NBB®-B-Am Broth Manual

English

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1. Introduction: Hygiene monitoring with NBB®-B-Am Broth

In the beverage industry, monitoring microbiological hygiene is an essential element of the hygiene concept to prevent microorganisms and their harmful substances from contaminating products. Microbial contamination in production is often caused by biofilms. They develop in many places in production and filling environments (e.g. on machine components) and are not always visible.

Biofilms are composed of a multitude of different microorganisms living in association with each other (see Figure 1). The mutual supply of nutrients and growth promoters within biofilms guarantees the survival of the microorganisms even under difficult conditions. Particular challenges are posed by persistent biofilms. They are usually resistant to simple cleaning methods. Therefore, it is important to constantly monitor biofilm formation and thus to prevent the formation of persistent biofilms.

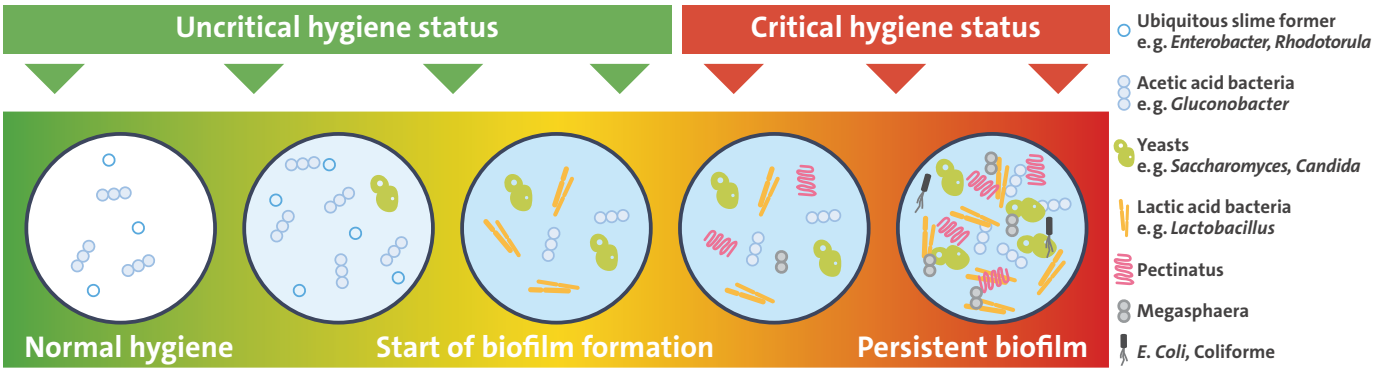


Figure 1: Sequential biofilm formation in a production environment (according to Back, W. 1994a,b; 2003).

NBB®-B-Am Broth is an optimized nutrient broth for the cultivation and qualitative detection of biofilm indicator microorganisms (e.g.: lactic acid and acetic acid bacteria) in the beverage industry, for example in breweries, wineries and filling plants for non-alcoholic beverages. The presence of biofilm indicator microorganisms can be detected quickly and easily by a specific colour change of the culture broth from red to yellow. Continuous recording of the microbial hygiene status with **NBB®-B-Am** Broth allows the analysis of microbial biofilm development and the use of targeted cleaning methods as well as the establishment of microbiological preservation methods in the production chain.

2. NBB®-B-Am Broth – Packaging formats

NBB®-B-Am Broth is available in two packaging formats:

Article no.	Doehler Microsafety Design®-product format	Description	Target bacteria	Packaging unit
2.04706.782	**NBB®-B-Am** Bottle	Ready-to-use **NBB®-B-Am** Broth used for detecting biofilm indicator bacteria by an indicator colour change	Biofilm indicator microorganisms: e.g.: Acetic acid and lactic acid bacteria	1x cardboard box (9x bottles of 250 ml)
2.04706.646	**NBB®-B-Am** Tubes			1x polystyrene box (20x glass tubes of 10 ml)

3. Storage

NBB®-B-Am Broth must be stored in a dark, dry place at a temperature of 4-8°C. The products must not be frozen. The unopened product can be used in the conditions outlined above until the expiration date. The expiration date can be found on the label on the packaging.

4. Intended purpose

The ready-to-use **NBB®-B-Am** Broth is intended for detecting biofilm indicator microorganisms in the beverage industry (such as: beer, wine, water, non-alcoholic beverages, juices, etc.).

