

# AMP-6000®

Powerful application for the enumeration of butyric acid producing Clostridia (BAPC)



## AmpMedia 666 – THE NEW STANDARD FOR RAW MILK TESTING

BAPC are undesired contaminants in cheese production due to their ability to cause late-blowing, a severe quality defect in long ripened hard and semi-hard cheese.

Contaminated raw milk is the major source of Clostridia. Far less than 100 spores per litre of raw milk may result in severe cheese deficiencies like irregular eye formation, slits and cracks or unpleasant off-flavors and therefore cause severe economic losses. Just for the well known Italian Grana Padano cheese 2% of the production is still affected by BAPC associated late blowing with an estimated annual loss of about € 36 million (1).

The 4 most common species isolated from milk and cheese are *Clostridium tyrobutyricum*, *Clostridium butyricum*, *Clostridium sporogenes* und *Clostridium beijerinckii*. *Clostridium tyrobutyricum* has been described as the major source of late blowing (2).

Clostridial spores are environmental born and enter the barn environment via feed. They can ideally replicate in silage and are able to passage the ruminant intestine without any damage. Raw milk is mainly contaminated via the faecal route.

Clostridia enumeration is therefore a valuable parameter to control hygienic conditions in stables of dairy cows.

In addition clostridia spores can act as useful indicator for the type of roughage used to feed the cows.

SY-LAB AmpMedia 666 is a new chromogenic microbiological broth enabling the selective and highly sensitive enumeration of even low levels of clostridial spores.

The media is provided as a liquid concentrate enabling the simple processing of large sample volumes to achieve detection levels down to less than 14 spores/litre.

SY-LAB is offering solutions for manual, semi-automated and fully automated testing tailored to the needs of almost any laboratory and testing set up.

Method characteristics for MPN-methods currently in use for BAPC testing are listed in a fact sheet "Enumeration of butyric acid forming (cheese spoiling) clostridia – methodical considerations", recently published by the International Dairy Federation (IDF), (3). See also table 1 below.

### METHOD HIGHLIGHTS

- **Highest specificity for BAPC** (> 95%)
- **Lowest limit of quantification** (< 14 spores/liter possible)
- **Chromogenic media** – ready to use
- **Shortest assay time** (48 hrs)
- **Minimum waste** (> 70% reduction per test)

### Matrix:

Milk (Cow, Sheep, Goat) and Cheese

For instrumentation as well as automation opportunities visit [www.sylab.com/microbiology](http://www.sylab.com/microbiology) or contact [SY-LAB at sales@sylab.com](mailto:sales@sylab.com)

#### RAPID:

Results within 48 hrs

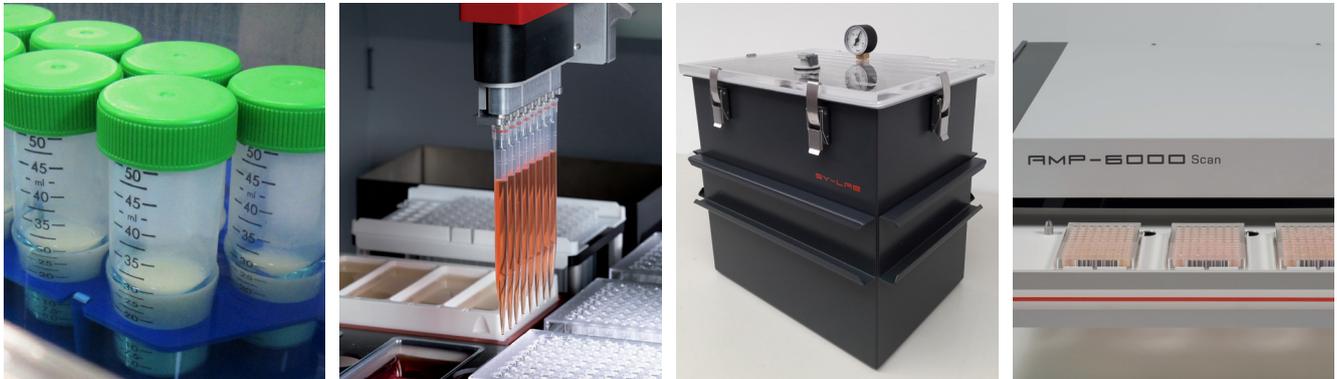
#### PRECISE:

large sample portions analysed and many replicates

#### SIMPLE:

result evaluation by colour change, instrumentation available

## SIMPLE WORKFLOW



Pasteurisation of Sample/Media mixture

Distribution of replicates to Microplates or Microtubes (different options of automation)

Anaerobic Incubation (48 hrs.)

Counting of wells/tubes with colour change (automated evaluation with AmpScan reader and software possible).

TABLE 1 COMPARISON OF BAPC ENUMERATION METHOD CHARACTERISTICS

(Reprint with permission from IDF Fact Sheet 22/2022 (3))

Method name	Bryant and Burkey (CNERNA)	Dutch Standard (NEN 6877)	RCM lactate (VDLUFA M7.18.3.1)	AMP-6000 Method
<b>Abbreviation</b>	<b>BB</b>	<b>NEN</b>	<b>RL</b>	<b>AMP</b>
<b>Medium</b>	Bryant and Burkey Broth (with resazurine)	Milk-glucose-lactate medium	Modified Reinforced Clostridial agar (pH 5.4)	Chromogenic AmpMedia 666
<b>Reaction vessel</b>	Glass tubes	Glass tubes	Glass tubes	Microtiter plates, Microtubes
<b>Pasteurisation</b>	75 °C, 10 min	80 °C, 10 min followed by 44-47°C 15 min	75 °C, 10 min	80 °C, 20 min
<b>Anaerobiosis</b>	Paraffin plug	Paraffin plug	Paraffin or agar plug	Anaerobic jar
<b>Incubation</b>	37°C ± 1°C, 7 days	37°C ± 1°C, 96 h ± 4 h	37°C ± 1°C, 3 to 5 days	37°C ± 1°C, <b>48 h ± 4 h</b>
<b>Evaluation</b>	Gas production leads to lifted paraffin plug	Gas production leads to lifted paraffin plug	Gas production leads to lifted paraffin plug or ruptured agar	Clostridial growth induces <b>colour change</b> of the broth from red to yellow
<b>Automation possible</b>	Sample preparation and inoculation	Sample preparation and inoculation	Sample preparation and inoculation	Sample preparation, inoculation and result evaluation
<b>Countries with accredited labs for the respective method</b>	France, Switzerland	Netherlands, Germany	Germany	Austria, Italy, Switzerland

- Morandi, S., Silvetti, T. & Brasca, M. (2022) Content and spatial distribution of dairy-related Clostridium spores in Grana Padano cheese during the ripening period. LWT-Food Science and Technology 167, 113850 <https://doi.org/10.1016/j.lwt.2022.113850>
- Brändle, J., Fraberger, V., Schuller, K., Zitz, U., Kneifel, W. & Domig, K.J. (2017). A critical assessment of four most probable number procedures for routine enumeration of cheese-damaging clostridia in milk. International Dairy Journal 73, 109-115, <http://dx.doi.org/10.1016/j.idairy.2017.05.011>
- IDF Factsheet 22/ 2022: Enumeration of butyric acid forming (cheese spoiling) clostridia – methodical considerations. IDF Factsheet 22/ 2022: Enumeration of butyric acid forming (cheese spoiling) clostridia – methodical considerations – FIL-IDF. <https://shop.fil-idf.org/collections/publications/products/idf-factsheet-22-2022-enumeration-of-butyric-acid-forming-cheese-spoiling-clostridia-methodical-considerations>