



milenia biotec



## Detection of Beer Spoilage Organisms

simple, rapid, reliable

**Milenia<sup>®</sup> GenLine**

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## About Us

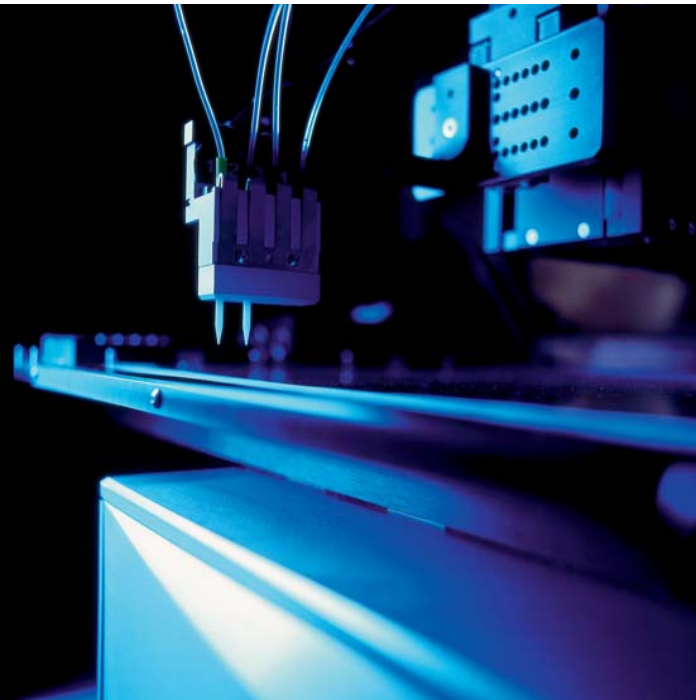
Milenia Biotec GmbH, founded in 2000, is a company dedicated to the development, production and distribution of point of care tests; renowned pregnancy test technology.

The products are easy to use; they can be read either visually or with simple instruments, and results are available within a matter of minutes.

In order to meet the expectations of our customers in terms of both quality and service, Milenia Biotec GmbH introduced a quality management system and have been certified according to EN DIN ISO 9001:2008 and EN DIN ISO 13485:2012 by TÜV Rheinland since 2003.

Milenia Genline has developed a new product line of molecular biology kits that work on a modular basis. Each test is based on a universal test strip capable of detecting any PCR product. This strip, and the corresponding buffer, is made available as a “Universal Module”. In addition to this, “PCR modules” are developed for the detection of each specific target, which allows for the rapid development of new test kits and to serve niche markets too.

The first products of the Milenia GenLine test range detect beer spoilage organisms.



## Milenia GenLine

### Background

Beer proves a difficult medium for microorganisms to thrive in due to the presence of alcohol and the low pH. Furthermore, the influence of carbon dioxide and bitter acids from the hops present additional growth barriers.

Nevertheless, some microorganisms have adapted to this inhospitable beer environment and are even capable of thriving in such a setting. Unfortunately, this microorganism growth has the potential to spoil the beer, altering the taste or making it cloudy.

For this reason breweries quality check the beer, including raw materials, by testing for the presence of beer spoiling organisms at regular intervals throughout the entire production cycle. The Milenia GenLine product line includes rapid, simple and reliable test systems for the molecular biology detection of such beer spoiling organisms.