When using the product, we recommend that you pay attention to the safety requirements outlined below and work particularly cautiously and hygienically to prevent secondary contamination during sampling.

5. Safety requirements

The basic rules for microbiological operations shall be followed when using this product. This includes using clean equipment such as lab coats, safety goggles and gloves. Such equipment not only contributes to your own personal safety, but also prevents any secondary contaminations being passed on by the user. The safety data sheet can be found at www.doehler-dmd.com.

NBB®-B-Am Broth is not suitable for consumption.

Special care is required when using Bunsen burners while wearing latex gloves. Always maintain a sufficiently safe distance from the flame. If the gloves catch fire, it can result in extremely severe injuries.

Fire hazard: Never use alcohol for sterilisation or decontamination purposes while working with an open flame (Bunsen burner).

6. Quality control

The quality of **NBB®-B-Am** Broth is carefully tested. In particular, the culture media's functionality is specifically tested, proved and confirmed using biofilm indicator microorganisms. You can obtain a certificate of analysis from Doehler GmbH.

7. Your benefits with the ready-to-use medium: NBB®-B-Am Broth

NBB®-B-Am Broth is a ready-to-use culture medium, eliminating the need for extensive and costly preparations such as weighing and mixing chemicals, in addition to adjusting the pH values and conducting subsequent autoclaving processes. This saves you both time and money so you can focus on your microbiological production hygiene straight away.

8. Recommended equipment

When using **NBB®-B-Am** Broth, the following items are recommended for carrying out the experiments:

Laboratory item	Use
Mobile Bunsen burner	Bunsen burner flames create a sterile work area (approx. 50 cm around the flame) in which microbiological experiments can be carried out. The heat kills off any germs present in the atmosphere. The Bunsen burner is also a useful tool for collecting sterile samples.
A sterile (serological) pipette (10 ml) with pipetting aid (e. g. Peleus ball or pipetting aid made of plastic)	(Serological) pipettes are suitable for transferring sterile liquids. **Warning:** **Never pipette fluids with your mouth!** **Always use a pipetting aid!**
Test tube racks	**NBB®-B-Am** Broth tube holder.
Incubator with thermostat	For incubating samples at defined temperatures.
Disposable gloves	Disposable gloves to prevent secondary contamination by the user.

Laboratory item	Use
Sterile deionised water	For filling the swab tubes. The liquid moistens the swab so that samples can be collected even from dry spots.
Sterile swab tubes (plastic) (e. g. smear swabs, article no. 80.625, Sarstedt)	For use with **NBB®-B-Am** Broth bottles (2.04706.782). **Not required** for use with **NBB®-B-Am** Broth tubes (2.04706.646).
Sterile workbench	Essential lab equipment for sterile work.
Waste container (> 250 ml)	For collecting water from the swab tubes during sample collection.

9. Important information

Clean microbiological work is the basis of all analysis. Following the steps below is highly recommended (see **10. Protocol**, p. 8) in order to ensure reliable results and adherence to the safety guidelines.

NBB®-B-Am Broth is not suitable for consumption.

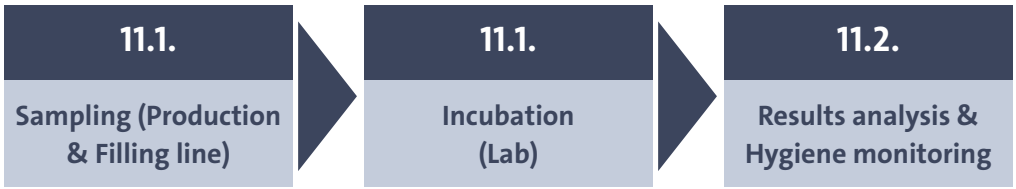
Following analysis, the incubated culture media should be deactivated using microorganisms before being disposed of. Autoclaving the culture media or disposing of them as hazardous waste is recommended for deactivation.

