media (media-independent).

### 1. Testing from Liquid Enrichment



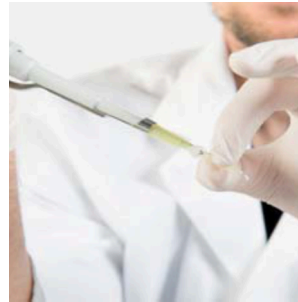
### 2. Direct Analysis of Single Colonies on Solid Media



## Test Procedure

1

Pipette 2  $\mu\text{l}$  of the prepared samples into PCR tubes, place in the thermal cycler and start the program.



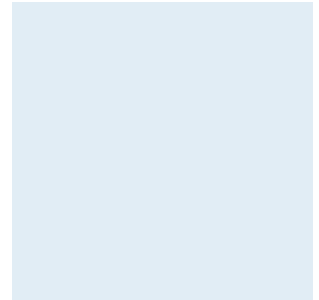
2

Remove the PCR tube from the thermal cycler and pipette 2  $\mu\text{l}$  onto the sample application site of the test strip.



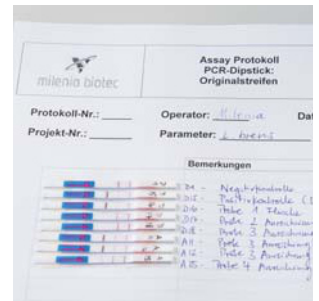
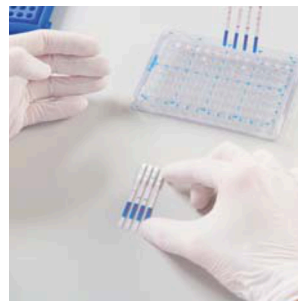
3

Place the strip in a container (e.g. microtiter plate) containing 80  $\mu\text{l}$  of running buffer.



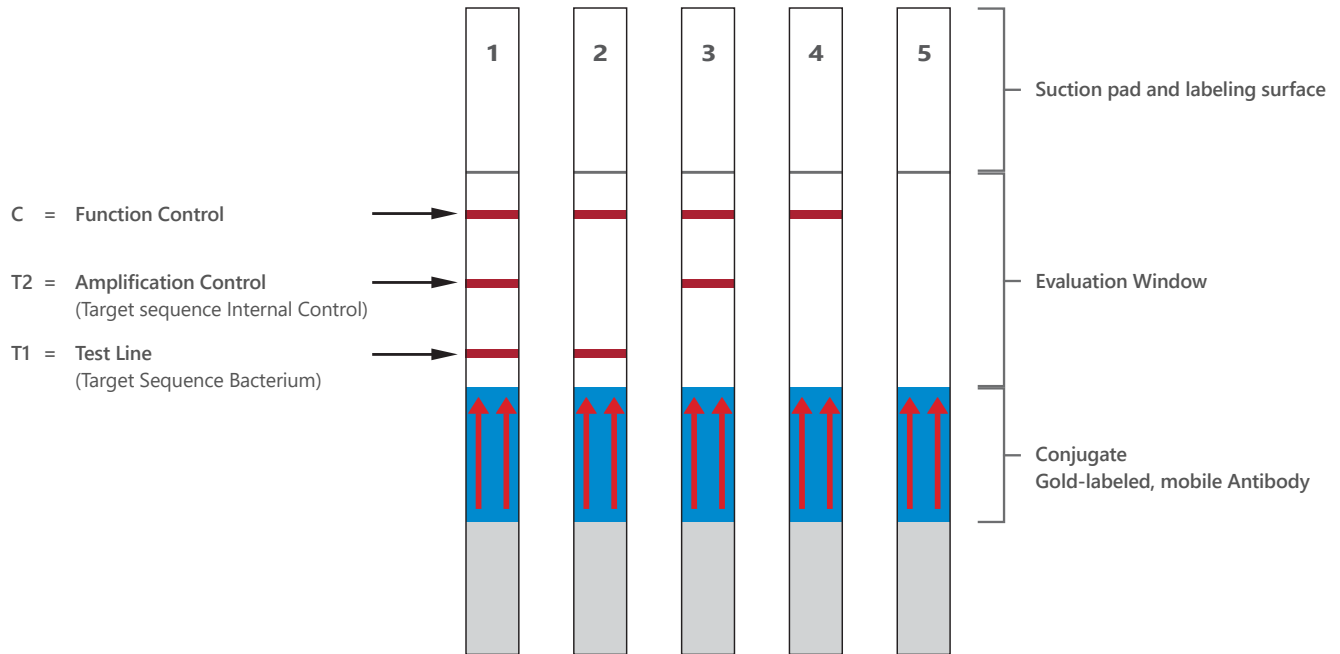
4

Read the test strips after 5 minutes.



