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# Safe – fast – universal: NBB-PCR – a reliable, fast and universal enrichment broth for the PCR detection of beer spoiling microorganisms

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## Introduction

Beer spoiling bacteria (BSB) represented by *Lactobacillus* spp., *Pediococcus* spp., *Pectinatus* spp. and *Megaspheara* spp. are responsible for around 80-90% of cases of spoiled beer[1]. They are normally detected using culture media, which takes more than five days. Faster methods are urgently needed. PCR is a less time consuming detection method, but pre-enrichment is always necessary to detect microbial trace contaminations.

This study aimed to evaluate a new developed, standardized, safe and fast ready-to-use pre-enrichment medium. The medium needed to be compatible with all commercially available PCR & real-time PCR solutions and improve the detection time to 48-72 hours.

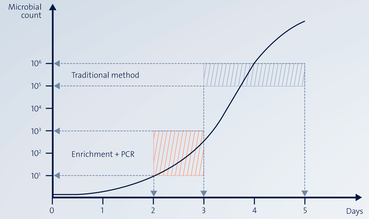
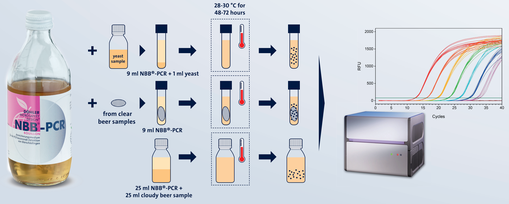



**Figure 1: Microbial growth curve for comparing the sensitivity of traditional cultural methods and PCR.**
Traditional culture media need approx. $10^3$-$10^5$ microorganisms/ml for optical detection. In comparison PCR detection is possible with $10^1$-$10^2$ microorganisms. The detection time is therefore reduced for PCR methods. For microbial trace contaminations ( e. g. one single vegetative cell) an enrichment step is still necessary for successful PCR detection.


## Methods




For yeast samples, 9 ml of NBB®-PCR Broth are added to 1 ml of yeast. Clear beer samples (e.g. pilsner) are filtered through a membrane filter and are then incubated in 10 ml of NBB®-PCR Broth. Non-filterable samples (e.g. wheat beer) are mixed with NBB®-PCR Broth with a 1:1 ratio. All samples are incubated for 48 h at 28 – 30°C and afterwards analysed with a PCR detection kit for beer spoiling bacteria.

## Results & Discussion

### Detection sensitivity of NBB®-PCR for beer spoiling bacteria

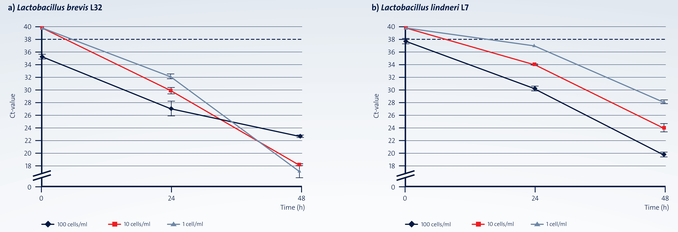



**Figure 2: Enrichment of microbial trace contaminations with NBB®-PCR Broth.**
a) Ct-values of *Lactobacillus brevis* L32 and b) Ct-values of *Lactobacillus lindneri* L7 spiked with 1; 10; 100 cells/ml in 15 ml NBB®-PCR Broth. The cells were counted microscopically. The sampling was performed after 0 h, 24 h and 48 h incubation at 28-30°C. The real-time PCR-detection was performed with 1 ml sample volume, purified and analysed according to the manufacturer´s instructions for the GeneDisc® Beer Spoilage Bacteria Kit (Pall Corporation). Data points are mean values from a threefold determination; the standard error is shown. A Ct-value of >38 means BSB detection is not possible (dotted line).

Two beer spoiling bacteria *L. brevis* L32 (fast growing) and *L. lindneri* L7 (more demanding) were incubated in various concentrations (1; 10; 100 cells/ml) with NBB®-PCR Broth for 0 h, 24 h and 48 h (see Fig. 2). Real-time PCR analysis was then performed with a commercially available kit for the detection of BSB. The decrease in the Ct-value indicates a increasing DNA content strictly correlated with the growth of BSB. Ct-values of at least 30 were measured in all samples after 48 h. These enable safe real-time PCR results and detection of BSB. Microbial trace contaminations such as 1 cell/ml are successfully enriched by NBB®-PCR Broth in 48 h even for slow growing *L. lindneri*.

### Universal detection of beer spoiling bacteria with NBB®-PCR

A panel of four beer spoiling bacteria including obligate anaerobic gram-negative *Pectinatus frisingensis* and slow growing *Pediococcus damnosus* was tested. These external validation tests were performed at the Research Center Weihenstephan for Brewing and Food Quality (Technical University Munich) [FZW BLQ TUM]. Four commercially available PCR kits were used in combination with NBB®-PCR Broth to detect these BSB. All real-time PCR systems succeeded in finding the respective spoiling bacteria in 48 h. In the case of the negative result for *L. lindneri* (PCR detection Kit B™ LB Screening), enough DNA was present after enrichment in NBB®-PCR Broth. This was proven using a different test kit.

