Technical Data Sheet

NutriSelect® prime

Bismuth sulfite (BS) agar acc. WILSON and BLAIR

Ordering number: 1.00191.0500

For the isolation and differentiation of *Salmonella* from food and animal feed and other materials, especially when the detection of *Salmonella* enterica subspecies *enterica* serovars Typhi and Paratyphi is of specific concern.

Bismuth sulfite (BS) agar acc. WILSON and BLAIR is also known as BSA, WILSON BLAIR agar and Wismuth sulfite agar.

This culture medium complies with the specifications given by FDA-BAM Medium M19, AOAC Official Method 967.25, GB 4789.4 and APHA. It complies with the specifications given for Medium XV Bismuth Sulfite Agar Medium by USP 31 (2008) <61> Microbial limit tests (non-harmonized method). The typical composition of this culture medium complies with the specifications given by EN ISO 6579-1.

Mode of Action

Bismuth sulfite and brilliant green are the selective agents and largely inhibit the accompanying bacterial flora. Iron(III) sulfate is an indicator for hydrogen sulphide production produced by H_2S positive *Salmonella*. Generally, this reaction causes a brown or black precipitate on the medium and a black or green metallic colony.

Peptone, meat extract and glucose allow good growth. Disodium phosphate acts as a buffer to maintain the pH and agar is the solidifying agent.

The freshly prepared medium is strongly inhibitory and it is thus especially suitable for heavily contaminated samples. The metallic lustre of the colonies usually only appears after 48 h on incubation. After 4 days storage at 4 °C the inhibitory action of the medium is not as strong and it should then be used for less heavily contaminated specimens; in this case the metallic lustre appears after a shorter period of incubation.

EN ISO, FDA-BAM, AOAC, APHA and GB recommend to prepare the plates on day before streaking and store in the dark; selectivity decreases after 48 h.

Typical Composition

There is no composition specified by APHA for Bismuth sulfite (BS) agar.

Specified by FDA-BAM M19, AOAC 967.25, USP 31 <61>		Specified by GB 4789.4		Specified by EN ISO 6579-1		NutriSelect® prime Bismuth sulfite (BS) agar acc. WILSON and BLAIR	
Polypeptone (or peptone)*	10.0 g/l	Peptone	10.0 g/l	Enzymatic digest of animal tissue	10.0 g/l	Enzymatic digest of animal tissue **	10.0 g/l
Beef extract	5.0 g/l	Beef extract	5.0 g/l	Meat extract	5.0 g/l	Meat extract ***	5.0 g/l
Dextrose	4.0 g/l	Glucose	5.0 g/l	Dextrose	5.0 g/l	D(+)Glucose* ***	5.0 g/l
Na_2HPO_4 anhydrous	4.0 g/l	Na_2HPO_4	4.0 g/l	Na_2HPO_4 anhydrous	4.0 g/l	Na_2HPO_4 anhydrous	4.0 g/l
$FeSO_4$ anhydrous	0.3 g/l	$FeSO_4$	0.3 g/l	$FeSO_4$ anhydrous	0.3 g/l	$FeSO_4$ anhydrous	0.3 g/l
Bismuth sulfite indicator	8.0 g/l	Ammonium bismuth citrate	2.0 g/l	Bismuth sulfite indicator	8.0 g/l	Bismuth sulfite indicator	8.0 g/l
		Sodium sulfite	6.0 g/l				
Brilliant green	0.025 g/l	Brilliant green	0.025 g/l	Brilliant green	0.025 g/l	Brilliant green	0.025 g/l
Agar	20.0 g/l	Agar	18 g to 20 g/l	Agar	20.0 g/l	Agar-agar *****	15.0 g/l
Water	1000 ml	Water	1000 ml	Water	1000 ml	Water	n/a
pH at 25 °C	7.7 ± 0.2 *****	pH at 80 °C	7.5 ± 0.2	pH at 25 °C	7.7 ± 0.2	pH at 25 °C	7.7 ± 0.2

* USP 31 <61> specifies Pancreatic digest of casein 5 g/l and Peptic digest of animal tissue 5 g/l.
** Enzymatic digest of animal tissue is equivalent to the term polypeptone (or peptone).
*** Meat extract is equivalent to the term Beef extract.
**** Glucose is equivalent to the term Dextrose.
***** Agar-Agar is equivalent to other different terms of agar.
***** AOAC 967.25 and USP 31 <61> specify a final pH of 7.6 ± 0.2.