11. Protocol for NBB®-B-Am Broth (tubes article no. 2.04706.646)

To use **NBB®-B-Am** Broth in tubes, you define within your production (e. g. for each filling line) a fixed number of control points for which you want to perform a microbiological test. A number of about 20 to 30 points is recommended. From these points, samples are taken in defined intervals (recommended: 2x per week in summer; 1x per week in winter), and used for analysing the production hygiene. The following points can be considered for sampling; more or different points can be chosen depending on local conditions:

	Washing machine	Bottle inspector	Filler	Recloser
Sampling points	• In the condensate zone of the bottle discharge	• Infeed, outfeed • Bottle conveyor • Other moistures sites	• (Control) valves • Bells • Lifters • Infeed worms • Star wheel surfaces • Infeed/outfeed star wheels (inside) • Baffles • Casings/girders • Plastic rail guides	• Guide rods (guides) • Piston • Crown cork infeed (plate) • Boarding • Infeed, outfeed • Bottle conveyor

As shown in the schematic diagram below, samples are taken in the production environment under the most sterile conditions possible. As the tubes are pre-filled with **NBB®-B-Am** Broth, sampling can be started immediately. The complete work process with **NBB®-B-Am** Broth can be broken down into three steps (11.1. – 11. 2.).



11.1. NBB®-B-Am Broth in tubes: Sampling and incubation

11.1.1. Sampling with NBB®-B-Am Broth in tubes

The ready-to-use glass tubes are pre-filled with 10 ml **NBB®-B-Am** Broth.

You can therefore use the **NBB®-B-Am** tubes and the sterile packed wooden swabs (see Chapter 14, p. 24) to collect samples directly at the sampling points in the production area.

You will need one **NBB®-B-Am** tube and one wooden swab for each sampling point.

Hold the wooden swab at its upper end as far as possible during sampling.

With moist sampling points, you can use the swab directly for taking samples.

With dry sampling points, it is recommened to moisten the swab in the **NBB®-B-Am** Broth tube prior to use.

Warning:
Never dip the swab multiple times into the NBB®-B-Am Broth tube because of the increased risk of contamination.

Gently swab the moistened swab across the sampling point to collect sample material.

After inserting the swab into the **NBB®-B-Am** Broth tube, break off the part of the swab handle that protrudes from the tube.

Seal the **NBB®-B-Am** Broth tube and discard the broken end of the wooden swab.

Mark or label the **NBB®-B-Am** Broth tubes.

11.1.2. Incubation of NBB®-B-Am Broth in tubes

Incubate all **NBB®-B-Am** Broth tubes with the swab at 27 ± 2 °C **under aerobic conditions** for **a maximum of 3 days.**

11.2. NBB®-B-Am Broth in tubes – Results analysis

11.2.1. NBB®-B-Am Broth in tubes – Sample analysis

The sampling points are analysed after **a maximum of 3 days** of incubation.

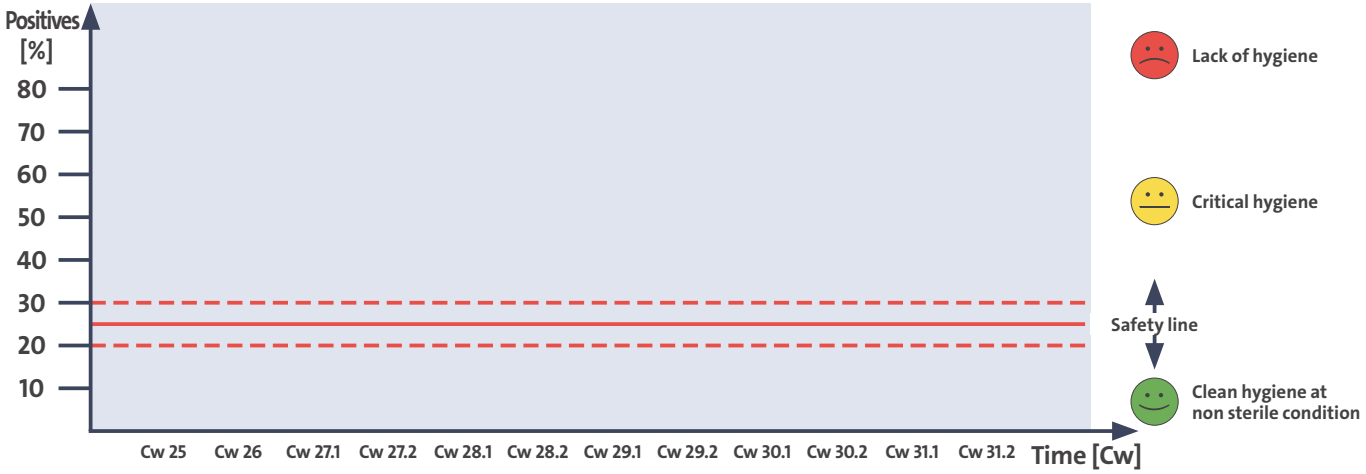
Strong biofilm formation occurs if an **NBB®-B-Am** Broth tube turns yellow after just one day and becomes very turbid. There may also be gas formation.

