12. Protocols NBB®-C Concentrate

DMD® product	Application	Frequency	Protocol
NBB®-C Concentrate	Enrichment of turbid beer samples	Regular (every production cycle)	12.1.
	Enrichment of wort samples (sterile beer addition)	Occasional	12.2.
	Yeast sampling	Occasional	12.3.

12.1. NBB®-C Concentrate: Enrichment of beer spoiling bacteria from turbid beer samples

Detection of beer spoiling bacteria with **NBB®-C** Concentrate is not based on a change in colour. The samples incubated with **NBB®-C** Concentrate are analysed under a microscope (e.g. in a counting chamber). The selectivity of the enrichment can be varied by mixing the beer sample with **NBB®-C** Concentrate and adding sterile water, which dilutes the bitter hop compounds. This makes it possible to detect everything from obligate beer spoiling bacteria to indicator microorganisms (see Figure 2 below). For indicator microorganisms air should be left in the bottle head space.

12.1.1. NBB®-C Concentrate: Preparation of swing top bottles

Turbid beer samples can be incubated in sterile, transparent swing top bottles for enrichment or for the detection of beer spoiling bacteria.

Warning: In order to produce sterile swing top bottles, fill the bottles with approx. 10 ml of deionised water and autoclave. The water does not have to be removed from the bottle before use.
This small amount of water can be left in the bottle due to the higher hop concentration of unfiltered beer samples.
The swing top on the bottle should be made from stainless steel to prevent corrosion during autoclaving.

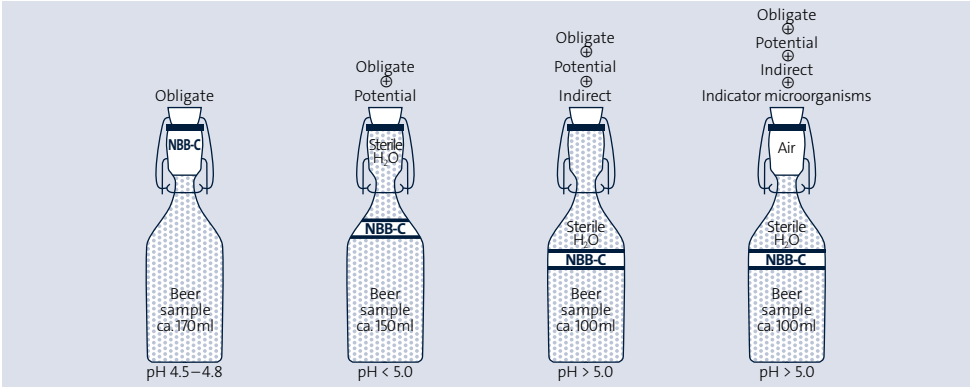


Figure 2: Selective detection of bacteria with **NBB®-C** Concentrate. The selectivity of the enrichment can be changed through the addition of sterile water. This allows enrichment of everything from obligate beer spoiling bacteria (e.g. *L. brevis, Pectinatus* spp. *& Megasphaera* spp.) to indicator microorganisms by adding air to the bottle headspace (e.g. acidic acid bacteria) (see Back, W., 2005). The volumes stated relate to a 180 ml swing top bottle and may need to be adapted to the actual bottle volume used.

12.1.2. Initiating enrichment with NBB®-C Concentrate in swing top bottles or beer bottles

A) Enrichment in swing top bottles

The beer volume to be investigated is placed into the pre-sterilised swing top bottles in a sterile workbench.

The bottle is then filled to 5 % of the total volume with **NBB®-C** Concentrate.

If needed, the selectivity of the enrichment can be modified through the addition of sterile deionised water or sterile tap water, as depicted in Figure 2, p. 32. For example: Adding more than 25 % (v/v) water enables the enrichment of potentially beer spoiling bacteria. The bottle should be filled to the top with water. If you want to enrich indicator microorganisms, the headspace has to be filled with air.

Close the sample bottle with the lid. For samples that contain yeast (e.g. yeast containing weiss beer), do not close the bottle tightly in order to allow gas diffusion.

Label the sample.

The lid can be sealed tight after three days of incubation (see 12.1.3, p. 33).

B) Enrichment in beer bottles

Enrichment can also be conducted directly in the beer bottle in order to test beer after filling.

Remove approx. 5 % of the bottle volume and fill the bottle almost to the top with approx. 5 % **NBB®-C** Concentrate.

Close the sample bottle with the lid. For samples that contain yeast (e.g. yeast containing weiss beer), do not close the bottle tightly in order to allow gas diffusion.

Label the sample.

The lid can be sealed tight after three days of incubation (see 12.1.3, p. 33).

12.1.3. Incubation of NBB®-C Concentrate

Incubate the bottle with NBB®-C Concentrate for 7 – 12 days at 27 ± 2 °C in an incubator or incubation room.

Please note that brief yeast growth can cause CO_2 formation, which puts the bottle under pressure. Yeast growth is quickly suppressed by inhibitors. The CO_2 formation replaces the anaerobic incubation.

The incubation time will need to be extended for some slow-growing beer spoiling bacteria (e.g.: *Lactobacillus lindneri*).

12.1.4. Evaluation of NBB®-C Concentrate

NBB®-C Concentrate does not contain a colour indicator.
Turbidity can display the growth of typical beer spoiling bacteria in **NBB®-C** Concentrate macroscopically. The sample should be constantly checked for the growth of beer spoiling bacteria during incubation. In very turbid samples, a microscope is the only reliable way to determine the growth of beer spoiling bacteria. In the microscopic evaluation (e. g. counting chamber; Neubauer improved), the number of bacteria in 16 microscopic fields (= group square) at a magnification of 600 must be significantly greater than 1. When the chambers have a layer thickness of 0.1 mm, there should be around 4 cells per group square. This corresponds to contamination of approx. 10^6 cells/ml. If there are only isolated cells visible, these are dead or harmless bacteria for the most part, which are almost always found in these kinds of samples.

