RPF Agar Base

M1736I

Intended Use

Recommended for the enumeration of coagulase positive Staphylococci from food and animal feeding stuffs. The composition and performance criteria are in accordance with ISO 6888-2:1999/ Amd 2:2018 and 11133:2014 (E). Amd.:2020 .

Composition**

ISO specification -Rabbit plasma fibrinogen agar (RPFA) medium

Ingredients	g / L
Pancreatic digest of casein	10.000
Meat extract	5.000
Yeast extract	1.000
L-Glycine	12.000
Sodium pyruvate	10.000
Lithium chloride	5.000
Agar	15.000
Final pH, after sterilization (at 25°C)	7.2±0.2

RPF Agar Base

Ingredients	g / L
Tryptone #	10.000
HM extract ##	5.000
Yeast extract	1.000
Glycine	12.000
Sodium pyruvate	10.000
Lithium chloride	5.000
Agar	15.000
Final pH, after sterilization (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Pancreatic digest of casein ## Equivalent to Meat extract

Supplements to be added after autoclaving per 90ml of medium

I Potassium tellurite solution	0.25ml
II Bovine fibrinogen solution	7.5 ml
III Rabbit Plasma and trypsin inhibitor solution	2.5 ml

FPT Inhibitor (FD195) per vial for 90ml of medium

Bovine fibrinogen	0.375g
Rabbit plasma	2.5 ml
Trypsin inhibitor	2.5 mg
Potassium tellurite	2.5 mg

Directions

Suspend 5.80 grams in 90 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 and aseptically add one vial of FPT Inhibitor (FD195). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Rabbit Plasma Fibrinogen Agar Base works on same principle as Baird Parker Agar with addition of Fibrinogen Plasma together with Trypsin and Potassium Tellurite as single supplement. Baird Parker Agar was developed by Baird Parker (1,2) from the Tellurite-glycine formulation of Zebovitz et al (3) for isolation and enumeration of Staphylococci in food and other material since it allows a good differentiation of coagulase positive strains. The composition laid down is as per ISO 6888-1 (4) and testing as per ISO 11133:2014 (5). Studies show that almost 100% of coagulase positive Staphylococci are capable of reducing tellurite, which produces black colonies, whereas other Staphylococci cannot always do so.

Tryptone, HM extract and yeast extract are sources of nitrogen, carbon, sulphur and vitamins. Sodium pyruvate not only protects injured cells and helps recovery but also stimulates *Staphylococcus aureus* growth destroying selectivity. Lithium chloride and potassium tellurite inhibit most of the contaminating microflora except *Staphylococcus aureus.* The tellurite imparts a black colour to the colonies. Glycine, pyruvate enhances growth of *Staphylococcus.* FPT Inhibitor allows allows detection of coagulase activity. A opacity halo and grey-black colonies on this medium are diagnostic for coagulase positive Staphylococci.

Type of specimen

Food samples and animal feeding stuffs

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (4,5).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested with the standard strains, slight variationin growth may be observed depending on the source from where the organism has been isolated.
3. It should be noted, however, that bovine strains, in particular, do not always produce this zone and confirmatory testing is needed.

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 1.5% agar gel.

Colour and Clarity of prepared medium

Yellow coloured clear to slightly opalescent gel forms in Petri plates..

Reaction

Reaction of 5.8% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Productivity : Cultural response was observed with added FPT Inhibitor (FD195) after an incubation at 35± 1°C for 24 ± 2 to 48 ± 2 hours. Recovery rate is considered as 100% for bacteria growth on Reference medium - Soyabean Casein Digest Agar.

Specificity : Cultural response was observed with added FPT Inhibitor (FD195) after an incubation at 35± 1°C for 24 ± 2 to 48 ± 2 hours.

Selectivity : Cultural response was observed with added FPT Inhibitor (FD195) after an incubation at 35± 1°C for 48 ± 2 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Characteristic reaction
Productivity				
Staphylococcus aureus subsp. *aureus ATCC 6538* (00032*)	50 -100	luxuriant	>=50 %	Black or grey colonies with opacity halo
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50 -100	luxuriant	>=50 %	Black or grey colonies with opacity halo
Selectivity				
E*scherichia coli* ATCC 8739 (00012*)	>=10^4	inhibited		
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited		
Specificity				
Staphylococcus epidermidis ATCC 12228 (00036*)	10^3-10^4	growth		Black or grey colonies without opacity halo

Staphylococcus saprophyticus ATCC 15305 (00159)*	10^3-10^4	growth	Black or grey colonies without opacity halo

Key : (*) - Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

1. Baird-Parker A. C., 1962, J. Appl. Bacteriol., 25:12.
2. Baird-Parker A. C. and Davenport E., 1965, J. Appl. Bacteriol., 28:390.
3. Zebovitz E., Evans J. B. and Niven C.F., 1955, J. Bacteriol., 70:686 .
4. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase positive Staphylococci (S*taphylococcus aureus* and other species). International Organization for Standardization (ISO), 1999 Ammd 2:2018-07, Draft ISO/DIS 6888-1.
5. Microbiology of food,animal feeding stuffs and water- Preparation, production,storage and performance culture media, EN ISO 11133:2014 (E). / Amd.:2020
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
7. 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.

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