Mueller Hinton Agar

M173

Intended Use:

Recommended for determination of susceptibility of microorganisms to antimicrobial agents isolated from clinical samples.

Composition**

Ingredients	Gms / Litre
HM infusion B from #	300.000
Acicase ##	17.500
Starch	1.500
Agar	17.000
Final pH (at 25°C)	7.3±0.1

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef infusion from

- Equivalent to Casein acid hydrolysate

Directions

Suspend 38.0 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates. Note: The performance of this batch has been tested and standardised as per the current CLSI (formerly, NCCLS) document M6-protocols for Evaluating Dehydrated Mueller Hinton Agar.

Principle And Interpretation

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria* species (6). Other media were subsequently developed that replaced the use of Mueller Hinton Agar for the cultivation of pathogenic *Neisseria* species, but it became widely used in the determination of sulfonamide resistance of gonococci and other organisms. Mueller Hinton Agar is now used as a test medium for antimicrobial susceptibility testing (9). Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gel as described in CLSI Approved Standard (3). Mueller Hinton Agar has been selected by the CLSI for several reasons:

i. It demonstrates good batch-to-batch reproducibility for susceptible testing.

ii. It is low in sulfonamide, trimethoprim and tetracycline inhibitors.

iii. It supports the growth of most non-fastidious bacterial pathogens and

iv. Many data and much experience regarding its performance have been recorded (7).

Kirby-Bauer et al recommended this medium for performing antibiotic susceptibility tests using a single disc of high concentration (4). WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility (11). Mueller Hinton Agar with 5% sheep blood and Mueller Hinton Agar with Hemoglobin have been recommended for antimicrobial susceptibility testing of *Streptococcus pneumoniae* and *Haemophilus influenzae*.

HM infusion B from and acicase provide nitrogenous compounds, carbon, sulphur and other essential nutrients. Starch acts as a protective colloid against toxic substances present in the medium. Starch hydrolysis yields dextrose, which serves as a source of energy. These ingredients are selected for low thymine and thymidine content as determined by MIC values for

Enterococcus faecalis with sulfamethoxazole trimethoprim (SXT).

The Kirby-Bauer procedure is based on agar diffusion of antimicrobial substances impregnated on paper discs. This method employs disc with a single concentration of antimicrobial agent and the zone diameters observed are correlated with minimum inhibitory concentration (MIC) values (2,6,9). A standardized suspension of the organism is swabbed over the entire surface

of the medium. Paper discs impregnated with specific amounts of antimicrobial agents are then placed on the surface of the medium, incubated and zones of inhibition around each disc are measured. The susceptibility is determined by comparing with CLSI standards (7). The various factors, which influence disc diffusion susceptibility tests, are agar depth, disc potency, inoculum concentration, pH of the medium and beta-lactamase production by test organisms (7,8).

Mueller Hinton Agar is not appropriate for assay by disc diffusion method with slow growing organisms, anaerobes and capnophiles. With slow growing organisms, increased incubation may cause deterioration of diffusing antibiotic and produce unprecise readings (5).

Type of specimen

Clinical samples : Isolated microorganisms from urine , stool, blood etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).

Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. This medium is recommended for susceptibility testing of pure cultures only.
2. Inoculum density may affect the zone size. Heavy inoculum may result in smaller zones or too less inoculum may result in bigger zones.
3. Fastidious organisms may not grow on this medium and may require supplementation of blood.
4. Fastidious anaerobes may not grow on this medium.
5. As antimicrobial susceptibility is carried with antibiotic disc, proper storage of the disc is desired which may affect the potency of the disc.
6. Under certain circumstances, the in vitro results of antibiotic susceptibility may not show the same in vivo.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.7% agar gel.
Colour and Clarity of prepared medium
Light amber coloured clear to slight opalscent gel froms in Petri plates.
Reaction
Reaction of 3.8% w/v aqueous solution at 25°C. pH : 7.3±0.1
pH
7.20-7.40
Cultural Response
Cultural characteristics observed after incubation at 30-35°C for 18 -24 hours for bacterial cultures. For testing S.pneumoniae : The medium was supplemented with 5% Sheep blood and incubated at 35°C for 16-18 hours at 5% CO2 For testing *H.influenaze* : The medium was supplemented with 5g/l of Yeast extract & 2 vials /l of Haemophilus Growth Supplement (FD117 containing 15 mg/l of Haematin + 15 mg/l of NAD) and incubated at 35°C for 20-24 hours at 5% CO2
Antibiotic Sensitivity test
Various discs were tested for standard ATCC strains and zone of inhibition were measured after an incubation 30-35°C for 18 hours. (As per the latest CLSI Protocol M6 & Standards as per the current CLSI M100)
Thymine/Thymidine Content
The zones for these discs are indicative of the Thymine/Thymidine content of the medium.
Divalent Cation Content