## Interpretation of the Test Results

Possible Results of the Evaluation of PCR Products using the Universal Test Strip ([REF MGUP 1](#))



- ▼ Strips 1 and 2 are to be interpreted as clearly positive findings.
- ▼ Strip 3 corresponds to a negative result.
- ▼ Strip 4 indicates a complete inhibition of the PCR.  
This is not a valid negative result, but a sample that cannot be evaluated; the sample must be repeated.
- ▼ Strip 5 cannot be evaluated.

↑↑ The red arrows on the test strip indicate the running direction.

## Milenia GenLine *Lactobacillus* / *Pediococcus* Screening

### Intended Use

The Milenia GenLine *Lactobacillus* / *Pediococcus* Screening is a confirmation and screening test for the most common beer spoilers. The test detects the most important spoilers of the genera *Lactobacillus* and *Pediococcus*, including *Lactobacillus brevis*, *Lactobacillus lindneri*, *Lactobacillus casei* and *Pediococcus damnosus*. The Lactobacilli used for biological acidification (e.g. *Lactobacillus delbrueckii*, *Lactobacillus amylovorus*, etc.) and the most important brewery-relevant yeasts are not detected.

Therefore, the Milenia GenLine *Lactobacillus* / *Pediococcus* Screening is a particularly useful tool for the assessment of microbiological sample material that is difficult to interpret, for example yeast-containing samples or non-selective enrichments, such as swab samples enriched in NBB-B-AM.

#### detectable ✓

*L. brevis*  
*L. backi*  
*L. casei*  
*L. paracasei*  
*L. collinoides*  
*L. paracollinoides*  
*P. damnosus*  
*L. lindneri*  
*L. plantarum*  
*L. harbinensis*  
*L. rossiae*  
*L. coryniformes*  
*L. acetotolerans*  
*L. frisingensis*  
*L. perolens*  
*P. clausenii*  
*P. inopinatus*

#### not detectable x

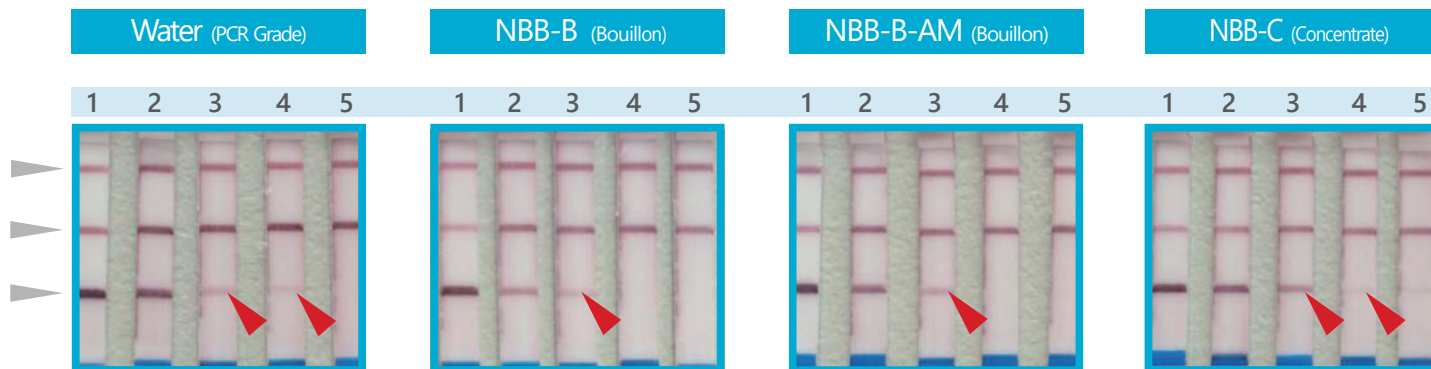
*L. delbrueckii*  
*L. amylovorus*  
*L. amylolyticus*  
*Megasphaera sp.*  
*Pectinatus sp.*  
*Acetobacteriaceae*  
*(Enterobacteriaceae)*  
*Torulaspora delbrueckii*  
*Wickerhamomyces anomalus*  
*Saccharomyces pastorianus* 34/70  
*Saccharomyces cerevisiae* 68  
*Saccharomycodes ludwigii*

## Milenia GenLine Lactobacillus / Pediococcus Screening

### Sensitivity / Media used

The Milenia GenLine tests can be used for direct analyses from enrichments. No DNA extraction is required. This considerably reduces the workload and complexity of processing.