Ambiguous samples can then be enriched with **NBB®-B** Broth to make sure. To do this, mix one drop of **NBB®-C** sample sediment with approx. 20 ml **NBB®-B** Broth and incubate for one day at 27 ± 2 °C.

Further analysis techniques, such as PCR analysis, can be used to identify the bacteria.

12.2. NBB®-C Concentrate: Enrichment of beer spoiling bacteria from wort samples

12.2.1. NBB®-C Concentrate: Preparation of swing top bottles

Wort samples with addition of sterile beer can be incubated in sterile, transparent swing top bottles for enrichment or for the detection of beer spoiling bacteria.

Warning:
In order to produce sterile swing top bottles, fill the bottles with approx. 10 ml of deionised water or tap water and autoclave it. The water does not have to be removed from the bottle before use. This small amount of water can be left in the bottle due to the higher hop concentration of wort and unfiltered beer samples. The swing top on the bottle should be made from stainless steel to prevent corrosion during autoclaving.

12.2.2. NBB®-C Concentrate: Enrichment of wort samples in swing top bottles

For a 200 ml swing top bottle, which holds around ca. 210 ml when filled to the top, place ca. 20 ml of sterile water in the swing top bottle.

Then fill the swing top bottle with ca. 50 ml wort and add approx. 5 % **NBB®-C** Concentrate (here ca. 10 ml).

Finally, fill the bottle to the top with pasteurised beer (e.g. samples from the shelf-life cabinet). The beer quantity is around 130 ml.

Loosely close the sample bottle with the lid in order to allow gas diffusion and label it.

12.2.3. Incubation of NBB®-C Concentrate

Incubate the bottle with the **NBB®-C** Concentrate for 7 – 12 days at 27 ± 2 °C in an incubator or incubation room.

The incubation time will need to be extended for some slow-growing beer spoiling bacteria (e.g.: *Lactobacillus lindneri*).

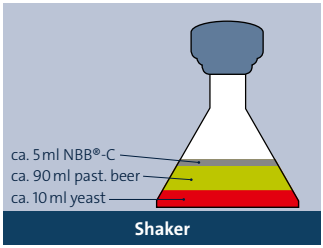
12.2.4. Evaluation of NBB®-C Concentrate

NBB®-C Concentrate does not contain a colour indicator. Turbidity can display the growth of typical beer spoiling bacteria in **NBB®-C** Concentrate macroscopically. The sample should be constantly checked for the growth of beer spoiling bacteria during incubation. In slightly turbid samples, a microscope is the only reliable way to determine the growth of beer spoiling bacteria. In the microscopic evaluation (e.g. counting chamber; Neubauer improved), the number of bacteria in 16 microscopic fields (= group square) at a magnification of 600 must be significantly greater than 1. When the chambers have a layer thickness of 0.1 mm, there should be around 4 cells per group square. This corresponds to contamination of approx. 10^6 cells/ml. If there are only isolated cells visible, these are dead or harmless bacteria for the most part, which are almost always found in these kinds of samples.

Ambiguous samples can then be enriched with **NBB®-B** Broth to make sure. To do this, mix one drop of **NBB®-C** sample sediment with approx. 20 ml **NBB®-B** Broth and incubate for one day at 27 ± 2 °C.

Further analysis techniques, such as PCR analysis, can be used to identify the bacteria.

12.3. NBB®-C Concentrate: Enrichment of beer spoiling bacteria from yeast samples (alternative protocol)



12.3.1. NBB®-C Concentrate: Preparation of sample enrichment

Autoclave a 200 ml Erlenmeyer flask with a cotton stopper.

In a sterile workbench, add approx. 10 ml of the yeast sample to be tested to the sterile flask.

Then add approx. 90 ml of sterile beer.

Pipette approx. 5 ml **NBB®-C** Concentrate into the flask.

Close the flask with the sterile cotton stopper.

Mix the liquids by gently swirling the closed flask.

12.3.2. Incubation of NBB®-C Concentrate with yeast samples

Incubate the flask with the yeast sample on a shaker (slow rotation) in an incubator for 5 – 12 days at 27 ± 2°C.

The brief yeast fermentation generates an anaerobic atmosphere in the flask, so anaerobic cultivation is not essential.

12.3.3. Evaluation of NBB®-C Concentrate with yeast samples

NBB®-C Concentrate does not contain a colour indicator. Turbidity can display the growth of typical beer spoiling bacteria in **NBB®-C** Concentrate macroscopically. The sample should be constantly checked for the growth of beer spoiling bacteria during incubation. In very turbid samples, a microscope is the only reliable way to determine the growth of beer spoiling bacteria. In the microscopic evaluation (e. g. counting chamber; Neubauer improved), the number of bacteria in 16 microscopic fields (= group square) at a magnification of 600 must be significantly greater than 1. When the chambers have a layer thickness of 0.1 mm, there should be around 4 cells per group square. This corresponds to contamination of approx. 10^6 cells/ml. If there are only isolated cells visible, these are dead or harmless bacteria for the most part, which are almost always found in these kinds of samples.

Ambiguous samples can then be enriched with **NBB®-B** Broth to make sure. To do this, mix one drop of **NBB®-C** sample sediment with approx. 20 ml **NBB®-B** Broth and incubate for one day at 27 ± 2 °C.

Further analysis techniques, such as PCR analysis, can be used to identify the bacteria.