$ The zones for these discs are indicative of the Divalent Cation content of the medium

Organism	Growth	Standard Zone	Zone of inhibition Observed
***Escherichia coli* ATCC 25922 (00013*)**	luxuriant		
Cephalothin CEP 30mcg		29-37 mm	29 -37 mm
Chloramphenicol C 30 mcg		21-27 mm	21 -27 mm
Co-Trimoxazole COT 25 mcg #		23-29 mm	23 -29 mm
Cefotaxime CTX 30 mcg		29-35 mm	29 -35 mm
Gentamicin GEN 10 mcg		19-26 mm	19 -26 mm
Sulphafurazole SF 300 mcg		15-23 mm	15 -23 mm
***Staphylococcus aureus* subsp. *aureus* ATCC 25923 (00034*)**	luxuriant		
Co-Trimoxazole COT 25 mcg #		# 20 mm (Clear zone)	>=20 mm
Cefoxitin CX 30 mcg		23-29 mm	23 -29 mm
Erythromycin E 15 mcg		22-30 mm	22 -30 mm
Linezolid LZ 30 mcg		25-32 mm	25 -32 mm
Oxacillin OX 1mcg		18-24 mm	18 -24 mm
Pristinomycin RP 15 mcg		21-28 mm	21 -28 mm
Tetracycline TE 30 mcg $		18-25 mm	18 -25 mm
Ciprofloxacin CIP 5mcg		22-30 mm	22 -30 mm
***Pseudomonas aeruginosa* ATCC 27853 (00025*)**	luxuriant		
Ceftazidime CAZ 30 mcg		22-29 mm	22 -29 mm
Ciprofloxacin CIP 5mcg		30-40 mm	30 -40 mm
Tobramycin TOB 10 mcg $		19-25 mm	19 -25 mm
Amikacin AK 30 mcg $		18-26 mm	18 -26 mm
Aztreonam AT 3mcg		23-29 mm	23 -29 mm
Cephotaxime CTX 30 mcg		18-22 mm	18 -22 mm
Gentamicin GEN 10 mcg $		16-21 mm	16 -21 mm
Imipenem IPM 10 mcg		20-28 mm	20 -28 mm
Piperacillin PI 100 mcg		12-18 mm	25 -33 mm
***Escherichia coli* ATCC 35218**	luxuriant		
Amoxyclav AMC 30 mcg		18-24 mm	18 -24 mm
Piperacillin/Tazobactam PIT 100/10 mcg		24-30 mm	24 -30 mm
Ticarcillin TI 75 mcg		6 mm	6 -6 mm
Ticarcillin/Clavulanic acid TCC 75/10mcg		20-28 mm	20 -28 mm
Ampicillin AMP 10 mcg		16-22 mm	16 -22 mm
Ampicillin/Sulbactam A/S 10/10 mcg		29-37 mm	29 -37 mm
***Enterococcus faecalis* ATCC 29212 (00087*)**	luxuriant		
Trimethoprim TR 5 mcg #		# 20 mm	>=20 mm
Vancomycin VA 30 mcg		17-21 mm	17 -21 mm
***Staphylococcus aureus* subsp. *aureus* ATCC 43300 (MRSA) (00211*)**	luxuriant		
Oxacillin OX 1 mcg		Very Hazy to No Zone	No zone

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Bauer A. W., Kirby W. M., Sherris J. L. and Turck M., 1966, Am. J. Clin. Pathol., 45:493.

2. Ericsson H. M. and Sherris J. L., 1971, Acta Pathol. Microbiol., Scand. Sect B Suppl., 217:1.

3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

5. Mueller J. H. and Hinton J., 1941, Proc. Soc. Exp. Biol. Med., 48:330.

6. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.

7. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore

8. National Committee for Clinical Laboratory Standards, 1986, Proposed Standards, M6-P, NCCLS, Villanova, Pa.

9. National Committee for Clinical Laboratory Standards, 2000, Approved Standard: M7-A5. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that grow aerobically, 5th Ed., NCCLS, Wayne, Pa.