**Table 1: NBB®-PCR Broth enrichment with subsequent real-time PCR-detection performed with kits for BSB from BIOTECON Diagnostics GmbH, GEN-IAL® GmbH, Pall Corporation and PIKA Weihenstephan GmbH.**
10 ml NBB®-PCR Broth was spiked with 100 cells/ml of *L. brevis*, *L. lindneri*, *P. damnosus* and 1000 cells/ml of *P. frisingensis*. The cells were counted microscopically and the PCR analysis was performed according to the manufacturers instructions after 48 h anaerobic incubation. Data points are mean values from a threefold determination.

| | *L. brevis* BLQ6 | *L. lindneri* L7 | *Pedi. damnosus* BLQ12 | *Pecti. frisingensis* DU2 |
|---|---|---|---|---|
| foodproof® Beer Screening Kit (BIOTECON Diagnostics GmbH) | + | + | + | + |
| First Beer PCR Kit / *L. lindneri* PCR / *P. damnosus* PCR (GEN-IAL® GmbH) | + | + | + | + |
| GeneDisc® Beer Spoilage Bacteria (Pall Corporation) | + | + | + | + |
| PCR detection Kit B™ LP Screening & PCR detection kit B™ Pectinatus sp. (PIKA Weihenstephan GmbH) | + | -* | + | + |

+ = Ct-value <38; - = Ct-value >38 (not detectable); *Growth of bacteria was determined using a different detection kit

Unlike the dehydrated culture medium MRS, NBB®-PCR Broth is ready-to-use and quality tested with a comprehensive test panel of beer spoiling bacteria for proven functionality (see Tab. 2). NBB®-PCR Broth is also free of any PCR inhibitor and the DNA of beer spoiling bacteria.

**Table 2: Comparison of different culture media for the enrichment of BSB.**
10 ml of each medium were spiked with 100 cells/ml *Lactobacillus brevis*, *Lactobacillus lindneri*, *Pediococcus damnosus* or 1000 cells/ml *Pectinatus frisingensis*. The cells were counted microscopically and the PCR analysis was performed using the foodproof® Beer Screening Kit (BIOTECON Diagnostics GmbH) after 48 h anaerobic incubation. Data points are mean values from a threefold determination.

| | *L. brevis* BLQ6 | *L. lindneri* L7 | *Pedi. damnosus* BLQ12 | *Pecti. frisingensis* DU2 | *Ready-to-use* |
|---|---|---|---|---|---|
| MRS | + | + | + | + | No, powder |
| NBB®-PCR | + | + | + | + | Yes |

+ = Ct-value <38; - = Ct-value >38 (not detectable)

### Field test of NBB®-PCR Broth in breweries

Tab. 3 shows the results in breweries using their own beer samples and in-house *L. brevis* strains for spiking. In all samples, NBB®-PCR Broth worked perfectly with the real- time PCR kits used after 48 h enrichment in spiked yeast and beer samples (yeast, pilsner, wheat beer).

**Table 3: Application of NBB®-PCR Bouillon in eight national and international breweries.**
Brewery-specific in-house *L. brevis* strains and yeast and different beer samples were used. Beer samples were spiked with 100 cells/ml *L. brevis* and analysed with the respective in-house real-time PCR system. Data points are mean values from a threefold determination.

| *L. brevis* | FZW BLQ TUM | Brewery A – H | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Yeast | + | + | + | n. a. | + | + | + | + |
| Pilsner | + | + | n. a. | n. a. | + | + | + | + |
| Wheat beer | + | + | n. a. | + | + | + | + | + |

+ = Ct-value <38; - = Ct-value >38 (not detectable); n. a. = not analysed

## Summary

- NBB®-PCR successfully enriches beer spoiling bacteria in 48-72 h to allow fast and safe PCR detection. Even microbial trace contaminations of slow growing bacteria can be detected.
- NBB®-PCR is compatible with all sample types in a brewery, such as yeast, clear and yeast-turbid beer.
- NBB®-PCR is compatible with all commercially available PCR & real-time PCR systems for beer spoiling bacteria and contains no PCR inhibitor.
- NBB®-PCR is a ready-to-use culture medium with a quality testing using a comprehensive panel of spoiling bacteria.

## Acknowledgments

We would like to thank the Research Center Weihenstephan for Brewing and Food Quality at the Technical University of Munich, especially Dr. Mathias Hutzler and his team. We would also like to thank the national and international breweries, for extensively testing the NBB®-PCR Broth and Prof. Dr. Werner Back for his technical consultation.

## References

[1] Back, W.: Colour Atlas and Handbook of Beverage Biology, Fachverlag Hans Carl, Nürnberg, 2005.
[2] Shimokawa M., Suzuki K., Yamagishi H.: Nachweis und Bestimmung von Bierschädlichen Milchsäurebakterien [detection & determination of beer spoiling lactic acid bacteria], BRAUWELT 3, 2016 p. 52-56.