For hygiene monitoring over time, you now add up all positive points (here, for example: 3 yellow tubes = 3 positive results).

To determine the relative positive results, you then calculate the quotient from the positive results and the full number of sampling points, and multiply it by 100 (for example: 3 positive results / 12 sampling points · 100 = 25 % positive results).

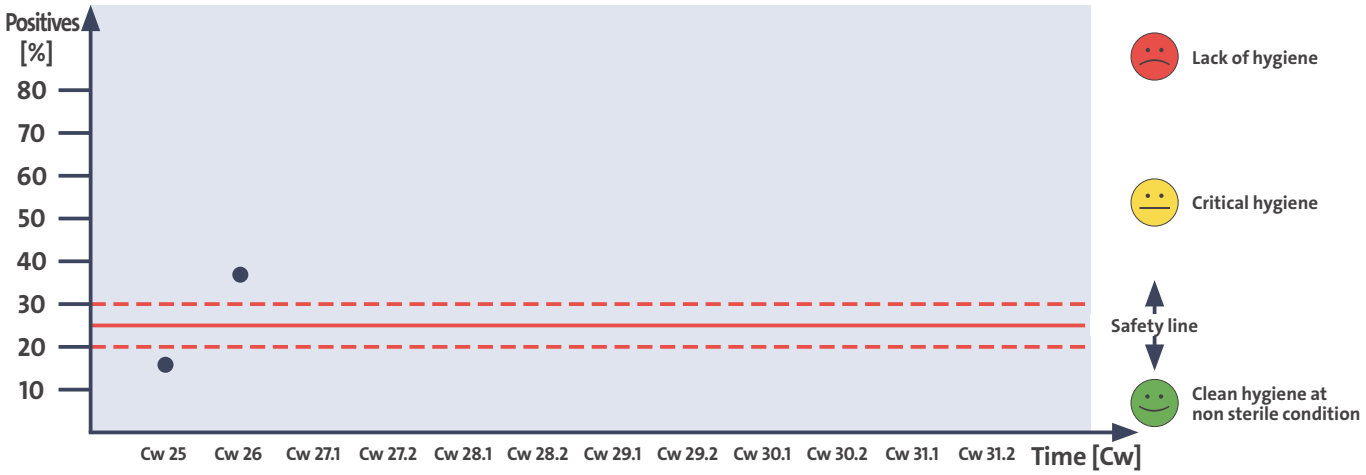
Due to the fact that you can never achieve a completely sterile production environment, there will always be a certain number of positive sampling points. This means that even with very thorough cleaning, there will be a colour change in the sample tubes at some points. This is why you define a safety line in 11.2.2. on p. 19.