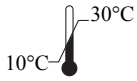
10. NCCLS Approved Standard: ASM-2, 1979, Performance Standards for Antimicrobic disc Susceptibility Tests, 2nd Ed., National Committee for Clin. Lab. Standards.

11. Present Status and Future Work, WHO Sponsored collaborative study, Chicago, Oct. 1967.

Revision : 04 / 2018

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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Technical Data

HiCulture ™ Transport Swabs w/ Cary -Blair Medium

MS202

Recommended for recovery of aerobic, anaerobic and fastidious bacteria from faecal specimens.

Composition**

Ingredients	Gms / Litre
Disodium phosphate	1.100
Sodium thioglycollate	1.500
Sodium chloride	5.000
Agar	5.000
Final pH (at 25°C)	8.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Remove cap from the tube. Collect sample using capped swab. Discard cap of the tube, replace with capped swab.

Principle And Interpretation

Proper collection and transportation of faecal specimens is vital for detection of faecal pathogens. Cary and Blair (1) devised this medium to provide conditions that will allow and increase survival of organisms without aiding multiplication due to minimal nutrients. Sodium thioglycollate in the medium provides a low oxidation reduction potential. An alkaline pH of the medium prevents bacterial destruction due to formation of acid. Sterile cotton swabs allow absorption of specimen material while polystyrene shaft allows semiflexibility to the swab stick, aiding in collection.

Quality Control

Appearance
Sterile Cary-Blair medium in tubes with sterile cotton swabs.

Colour
Light amber coloured medium

Quantity of Medium
8ml of medium in tubes

Reaction
8.20- 8.60

Cultural response
Viability of following organisms was established for a period of 48 hours.Organisms grew luxuriantly when recovered on Tryptone Soya Agar (M290) and incubated at 35 - 37°C for 18-24 hours.

Sterility test
Passes release criteria

Cultural Response

Organism	Recovery	Recovery
Enterobacter aerogenes ATCC 13048	Good - Luxuriant	
Escherichia coli ATCC 25922	Good - Luxuriant	
Klebsiella pneumoniae ATCC 13883	Good - Luxuriant	
Neisseria meningitidis ATCC 13090	Good - Luxuriant	
S. serotype Typhimurium ATCC 14028	Good - Luxuriant	

Shigella flexneri ATCC 12022	Good - Luxuriant
Vibrio parahaemolyticus ATCC 11344	Good - Luxuriant
Vibrio cholerae ATCC 15748	Good - Luxuriant

Storage and Shelf Life

Store between 5 – 25°C with caps firmly screwed. DO NOT FREEZE. Use before expiry date on label.

Reference

1. Cary and Blair, 1964, J. Bact., 88:96.

Revision : 1 / 2011

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Certificate of Analysis, Quality and Conformity

Material Code : MS202	**Material Name :** HiCulture ™ Transport Swabs w/ Cary-Blair medium	**Lot No** : MSI104 **Mfg. Date** :2020-06
Report No.: 40001042420	**Date of Release & Report :** 2020-08-20	**Expiry Date :** 2022-06

Appearance
Sterile Cary-Blair medium in tubes with sterile viscous swabs.

Colour of medium
Light amber coloured medium

Quantity of medium
8ml of medium in tubes

pH
pH Range : 8.20- 8.60 Observed : 8.42

Cultural response
Viability of following organisms was established for a period of 48 hours. Organisms grew luxuriantly when recovered on Tryptone Soya Agar (M290) and incubated at 35 - 37°C for 18-24 hours.

Organism	Recovery
Recovery	
Enterobacter aerogenes ATCC 13048	Good - Luxuriant
Escherichia coli ATCC 25922	Good - Luxuriant
Klebsiella pneumoniae ATCC 13883	Good - Luxuriant
Neisseria meningitidis ATCC 13090	Good - Luxuriant
S. serotype Typhimurium ATCC 14028	Good - Luxuriant
Shigella flexneri ATCC 12022	Good - Luxuriant
Vibrio parahaemolyticus ATCC 11344	Good - Luxuriant
Vibrio cholerae ATCC 15748	Good - Luxuriant

Sterility Check
Passes release criteria

. ATCC is a registered trade mark of the American Type Culture Collection
. NCTC and National Collection of Type Culture are registered trade mark of the Health Protection Agency

Control Media :
. For Bacteria : Soyabean Casein Digest Agar / Columbia Blood Agar base enriched with 5% v/v Sheep/Horse blood.
. For Yeast & Mold : Sabouraud Dextrose Agar.