All Milenia GenLine detections are compatible with common detection media. Processing by direct analysis works both from liquid enrichments and as colony analysis of solid media.



#### L. brevis: 1821B

1	ca. $1 \times 10^6$ cfu/mL
2	ca. $1 \times 10^5$ cfu/mL
3	ca. $1 \times 10^4$ cfu/mL
4	ca. $1 \times 10^3$ cfu/mL
5	ca. $1 \times 10^2$ cfu/mL

#### Sensitivity:

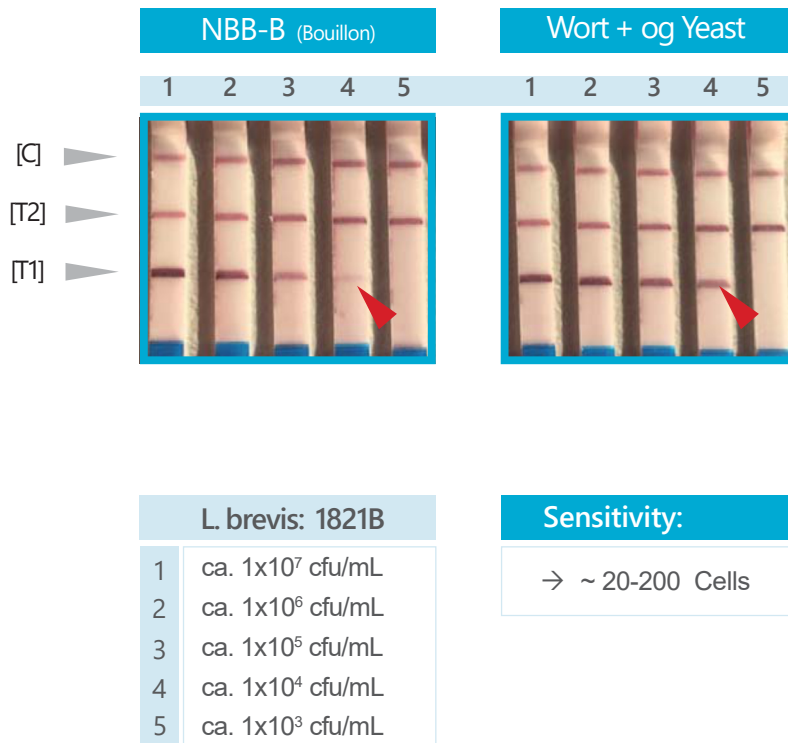
PCR Water	→	~2-20	Cells
NBB-B	→	~20-200	Cells
NBB-B-AM	→	~20-200	Cells
NBB-C	→	~20-200	Cells

# Milenia GenLine Lactobacillus / Pediococcus Screening

## Sensitivity / Yeast containing Samples

The analysis of yeast-containing samples is often particularly difficult in breweries. With the Milenia GenLine tests, it is possible to carry out direct measurements from yeast-containing samples with very good sensitivity.

The detections are therefore a valuable tool for analyzing sample matrices that are difficult to interpret.



## Performance Data Milenia GenLine Lactobacillus / Pediococcus

		Milenia (LB-PC-Screen)		
		+	-	Total
RT-PCR (sLP600, Brandl 2006)	+	16	0	16
	-	3	31	34
Total		19	31	50
				Concordance index 0,91

# Milenia GenLine Hop Resistance Gene Screening

## Background information

Beer is a difficult habitat for microorganisms to colonize. However, some specialists have managed to tolerate this environment and grow in beer. An often decisive factor for microbial growth in beer is the ability to tolerate hop bitters (primarily iso-alpha-acids). The Milenia GenLine Hop Resistance Screening identifies two of the most important cross-species genetic markers for resistance to hop bitterness.

If the bacteria detected have the corresponding genes, it is highly probable that they are strong spoilage organisms with a considerable product damage potential.

## Use and comparison with the product spoilage test

The Milenia GenLine Hop Resistance Screening is suitable as a confirmation and screening test for obligate beer spoilage members of the genera *Lactobacillus* and *Pediococcus*. This test can be used to assess the harmful potential of potentially beer spoiling bacteria. This is a new type of tool that provides information in less than two hours, which previously took more than 14 days using the classic test.