## Technology

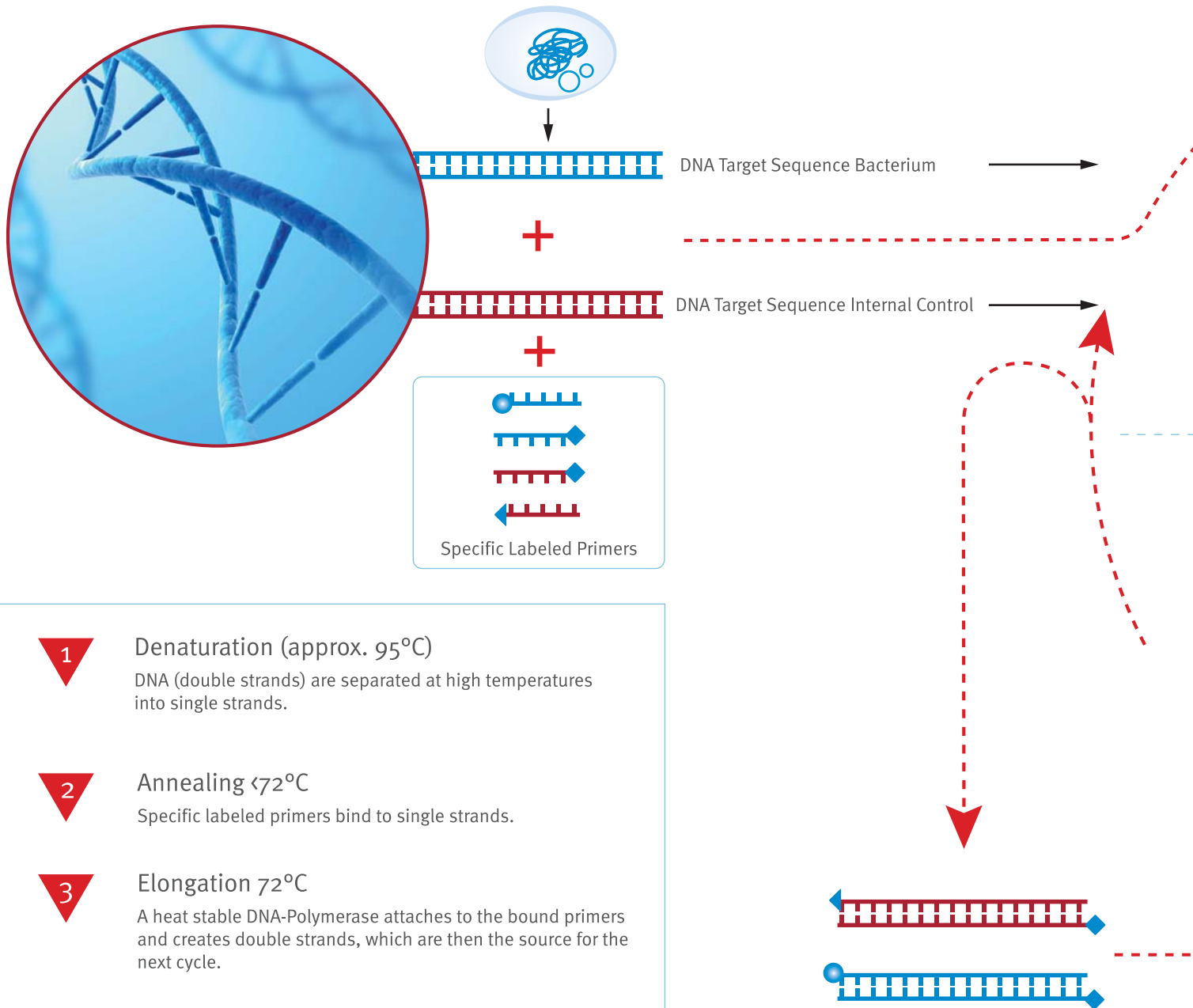
All Milenia GenLine tests are based on PCR (Polymerase Chain Reaction) technology.

PCR copies targeted double strands of DNA in vitro. A DNA polymerase, short target-specific DNA single strands, so-called primers and nucleotides must be present in the reaction mix. The basis of PCR is a temperature protocol which runs over a number of consecutive cycles. In each cycle a duplication of the specific DNA target sequence is achieved. Based on this principle, more than 1 billion copies can be created after 30 cycles from the one original copy.