. All ISO 11133 : 2014/Amd.1:2018(E) control strains are included in the Quality parameter
. HiMedia Laboratories Pvt Ltd is Certified for ISO 9001:2015, ISO 13485:2016 and WHO GMP

. The Quality Assurance Parameters are as per the guidelines specified in CLSI (NCCLS) document M22-A3 wherever applicable.

Storage & Shelf Life

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Certificate of Analysis, Quality and Conformity

Material Code : MS202	**Material Name :** HiCulture ™ Transport Swabs w/ Cary-Blair medium	**Lot No** : MSI104 **Mfg. Date** :2020-06
Report No.: 40001042420	**Date of Release & Report :** 2020-08-20	**Expiry Date :** 2022-06

Store between 5 – 25°C with caps firmly screwed. DO NOT FREEZE. Use before expiry date on label.

STATUS OF THE MATERIAL : APPROVED

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This document has been produced electronically and is valid

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2020-08-20

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Certificate of Analysis, Quality and Conformity

Material Code : M173	**Material Name :** Mueller Hinton Agar	**Lot No :** 0000453788
Report No.: 40001064394	**Date of Release & Report :** 2020-10-27	**Expiry Date :** 2025-09

Appearance
Cream to yellow homogeneous free flowing powder . Observed : Light yellow

Gelling
Firm, comparable with 1.7% agar gel.

Colour and Clarity of prepared medium
Light amber coloured clear to slight opalscent gel froms in Petri plates.

Reaction
Reaction of 3.8% w/v aqueous solution at 25°C.

pH
pH Range :7.20-7.40 Observed : 7.37

Cultural Response
Cultural characteristics observed after incubation at 30-35°C for 18 -24 hours for bacterial cultures. For testing S.pnuemoniae : The medium was supplemented with 5% Sheep blood and incubated at 35°C for 16-18 hours at 5% CO2 For testing H.influenaze : Yeast extract & 2 vials /l of Haemophilus Growth Supplement (FD117 containing 15 mg/l of Haematin + 15 mg/l of NAD) and incubated at 35°C for 20-24 hours at 5% CO2

Antibiotic Sensitivity test
Various discs were tested for standard ATCC strains and zone of inhibition were measured after an incubation 30-35°C for 18 hours. (As per the latest CLSI Protocol M6 & Standards as per the current CLSI M100)

Thymine/Thymidine Content
 # The zones for these discs are indicative of the Thymine/Thymidine content of the medium.

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Standard Zone	Zone of inhibition Observed
Escherichia coli ATCC 25922						
Growth promoting	89	luxuriant	80	89%	-	-
Amoxyclav AMC 30 mcg	-	-	-	-	18-24 mm	23mm
Ampicillin AMP 10 mcg	-	-	-	-	16-22 mm	21mm
Cefotaxime CTX 30 mcg	-	-	-	-	29-35 mm	35mm
Cefoxitin CX 30 mcg	-	-	-	-	23-29 mm	28mm
Cephalothin CEP 30mcg	-	-	-	-	15-21 mm	20mm

