Also known as
BAT Medium

Specification
Solid medium for the detection and isolation of *Alicyclobacillus*, in fruit juices and other acidic food, according to IFU standard Method No. 12.

Formula * in g/L

Yeast extract	2,00000		
Dextrose	5,00000	Copper sulfate	0,00016
Potassium phosphate	3,00000	Manganese sulfate	0,00015
Calcium chloride	0,25000	Sodium molybdate	0,00030
Magnesium sulfate	0,50000	Agar	20,00000
Ammonium sulfate	0,20000		
Zinc sulfate	0,00018	Final pH 4,0 ±0,2 at 25 ºC	

* Adjusted and /or supplemented as required to meet performance criteria

Directions
Suspend 30.95 g of BAT Agar in 1 L of distilled water and bring to the boil to dissolve. Distribute in suitable containers and sterilise in the autoclave at 121ºC for 15 minutes. Cool to 45-50ºC and adjust the pH to 4,0 ± 0,2 by adding 1N H_2SO_4 , or 1M HCl. Mix well to homogenise and pour into sterile Petri dishes. Avoid heating or remelting the medium after the pH adjustment.

Description
Since the early 1980's, when spoilage of fruit juices by acid dependent thermotolerant spore-forming bacteria was recognized (Cerny *et al.*, 1984) members of the genus *Alicyclobacillus* have been identified as food spoilage organisms of major significance to the fruit juice industry (Baumgart & Menje, 2000). Spoilage is generally manifested as the formation of off flavours and odours from compounds such as guaiacol and the halogenated phenols. The economic impact of such incidents can be very high, nevertheless, to date, no human risk to be associated with the consumption of juices and other food products containing *Alicyclobacillus* bacteria.
An acidic environment is required to grow alicyclobacilli and BAT (*Bacillus AcidoTerrestris*) media supports the growth of all currently known species of *Alicyclobacillus* (*A. acidocaldarius*, *A. acidoterrestris*, *A. cycloheptanicus* and *A. hesperidium*). These media comply with the Standard IFU Method for the detection of organisms that taint fruit juices (No. 12).
The low pH-value of the media, in combination with the high incubation temperature inhibits the growth of contaminating microbiota.
K Agar when incubated at 45ºC supports the growth of predominantly *A. acidoterrestris* and limited growth of other species of the genus. Therefore, K Agar can be used to detect predominantly *A. acidoterrestris* strains.
In the latest revision of the IFU standard n. 12: 2019, the components cobalt chloride and boric acid have been removedform teh formulation due to toxicity.

Technique
The IFU Standard describes three methods of detection depending on the sample composition and the time elapsed since processing:

1. Raw materials (including processed water): A heat shock treatment is required followed by direct plating (optional), filtration or enrichment in liquid medium, of the heated material.
2. Final products: sampled directly after (heat) processing where an additional heat shock is unnecessary: Pre-incubation of the sample in liquid medium is required.
3. Final products taken from the market: Pre-incubation of the sample, and heat shock treatment are optional. However if spoilage is suspected and no alicyclobacilli detected after direct plating, a heath shock and enrichment is recommended.

In all methodology incubation for 3-5 days at 45 ± 1ºC is recommended. Count all colonies growing on the BAT Agar as presumptive alicyclobacilli. Confirm these colonies by further testing.

Note: A. acidocaldarius require incubations above 50 ° C.

Quality control
Incubation temperature: 45°C ±1,0 **Incubation time:** 3 - 5 days
Inoculum: Practical range 100 ± 20 CFU. Min. 50 CFU (Productivity) / 10^4-10^6 CFU (Selectivity) according to ISO 11133:2014/Amd 1:2018.

Microorganism	Growth	Remarks
Escherichia coli ATCC® 25922	Inhibited	-
Bacillus cereus ATCC® 11778	Inhibited	-
Alicyclobacillus acidoterrestris ATCC® 49025	Productivity > 0.70	-

References

· BAUMGART, J. (2003) Media for detection and enumeration of Alicyclobacillus acidoterrestris and Alicyclobacillus acidocaldarius in foods. In Handbook of Culture Media for Food Microbiology. J.E.L. Corry et al. (Eds.) Elsevier Sci B.V. Amsterdam.
· BAUMGART, J. & S. MENJE (2000) The impact of Alicyclobacillus acidoterrestris on the quality of juices and soft drinks. Fruit Processing 7:251-254.
· CERNY, G., W. HENNLICH & K. PORALLA (1984) Fruchsaftverdeb durch Bazillen: Isolierung und Charakterisierung des Verdebserregers. Z. Lebens. Unter Forsch. 179:224-227.
· IFU STANDARDS (2004) Method No. 12 on the detection of taint producing Alicyclobacillus in fruit juices. Revision march 2007.
. IFU STANDARDS (2019) Method No. 12 on the detection and enumeration of spore-forming thermo-acidophilic spoilage bacteria (*Alicyclobacillus* spp.).
. SMITH, Y., M. CAMERON, P. VENTER & R.C. WITTHUHN (2011) *Alicyclobacillus* spoilage and isolation – A review. Food Microbiol. 28(3):331-349.
. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).