The primers applied in the Milenia GenLine tests are all marked with a specific label. These labels are detected by the universal test strip if PCR products are run. The Milenia GenLine tests allow for the simultaneous detection of 2 different gene products on an individual test strip; a feature which permits the addition of a control gene to the PCR mixes and therefore enables the user to check whether or not the PCR has worked correctly in the sample matrix applied in the test.



# Technology



1

**Denaturation (approx. 95°C)**

DNA (double strands) are separated at high temperatures into single strands.

2

**Annealing <72°C**

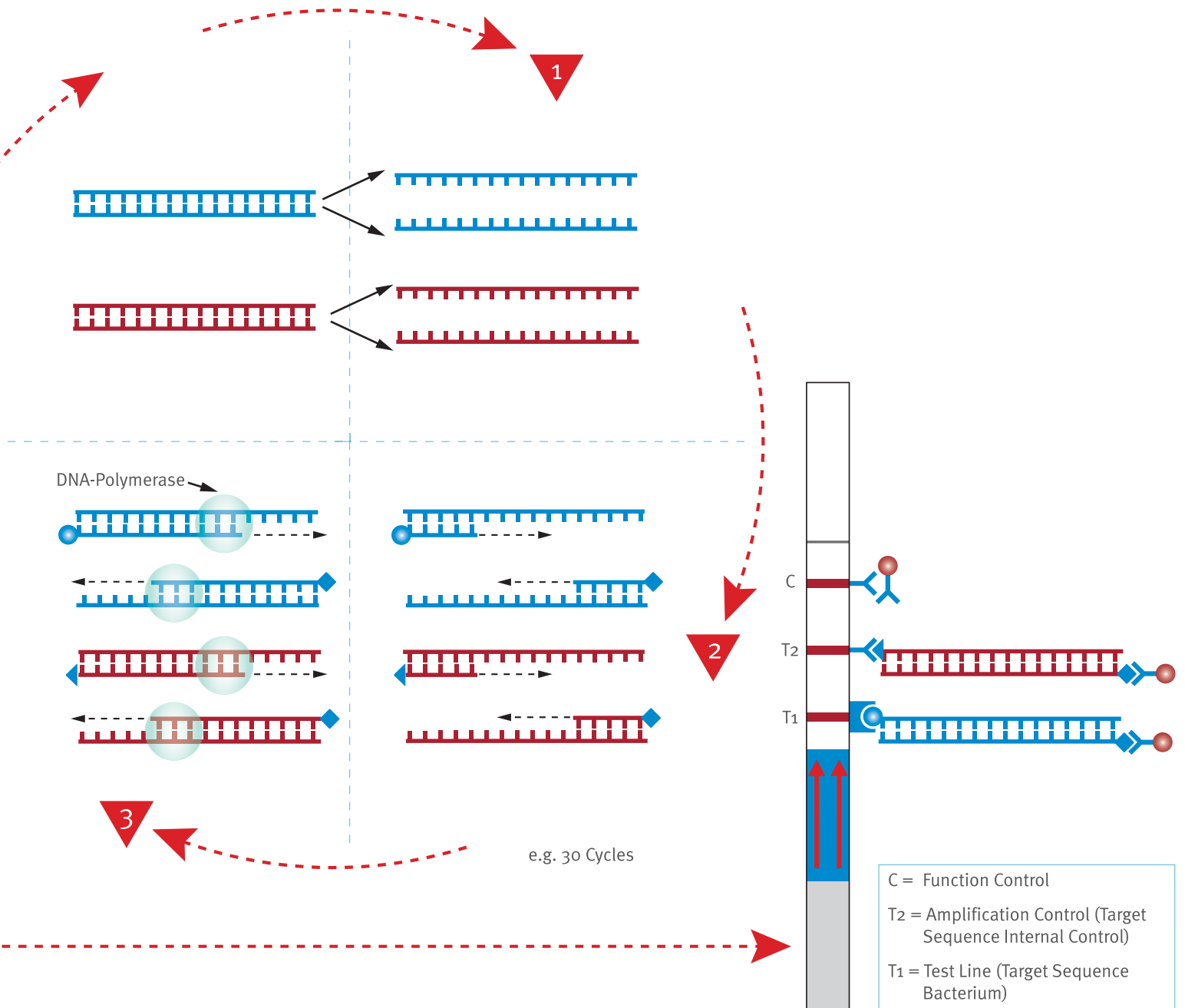
Specific labeled primers bind to single strands.