11.2.2. NBB®-B-Am Broth hygiene monitoring



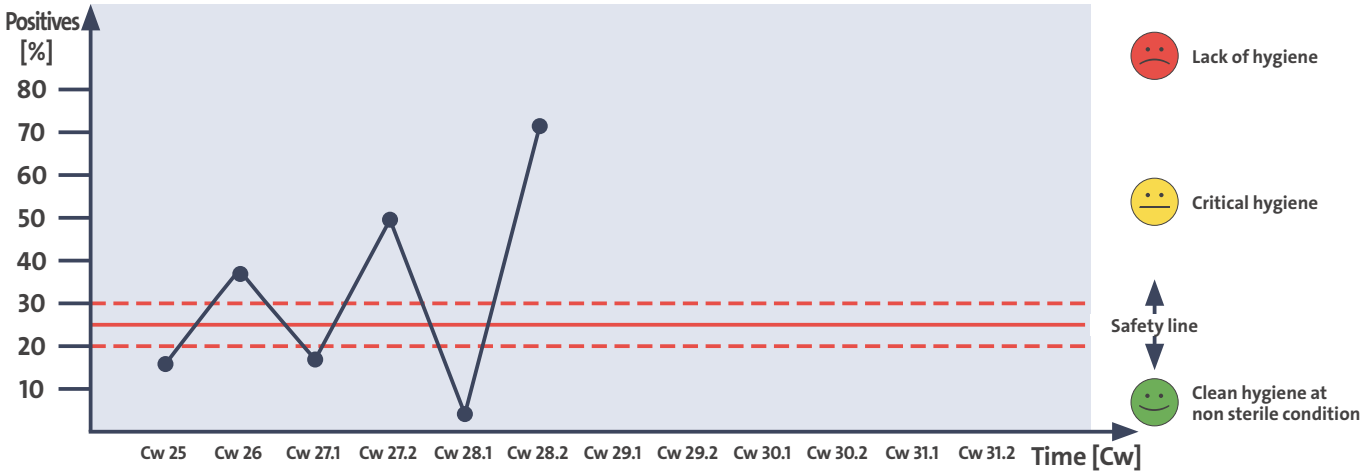
a)

- ▶ Plot an XY diagram.
- ▶ On the y axis, you plot the relative positive results.
- ▶ On the x axis, you plot the corresponding interval time (e. g. CW = calendar weeks).
- ▶ Now you define a safety line somewhere between 20 and 30 %.
- ▶ All points below the safety line represent "clean" production hygiene. Above the line is a critical zone of insufficient hygiene in which persistent biofilms develop.

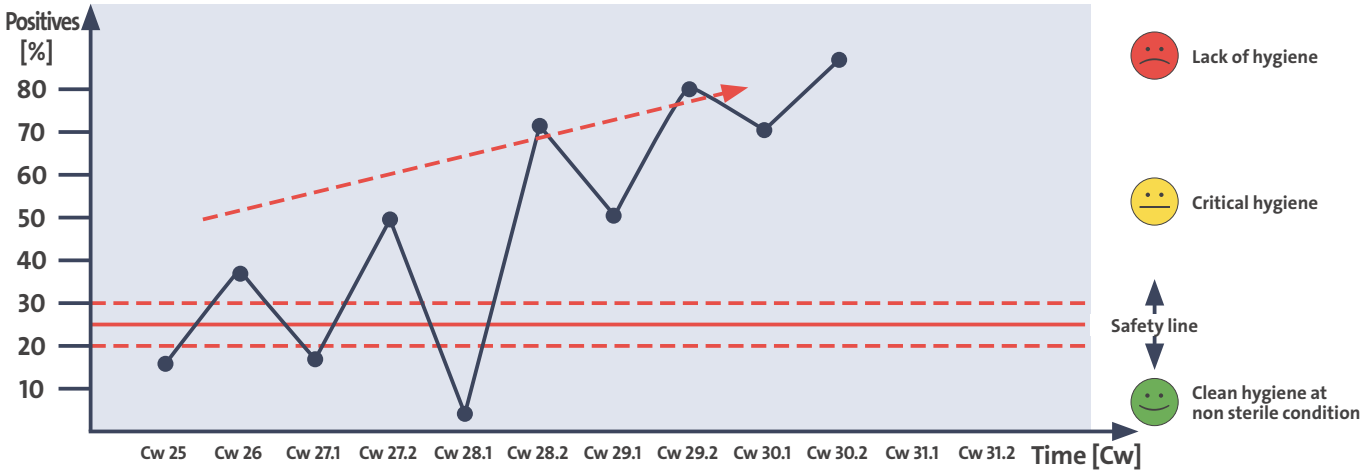


b)