Preparation

Dissolve 47.5 g in 1 liter of purified water. Heat in boiling water and agitate frequently until completely dissolved. Do not autoclave! Remove from heat within 2 min after dissolution is completed. Mix the formed precipitate homogenously and pour the medium to plates to give a thick layer.

Acc. to EN ISO 6579-1, pour 20 to 25 ml into Petri dishes with a diameter of approximately 90 mm and allow to set.

The dehydrated medium is a powder with green color.

The prepared medium is turbid and green. The pH value at 25 °C is in the range of 7.5 - 7.9.

EN ISO 6579-1, FDA-BAM, AOAC 967.25, APHA and GB 4789.4 recommend to prepare the plates on day before streaking and store in the dark at ambient temperature; selectivity decreases in 48 h.

Before inoculation, allow the prepared medium to equilibrate at room temperature if it was stored at a lower temperature.

There should be no visible moisture on the plates before use. When moisture is present, the plates should be dried for the minimum time required to remove visible moisture, following the procedure as described by EN ISO 11133.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.
If the detection of *Salmonella enterica* subspecies *enterica* serovars Typhi and Paratyphi is of specific concern, mostly Bismuth Sulfite (BS) agar and one or more solid selective media are inoculated from the selective enriched cultures.

Following the procedure given by EN ISO 6579-1, FDA-BAM and APHA, inoculate the surface of the medium from the selective enriched cultures so that well-isolated colonies will be obtained.

Incubate the inoculated plates inverted under aerobic conditions, e.g.

- acc. to EN ISO 6579-1 between 34 °C and 38 °C for (24 ± 3 h), if no typical or atypical colonies are detected, re-incubate the plates for a further 24 h between 34 °C and 38 °C;
- acc. to FDA-BAM Chapter No. 5 at (35 ± 2 °C) for (24 ± 2 h), pick typical colonies for further confirmation and re-incubate the plates an additional (24 ± 2 h);
- acc. to GB 4789.7 at (36 ± 1 °C) for (40 - 48 h);
- acc. to APHA Chapter No. 36 at (35 ± 2 °C) for (24 ± 2 h), plates with no suspect colonies or with no growth should be incubated for an additional 24 h.

Salmonella colonies often display blackening after 18 h of incubation, the metallic sheen appears several hours later depending on the age of the medium.

Colonies of the most important bacteria usually have the appearance described below:

Appearance of colonies	Microorganisms
Black centre, light edges surrounded by a black precipitate with metallic sheen (so-called rabbit's or fish-eye)	*Salmonella* with the exception of *S.* Paratyphi A and *S.* Pullorum
Small, green to brown, sometimes mucoid	Coliform bacteria, *Serratia*, *Proteus* and others

This presumptive evidence must be confirmed by carrying out the usual tests.

Storage

Store at +2 °C to +8 °C, dry and tightly closed. Do not use clumped or discolored medium. Protect from UV light (including sun light). For *in vitro* use only.

EN ISO 6579-1, FDA-BAM, AOAC 967.25, APHA and GB 4789.4 recommend to prepare the plates on day before streaking and store in the dark at ambient temperature; selectivity decreases in 48 h.

Microbiological Performance

Test method: Performance testing of solid culture media - Qualitative testing

Test strain	Specification	
	Growth	Typical reaction
Salmonella Typhimurium ATCC® 14028 [WDCM 00031]	good	black colonies with metallic luster
Salmonella Enteritidis ATCC® 13311 [WDCM 00121]	good	black colonies with metallic luster
Salmonella Abony NCTC 6017 [WDCM -]	good	black colonies with metallic luster
Salmonella Enteritidis NCTC 5188 [WDCM 00119]	good	black colonies with metallic luster
Escherichia coli ATCC® 25922 [WDCM 00013]	growth or partial inhibition	green-brown colonies without metallic luster
Escherichia coli ATCC® 8739 [WDCM 00012]	growth or partial inhibition	green-brown colonies without metallic luster

Incubation: 2 x 24 ± 3 h at 37 ± 1 °C, aerobic.

Please refer to the actual batch related Certificate of Analysis.

Literature

AOAC (2005): Official Method 467.25 *Salmonella* in Foods. Preparation of Culture Media and Reagents. AOAC International, Rockville, MD, USA.