3

**Elongation 72°C**

A heat stable DNA-Polymerase attaches to the bound primers and creates double strands, which are then the source for the next cycle.

# Technology



## Equipment

- ▼ Thermocycler
- ▼ Pipettes (2-20  $\mu\text{L}$ ; 20-200  $\mu\text{L}$ )
- ▼ Pipette Tips
- ▼ PCR Tubes
- ▼ Eppendorf Tubes
- ▼ Tube Holder
- ▼ Mini Centrifuge
- ▼ Vortexer
- ▼ Freezer





## Sample Preparation

Milenia GenLine Tests detect the presence of beer spoilage microorganisms, achievable without the DNA isolation stage prior to running the test. All commonly used liquid culture media can be applied in the tests, including NBB-B, NBB-B-AM, NBB-C form Döhler or media on MRS basis or Würze and beer.

Home-brewed media can also be easily adapted to the test. Yeast present in the samples has no impact on the test performance.

Furthermore, direct analysis of individual colonies on solid media can be completed.

### 1. Testing in liquid media



### 2. Testing of single colonies on solid media



## Test Procedure

1

Pipette 2  $\mu$ L sample in to the PCR tubes, transfer them to the thermocycler and start the program.



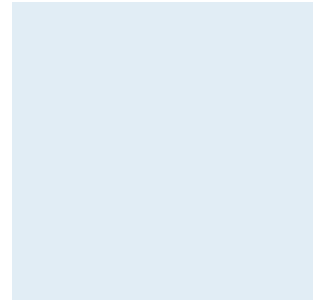
2

Take the PCR tubes out of the thermocycler and transfer 2  $\mu$ L to the sample application pad of the test strip.



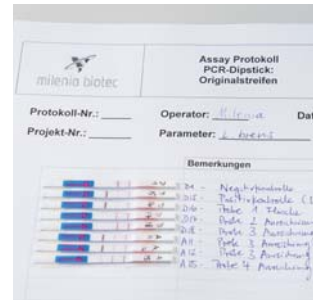
3

Transfer the strip to a well (e.g. microtiter plate), containing 80  $\mu$ L of chase buffer.