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Chloramphenicol C 30 mcg	-	-	-	-	21-27 mm	26mm
Ciprofloxacin CIP 5mcg	-	-	-	-	30-40 mm	38mm
Gentamicin GEN 10 mcg	-	-	-	-	19-26 mm	25mm
Sulphafurazole SF 300 mcg	-	-	-	-	15-23 mm	22mm
*Tetracycline TE 30 mcg **	-	-	-	-	18-25 mm	22mm
Co-Trimoxazole COT 25 mcg #	-	-	-	-	23-29 mm	28mm
Staphylococcus aureus ATCC 25923						
Growth promoting	84	luxuriant	75	89%	-	-
Amoxyclav AMC 30 mcg	-	-	-	-	28-36 mm	35mm
Ampicillin/Sulbactam A/S 10/10 mcg	-	-	-	-	29-37 mm	35mm
Cephalothin CEP 30mcg	-	-	-	-	29-37 mm	35mm
Ciprofloxacin CIP 5mcg	-	-	-	-	22-30 mm	28mm
Erythromycin E 15 mcg	-	-	-	-	22-30 mm	28mm
Linezolid LZ 30 mcg	-	-	-	-	25-32 mm	30mm
Oxacillin OX 1mcg	-	-	-	-	18-24 mm	23mm
Pristinomycin RP 15 mcg	-	-	-	-	21-28 mm	28mm
*Tetracycline TE 30 mcg **	-	-	-	-	24-30 mm	29mm
Vancomycin VA 30 mcg	-	-	-	-	17-21 mm	20mm
Co-Trimoxazole COT 25 mcg #	-	-	-	-	24-32 mm	30mm
Cefoxitin CX 30 mcg	-	-	-	-	23-29 mm	28mm
Pseudomonas aeruginosa ATCC 27853						
Growth promoting	81	luxuriant	72	88%	-	-
*Amikacin AK 30 mcg **	-	-	-	-	18-26 mm	25mm
Aztreonam AT 3mcg	-	-	-	-	23-29 mm	28mm
Cephotaxime CTX 30 mcg	-	-	-	-	18-22 mm	21mm
Ceftazidime CAZ 30 mcg	-	-	-	-	22-29 mm	28mm
Ciprofloxacin CIP 5mcg	-	-	-	-	25-33 mm	32mm
*Gentamicin GEN 10 mcg **	-	-	-	-	16-21 mm	20mm
Imipenem IPM 10 mcg	-	-	-	-	20-28 mm	27mm
Piperacillin PI 100 mcg	-	-	-	-	25-33 mm	32mm
Ticarcillin/Clavulanic acid TCC 75/10mcg	-	-	-	-	20-28 mm	27mm
*Tobramycin TOB 10 mcg **	-	-	-	-	19-25 mm	24mm
Escherichia coli ATCC 35218						

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Growth promoting	88	luxuriant	70	79%	-	-
Amoxyclav AMC 30 mcg	-	-	-	-	17-22 mm	21mm
Ampicillin AMP 10 mcg	-	-	-	-	6 mm	6mm
Ampicillin/Sulbactam A/S 10/10 mcg	-	-	-	-	13-19 mm	18mm
Piperacillin PI 100 mcg	-	-	-	-	12-18 mm	17mm
Piperacillin/Tazobactam PIT 100/10 mcg	-	-	-	-	24-30 mm	28mm
Ticarcillin TI 75 mcg	-	-	-	-	6 mm	6mm
Ticarcillin/Clavulanic acid TCC 75/10mcg	-	-	-	-	21-25 mm	24mm
Enterococcus faecalis ATCC 29212						
Growth promoting	87	luxuriant	77	88%	-	-
Co-Trimoxazole COT 25 mcg #	-	-	-	-	# 20 mm (Clear zone)	32mm
Trimethoprim TR 5 mcg #	-	-	-	-	# 20 mm	27mm
Vancomycin VA 30 mcg	-	-	-	-	# 17 mm	21mm
Staphylococcus aureus ATCC 43300 (MRSA)						
Growth promoting	83	luxuriant	75	90%	-	-
Oxacillin OX 1 mcg	-	-	-	-	Very Hazy to No Zone	No zone
Sterptococcus pneumoniae ATCC 49619 (On Medium with 5% Sheep Blood)						
Growth promoting	82	luxuriant	72	87%	-	-
Neisseria gonorrhoeae ATCC 49226 (Incubated w/5% CO2)						
Growth promoting	87	luxuriant	75	86%	-	-
Haemophilus influenzae ATCC 49247 (On medium w/ Y.E.,NAD & Hematin)						
Growth promoting	86	luxuriant	77	89%	-	-

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. NCTC and National Collection of Type Culture are registered trade mark of the Health Protection Agency

Control Media :
. For Bacteria : Soyabean Casein Digest Agar / Columbia Blood Agar base enriched with 5% v/v Sheep/Horse blood.
. For Yeast & Mold : Sabouraud Dextrose Agar.

. All ISO 11133 : 2014/Amd.1:2018(E) control strains are included in the Quality parameter
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. Information for BSE/TSE Risk: The material was subjected to pH <= 7.0 and/or a temperature in excess of 75ºC for no less than 2 hours during the manufacturing process. The bovine raw material for this product was collected entirely from Indian Origin animals in a licensed based establishment. The animals are inspected under a Govt. approved veterinarian's supervision and were apparently free from infectious and contagious diseases. BSE (Bovine Spongiform Encephalopathy)/ TSE (Transmissible Spongiform Encephalopathy) and dioxine are not known to exist in India. This material does not contain, nor is derived from the specific risks material as defined in The Maharashtra Animal Preservation Act Govt. of Maharashtra, India.

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2020-10-27