APHA (2018): Part 9260: Detection of pathogenic bacteria. Standard Methods for the Examination of Water. 23rd ed. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, D.C.

APHA (2015): Chapter No. 36 *Salmonella*. and Chapter No. 67 Microbiological media, reagents and stains. Compendium of Methods for the Microbiological Examination of Foods. 5th ed. American Public Health Association, Washington, D.C.

APHA (1978): Chapter No. 5 Pathogens in Milk and Milk Products. Standard Methods for the Examination of Dairy Products. 17th ed. American Public Health Association, Washington, D.C.

ISO International Standardisation Organisation. Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Part 1: Detection of *Salmonella* spp. + Amendment 1 Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC. EN ISO 6579-1:2027/ Amd1:2020.

FDA-BAM (2017): Chapter No. 5: *Salmonella*. Food and Drug Administration - Bacteriological Analytical Manual.

FDA-BAM (2018): Media Index for BAM - BAM Media M19: Bismuth Sulfite Agar (Wilson and Blair). Food and Drug Administration - Bacteriological Analytical Manual.

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China Food and Drug Administration. National Standard of the People's Republic of China. National food safety standard — Food microbiological examination: Examination of *Salmonella*. GB 4789.4-2016.

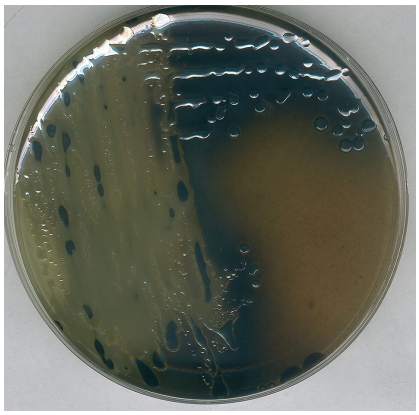
United States Pharmacopeial Convention. (2008): The United States Pharmacopeia 31/National Formulation 26. Chapter <61> Microbial limit test. Rockville, Md., USA.

Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. (2012): Bismuth sulfite agar. In: Handbook of Culture Media for Food and Water Microbiology, pp. 683-686. Royal Society of Chemistry, Cambridge, UK.

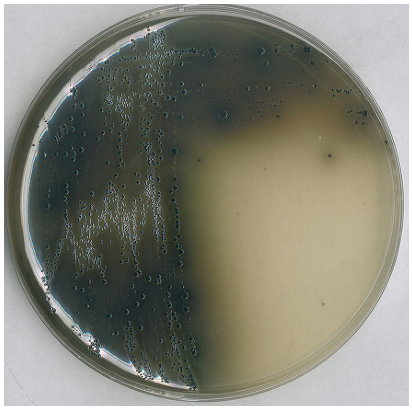
Mooijman, K.A. (2012): Culture media for the isolation of *Salmonella*. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds), pp. 261-286. Royal Society of Chemistry, Cambridge, UK.

Wilson, W.J. and Blair, E.M.M.V. (1927): Use of a glucose bismuth sulfite iron medium for the isolation of *B. typhosa* and *B. proteus*. J. Hyg. **26**: 374-391.

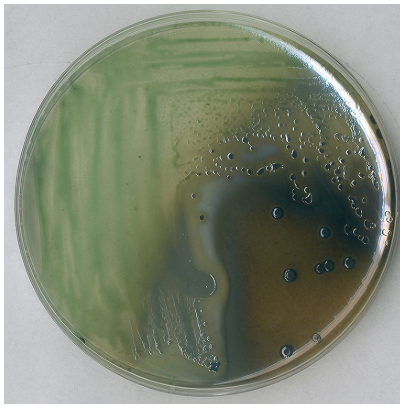
Wilson, W.J., and Blair, E.M. McV. (1931): Further experience of the bismuth sulfite media in the isolation of *Bacillus typhosus* and *Bacillus paratyphosus* B from faeces, sewage and water. J. Hyg. **31**: 138-161.



Salmonella Enteritidis
NCTC 5188 [WDCM 00119]



Salmonella Arizonae
ATCC® 13314



Salmonella Abony
NCTC 6017

Ordering Information

Product	Cat. No.	Pack size
NutriSelect® prime Bismuth sulfite (BS) agar acc. WILSON and BLAIR	1.00191.0500	500 g