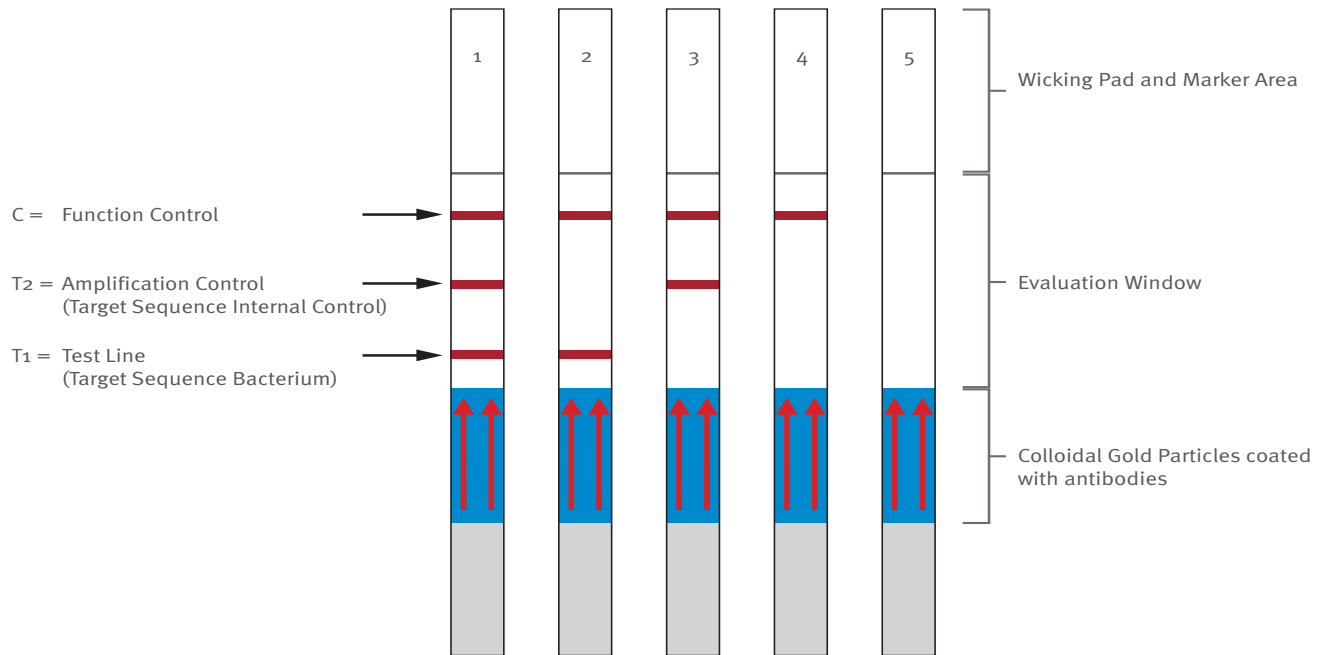
4

Read test results visually after 5 minutes.



## Interpretation of Test Results

Possible universal test strip results after a run of PCR-products.



- ▼ Strip 1 and 2 show clear positive results.
- ▼ Strip 3 corresponds to a negative result.
- ▼ Strip 4 is related to complete inhibition of the PCR. This is not a valid negative result! This sample cannot be analyzed and needs to be repeated.
- ▼ Strip 5 shows an invalid result.

The red arrow shows the direction of the liquid flow.

## Milenia GenLine Lactobacillus / Pediococcus Screen

### Application

The Milenia GenLine Lactobacillus / Pediococcus Screen is a confirmation and screening test for the most frequent beer spoilage organisms. This test detects the most prominent members of the genus Lactobacillus and Pediococcus, including Lactobacillus brevis, Lactobacillus lindneri, Lactobacillus casei, and Pediococcus damnosus. Interestingly, the test does not detect bacteria used by brewers for acidification (e. g. Lactobacillus delbrueckii, Lactobacillus amylovorus etc.) and the most important yeasts used in breweries.

Therefore Milenia GenLine Lactobacillus /Pediococcus Screen is an incredibly useful tool for the interpretation of microbiological sample material which is difficult to analyze, like samples containing high amounts of yeast or enriched samples from unselective media, like swabs 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. claussenii*  
*P. inopinatus*

#### Not Detectable ✕

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

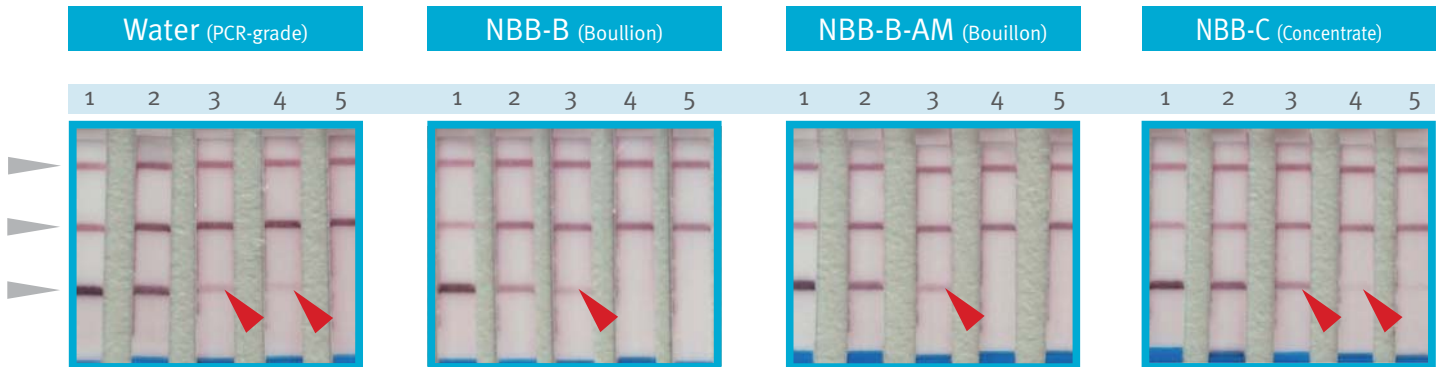
# Milenia GenLine Lactobacillus / Pediococcus Screen