No.	Organism	Milenia GenLine Tests		Cultivation						
		Lacto-Pedio-Screen	Hop Resistance Screen	NBB-B-AM (Bouillon)	NBB-B (Bouillon)	Hefeweizen	Lager	Pilsener	Pale Ale	Double IPA
	Alcohol Content					<0,04	5,2	5,1	5,7	8,3
	pH Value					4,36	4,73	4,46	4,79	4,91
	IBU's					15	24	32	36	83
-	Negative Control	-	-	-	-	-	-	-	-	-
1	<i>Lactobacillus brevis</i> I	+	+	+	+	+	+	+	+	+
2	<i>Lactobacillus brevis</i> II	+	+	+	+	+	+	+	-	-
3	<i>Lactobacillus lindneri</i> I	+	+	+	+	+	+	+	+	+
4	<i>Lactobacillus lindneri</i> II	+	+	+	+	+	+	+	-	+
5	<i>Lactobacillus backi</i> 2334	+	+	+	+	+	-	+	+	+
6	<i>Pediococcus damnosus</i>	+	+	+	+	+	+	+	+	+
7	<i>Lactobacillus rossiae</i> I	+	+	+	+	+	+	-	-	-
8	<i>Lactobacillus rossiae</i> II	+	-	+	+	-	-	-	-	-
9	<i>Lactobacillus casei</i> 610	+	-	+	+	-	(+)*	(+)*	-	(+)*
10	<i>Lactobacillus parabuchneri</i>	+	-	+	+	n.a.	-	-	-	-
11	<i>Lactobacillus plantarum</i>	+	-	+	+	-	-	-	-	-
12	<i>Leuconostoc mesenteroides</i>	-	-	+	+	-	-	-	-	-
13	<i>Lactococcus lactis</i>	-	-	+	+	n.a.	-	-	-	-

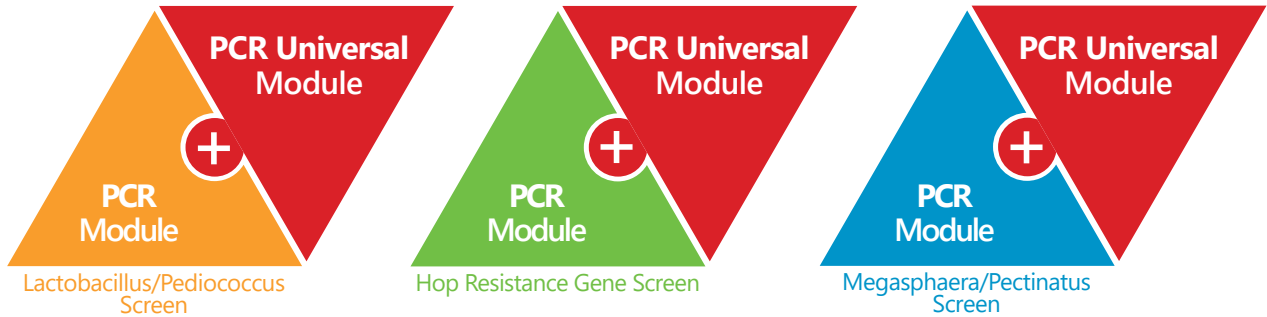
Analyses were carried out in duplicate, \* bacterial growth was only recorded in 1/2 cultures; in these samples a renewed test for hop resistance was positive



## Performance Data Milenia GenLine Hop Resistance Gene Screening

		Milenia (HOR)		
		+	-	Total
RT-PCR (TUM FZWBLQ, Hor A or Hor C Gene)	+	10	0	10
	-	0	7	7
Total		10	7	17
				Concordance index 1

## Available Products



### 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

## Features and Advantages of Milenia GenLine

- ▼ Sensitive and Specific
- ▼ Internal PCR Control
- ▼ Positive Controls included in the Test Kit
- ▼ Direct Sample Application without DNA Purification
- ▼ Low Costs in Devices
- ▼ Low influence of yeast
- ▼ Results in 60 Minutes





**Milenia Biotec GmbH**

Versailler Str. 1  
35394 Gießen  
Germany

Tel.: +49 641-94 888 3-0  
Fax: +49 641 888 3-80  
E-Mail: [info@milenia-biotec.de](mailto:info@milenia-biotec.de)  
Web: [www.milenia-biotec.com](http://www.milenia-biotec.com)

design: VISUV

fotos: Hendrik Roggemann, Matthias Hoffmann