Agar medium M
(Triple Sugar Iron agar medium)

356-4384

DEFINITION
Medium used for the biochemical characterization of *Enterobacteria* in general and of *Salmonella* in particular in the analysis of food products and in screening for contamination of non-sterile Pharmacopeia products.

Equivalent USP 30/NF 25: Medium XVI

STANDARDS
• ***European Pharmacopeia* 6.0 -** Biological methods - **2.6.13.**: Microbiological test of non-sterile products (Detection of specified micro-organisms)

• **USP 30/NF 25 US Pharmacopeia and National Formulary (2007)**: Microbial Limit Tests **(61)** - Microbiological Tests

PRINCIPLE
The principle of the medium relies on the ability or otherwise of enterobacteria to ferment glucose (with or without production of gas), lactose, and saccharose and to reduce sulfates to sulfides which, in the presence of iron, produces a black precipitate of iron sulfide.

PRESENTATION
Dehydrated
500 g **code 356-4384**

STORAGE
- +15-25℃, in carefully-sealed bottles in a cool, dry place
- Expiration date and batch number are shown on the package.

TYPICAL FORMULA

Meat extract	3 g
Yeast extract	3 g
Peptone	20 g
Sodium chloride	5 g
Lactose	10 g
Saccharose	10 g
Glucose	1 g
Ferric ammonium sulfate	300 mg
Phenol red	24 mg
Sodium thiosulfate (anhydrous)	300 mg
Agar	11 g
Distilled water	1,000 ml

Final pH (25℃) = 7.4 ± 0.2

NB: the formula has been adapted to attain the required performance criteria.

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)
- Diluent(s)
- Distilled water

EQUIPMENT REQUIRED (NOT SUPPLIED)
(non-exhaustive)
- Scales
- Sterile weighing bags
- Grinder
- Hotplate
- Mixer-homogenizer
- Test tubes (16 x 160 mm) with autoclave-proof stoppers
- Sterile Pasteur pipettes or inoculating loops
- Thermostatically-controlled incubator or incubation room, precise to ±1℃
- Autoclave
- All usual laboratory equipment

PREPARATION OF DEHYDRATED MEDIUM
Always shake well before use.
Dissolve 63.5 g of powder in 1 liter of distilled water, mix until a homogenous suspension is obtained.
Heat gently, swirling frequently, then bring to the boil until completely dissolved.
Dispense 10 ml per tube and sterilize in autoclave at 115℃ ± 1℃ for 15 minutes.
Leave to rest in an inclined position so as to obtain a sediment 2.5 cm deep.

Reconstitution ratio: 63.5 g/l
500 g of powder makes 7.9 liters of medium.

PROTOCOL
• **Preparation of samples**
According to the standards applicable to the product concerned.

• **Inoculation and incubation**
From each dish of selective medium, collect the recommended number of colonies and re-isolate on an ordinary nutrient agar (code 356-4485) in order to obtain pure strains.
Incubate at 37℃ ± 1℃ for 24 hours.
Using the pure strains, streak-inoculate the slope of the medium and stab the pellet with a depth inoculation using a previously flame-sterilized Pasteur pipette or inoculating loop.

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READING AND INTERPRETATION

Interpretation of resulting phenomena is as follows:

Sediment:
• yellow: glucose-positive (fermentation of glucose)
• red or unaltered: glucose-negative
• black: formation of hydrogen sulfide
• bubbles or cracking: production of gas from glucose

Slope:
• yellow: lactose- and/or saccharose-positive (utilization of lactose and/or of saccharose)
• red or unaltered: lactose- and/or saccharose-negative

Bacteria	Lactose and/or saccharose slope	Gas sediment [1]	H_2S
Citrobacter	+	+	+ [-]
Edwardsiella	-	+	+
Hafnia	- +	+ + **[2]**	- -
Escherichia	+ [-]	+	-
E. coli (biotype A.d.)	-	-	-
Klebsiella	+	+ [-]	-
Levinea	- or +	+	-
Proteus • *vulgaris* • *mirabilis* • *morganii* • *rettgeri*	 + or - - or + - -	 + or - + or - + or - -	 + + - -
Providencia	-	- [+]	-
Salmonella sp. *S.typhi* *S.paratyphi A* *S. arizona*	- - - -	+ - + +	+ traces - +
Serratia	+ or -	- or +**[3]**	-
Shigella	-	-	-
Yersinia	**d [4]**	-	-

[1] The pellet must be yellow (except in the case of H_2S release causing intense blackening), since all *Enterobacteria* ferment glucose.
[2] Apart from *E. agglomerans*
[3] Only small quantities of gas are produced by *Proteus*, *Providencia* and *Serratia* strains.
[4] d variable results depending on the strains

PRECAUTIONS

- The time lapse between the end of preparation of the stock solution (or the 10^{-1} dilution in the case of a solid product) and the moment when the dilutions come into contact with the culture medium must not exceed 15 minutes.
- Comply with Good Laboratory Practice.

QUALITY CONTROL

In view of the current harmonization of pharmacopeias, we recommend that you refer to the certificates of analysis for procedures relating to the quality control (performance and selectivity) of media produced by Bio-Rad.

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

KEY WORDS

TSI/*Enterobacteria*/*Salmonella*/
Food products/Identification/Triple sugar/Ferric citrate/Medium

BIBLIOGRAPHY

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