## Sensitivity / Media applied

Milenia GenLine tests can be used to run enriched samples; no DNA extraction is required. Consequently, work load and handling complexity is reduced.

All Milenia GenLine tests can be performed with the most frequently used media in breweries. The analysis can be done directly from liquid media as well as from single colonies grown on solid media.

Water (PCR-grade) NBB-B (Boullion), NBB-B-AM (Boullion), NBB-C (Concentrate)



### *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

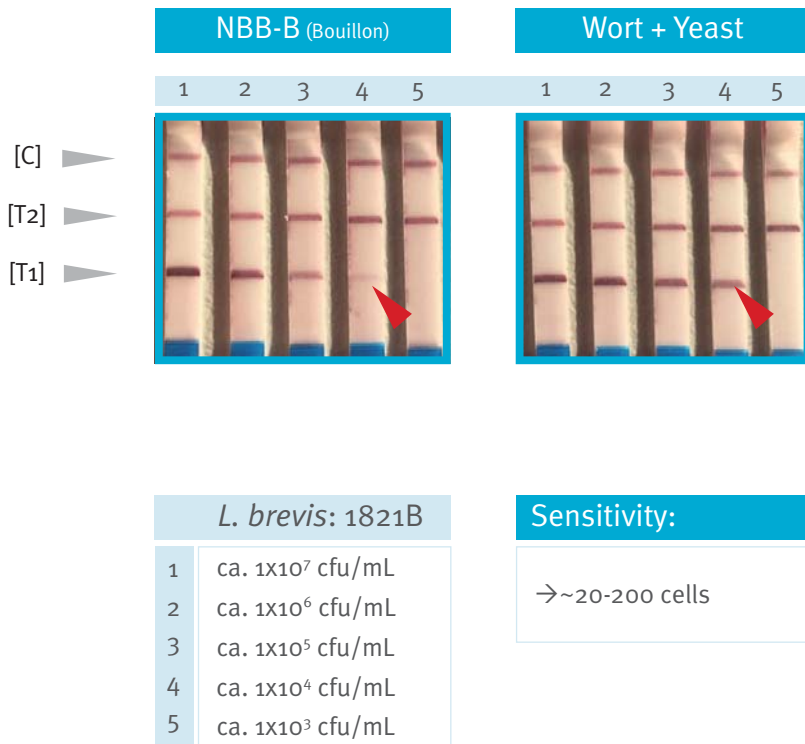
### Sensitivity:

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 Screen

## Sensitivity / Samples containing Yeast

In breweries, samples which contain high amounts of yeast are difficult to analyze. However, Milenia GenLine tests allow sensitive and direct detection of beer spoiling organisms from samples containing high levels of yeast. For this reason, these tests are invaluable tools for the analysis of such sample matrices which are hard to interpret by other methods, including microscopy.