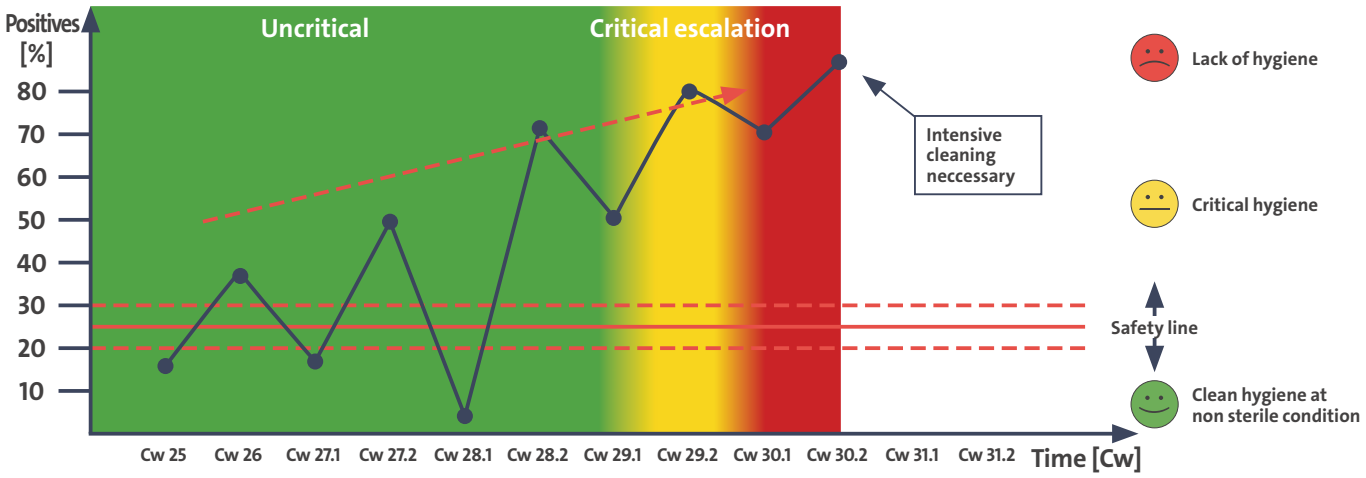
- ▶ Each result of one series of samples with **NBB®-B-Am** Broth is now added to the XY diagram.



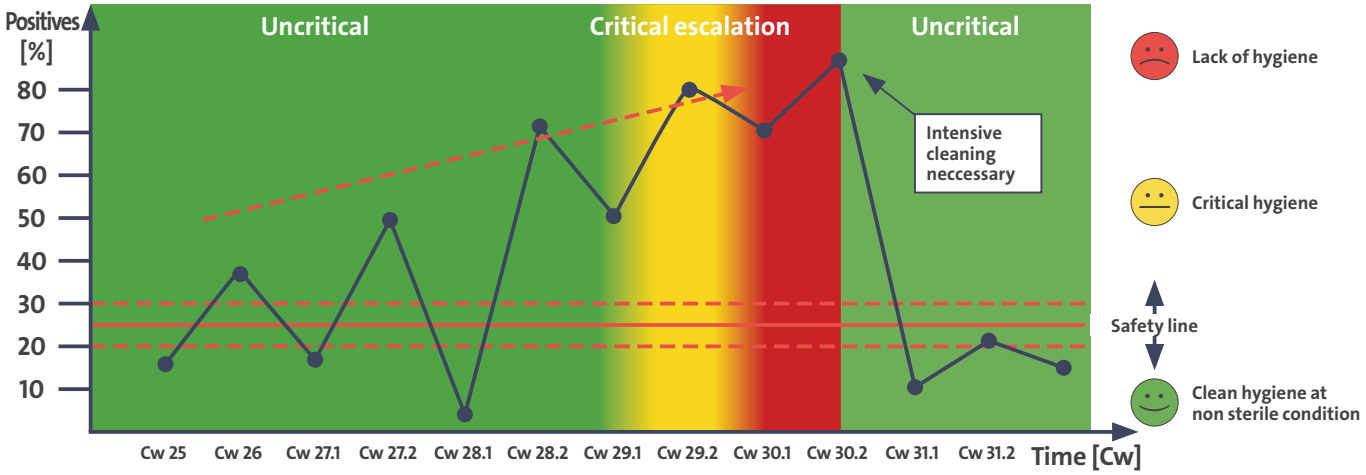
c) ▶ The individual measuring points can be connected by lines.



d) ▶ If you find that the **NBB®-B-Am** Broth samples are clearly above the safety line over several weeks, your hygiene status is reaching the critical zone that is characterised by the establishment of biofilms.



e) ▶ In order to avoid microbial damage to your product, you should carry out an in-depth cleaning if you observe such a condition.



f) ▶ If your cleaning has been successful, the measuring points will return below the safety line.