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Regulatory News 2019

New IFU Method No.12 for the detection and enumeration of spore-forming thermo-acidophilic spoilage bacteria (*Alicyclobacillus* spp.) in juice and juice-related products and their ingredients intended for human consumption.

The International Fruit and Vegetable Juice Association (IFU) published a revised Method No. 12 for detection and enumeration of spore-forming, thermo-acidophilic bacteria (*Alicyclobacillus* spp.) in 2019.

The method is applicable to:

- juice and juice-related products and their ingredients intended for human consumption
- environmental samples including process water in the area of juice, juice-related production, and handling
- other beverages not containing juices and their ingredients including syrups

This third edition from 2019 cancels and replaces the second edition of IFU Method No. 12: 2007, which has been technically revised. The main changes introduced in the third version, compared to IFU Method No. 12:2007, are considered as major.

New IFU Method No.12:2019 at a glance...

- The title of the method has been changed.
- The optional usage of several media has been changed to the usage of one liquid (BAT broth) and one solid medium (BAT agar).
- Reduced toxicity of BAT media due to exclusion of cobalt chloride and boric acid from media composition.
- New pour plating technique for enumeration (1 g) in non-filterable samples is introduced.
- Simple confirmation method of presumptive positive colonies from BAT agar introduced.
- Performance testing for the quality assurance of the culture media has been added to Annex B.
- Method validation and performance characteristics for detection and enumeration methods have been added to Annex D.
- Optional pre-incubation of packed ready-to-drink products has been added to Annex E.
- Matrix-dependent special processes have been added to Annex F.

Procedure A: Enumeration by pour plate technique acc. new IFU Method No. 12:2019

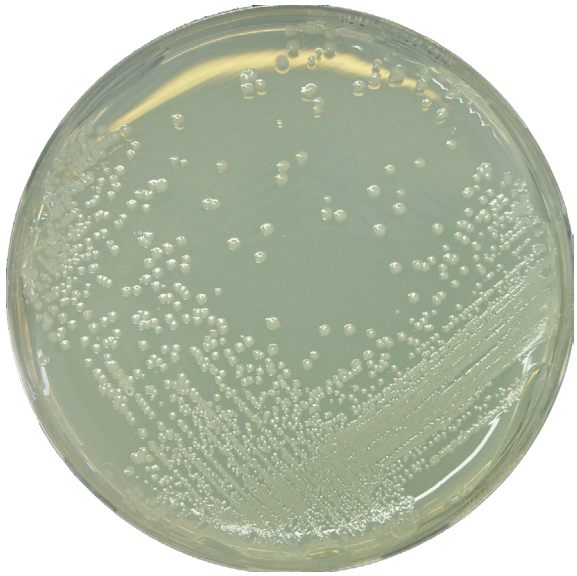
Day	Procedure Step	
1	Sample preparation	10 g or mL test portion + 90 mL BAT broth (1:10 dilution) Heat treatment at 80 °C ±1 °C for 10 min
2	Plating (pour plate)	- Pour 10 mL of diluted sample + 90 mL molten BAT agar 44 - 47 °C in large petri dishes (140 mm) - (alternatively, 2 mL of sample + 15-20 mL molten BAT agar in 5× empty 90 mm plates) - Incubate for 5 days at 45 °C ± 1 °C aerobic
3	Enumeration and confirmation	- Count presumptive *Alicyclobacillus* spp. colonies on BAT agar - Confirm colonies by streaking out on BAT agar and Plate Count agar Incubate 72 h ± 4 h at 5 °C ± 1 °C Colonies grown on BAT agar but NOT on Plate Count agar confirmed as *Alicyclobacillus* spp.

Procedure B: Enumeration by filtration technique acc. IFU Method No. 12:2019

Day	Procedure Step	
1	Sample preparation	10 g or mL test portion + 90 mL BAT broth (1:10 dilution). If necessary, prepare further dilutions. Filterable sample and water samples (e.g. process water) need no dilution. Heat treatment at 80 °C ± 1 °C for 10 min
2	Plating	- Pass total volume of 100 mL through 0.45 µm membrane filter - Place membrane filter on 90 mm BAT agar plate - Incubate for 5 days 45 °C ± 1 °C, aerobic
3	Enumeration & confirmation	- Count presumptive *Alicyclobacillus* spp. colonies on BAT agar. - Confirm colonies by streaking out on BAT agar and Plate Count agar - Incubate 72 ± 4 h at 45 °C ± 1 °C - Colonies grown on BAT agar but Plate Count agar confirmed as *Alicyclobacillus* spp.

Procedure C: Detection of *Alicyclobacillus* spp. by enrichment acc. new IFU Method No. 12:2019

Day	Procedure Step	
1	Sample preparation	10 g or mL test portion + 90 mL BAT broth (1:10 dilution). Heat treatment at 80 °C ± 1 °C for 10 min
2	Enrichment	Incubate at 45 °C at 45 °C ± 1 °C for 5 days, aerobic
3	Plating	- Spread 0.1 mL on 90 mm BAT agar plate or - Pour 1 mL + 15-20 mL molten BAT agar in 90 mm dish (pour plate technique) 45 °C ± 1 °C for 48 h ± 4 h; if negative, additional 72 h ± 4 h at 45 °C ± 1 °C
4	Confirmation	- Confirm colonies by streaking out on BAT agar and Plate Count agar - Incubate 72 h ± 4 h at 45 °C ± 1 °C - Colonies grown on BAT agar, but on Plate Count agar confirmed as *Alicyclobacillus* spp.



Alicyclobacillus acidoterrestris DSM 2498 on BAT agar

As a worldwide leading provider of a broad range of granulated and ready-to-use culture media for food, beverage and water microbiology, we closely track and support the development of relevant standards aimed at increasing consumer confidence and safety.

Our GranuCult® granulated media, Readybag® pre-weighed granulated media in ready-to-use bags, ReadyPlate™ and ReadyTube® ready-to-use media are all compliant with EN ISO 11133:2014. All information regarding compliance with additional reference standards are displayed on the product label and in the product's technical information available on our website.

Compliance to New IFU Method No. 12:2019

We have implemented all the requirements described in the new IFU Method No. 12:2019. For more information, please visit our webpage **SigmaAldrich.com/Beverages-Microbiology.** The following culture media and accessories described in the new IFU Method No. 12:2019 are available:

Ordering Information

Product	Pack size	Cat. No.
GranuCult® BAT broth acc. IFU Method No. 12	500 g	**1079930500**
GranuCult® BAT agar acc. IFU Method No. 12	500 g	**1079940500**
GranuCult® Plate Count agar acc. ISO 4833, ISO 17410 and FDA-BAM	500 g	**1054630500**
ReadyPlate™ 55 Plate Count Agar	20 x 55 mm plates	**1467630020**
Cellulose mixed ester filter: S-Pak® filters 0,45 µm, 47 mm, white gridded	600 individually sealed filters, sterile	**HAWG047S6**
Cellulose mixed ester filter: EZ-Pak® filters 0,45 µm, 47 mm, white gridded	4 bands of 150 sterile filters	**EZHAWG474**
EZ-Stream® Vacuum Pump		**EZSTREAM1**
EZ-Fit® Manifold		**EZFITBASE 1,3,6**
EZ-Pak® Dispenser Curve		**EZCURVE01**

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