## Performance Data of Milenia GenLine Lactobacillus / Pediococcus Screen

		Milenia (LB-PC-Screen)		
		+	-	Sum
RT-PCR (sLP 600, Brandl 2006)	+	16	0	16
	-	3	31	34
Sum		19	31	50
		Concordance Index 0,91		

# Milenia GenLine Hop Resistance Screening

## Background Information

Most microorganisms struggle to multiply in the hostile environment that is beer. However, some have evolved to tolerate this medium and are capable of growing. A frequently described feature of microbial growth is the ability of microorganisms to tolerate bitter hop acids (e. g. iso-alpha-acids). The Milenia Hop Resistance test detects two of the most important genes coding for resistance against hop bitter acids. If these genes are present in bacteria, it is likely that they are true beer spoilers and therefore pose a real threat to product quality.

## Application and Comparison to the Product Spoilage Test

The Milenia Hop Resistance Screen is designed for the confirmation and screening of obligate beer spoilage bacteria of the genus *Lactobacillus* and *Pediococcus*. The test estimates the spoilage ability of any potential beer spoilage bacteria. This great new tool also delivers results in less than 2 hours, whereas classic product spoilage tests take up to 14 days to provide such information.

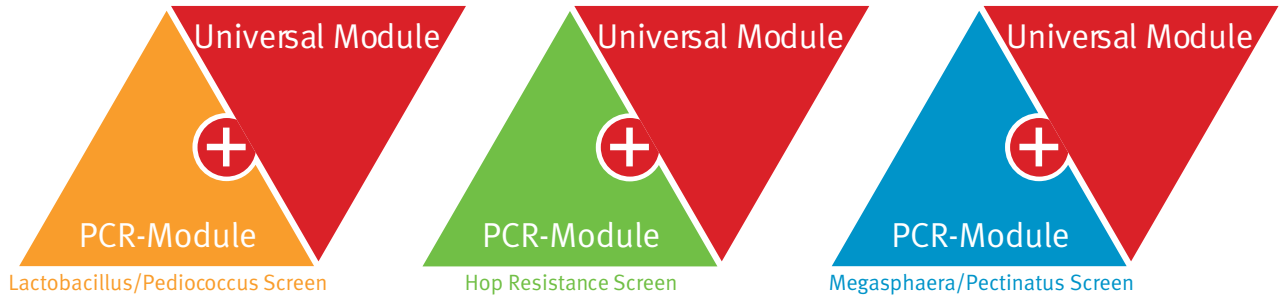
No.	Organism	Milenia® GenLine Tests		Culture						
		Lacto-/Pedi-Screen	Hop Resistance Screen	NBB-B-AM (Bouillon)	NBB-B (Bouillon)	Wheat Beer	Lager	Pilsener	Pale Ale	Double IPA
	Alcohol Content					10,04	5,2	5,1	5,7	8,3
	pH-Value					4,36	4,73	4,46	4,79	4,91
	IBU's					15	24	32	36	83
-	Negative Control	-	-	-	-	-	-	-	-	-
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.	-	-	-	-

Analysis has been made in duplicate, \*Only in 50% of the culture growth could be observed, in these cases a repeated test for hop resistance was negative

## Performance Data of Milenia GenLine Hop Resistance Screen

		Milenia (HOR)		
		+	-	Sum
RT-PCR (TUM FZW BLQ, Hor A or Hor C Gen)	+	10	0	10
	-	0	7	7
Sum		10	7	17
				Concordance Index

## Product Menu



### Universal Module

MGUP 1

48 tests

The Universal Module can only be used in combination with PCR-Modules.

### PCR Modules



Lactobacillus / Pediococcus Screen

MGSCLP 1

48 tests



Hop Resistance Screen

MGSCHOR 1

48 tests



Megasphaera / Pectinatus Screen

MGSMP 1

48 tests



Lactobacillus brevis

MGLBR Z

24 tests

## Features and Benefits of Milenia GenLine

- ▼ Sensitive and Specific
- ▼ Internal PCR Control
- ▼ Positive Control included in the kit
- ▼ Direct application of the sample – No DNA preparation necessary
- ▼ Low equipment investment
- ▼ Low interference of yeast
- ▼ Time to Result = just 60 minutes





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