

HECTOEN ENTERIC AGAR

INSTRUCTIONS FOR USE THE READY-TO-USE PLATED MEDIUM

1. Intended use

Hektoen Enteric Agar is a semi-selective and differential medium intended for qualitative detection and isolation of Gram-negative *Enterobacteriales* especially for isolation of *Shigella* and *Salmonella* from specimens of human biological materials, mainly stool and rectal swabs, and other clinical and non-clinical specimens.

The function of the medium is to support the diagnosis of patients with symptoms indicative of a potential infection with Gram-negative enteric pathogens, particularly those of the *Shigella* and *Salmonella* genera.

Salmonella is one of the most common causes of food poisoning. The pathogenicity of these microorganisms varies from one serovar to another, and can vary within the same subspecies. Some serovars are known to cause invasive diseases, while some cause self-limiting food poisoning. The most commonly isolated serovars of *Salmonella* are *Salmonella enterica* subsp. *enterica* *S. enteritidis*, *S. typhimurium*, *S. virchow*, *S. badar* or *S. infantis*.

The genus *Shigella* includes four species: *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*. All species are obligate pathogens and cause bacterial dysentery.

Cat. no:	Medium type:	Packaging:
1060PD90; 201060	Solid medium on a plate	1x10 pcs (90 mm)

2. Principle of the procedure

Enzymatic digest of animal tissues and yeast extract are the source of nutrients in the medium. Bile salts mixture and acid fuchsin inhibits the growth of Gram-positive bacteria and most other non-pathogenic coliform bacteria that are the natural intestinal microbiome. Lactose, sucrose and salicin included in the medium allow differentiation of bacteria based on their ability to ferment carbohydrates. Non-fermenting bacteria (e.g., *Salmonella*, *Shigella*) do not cause a change in the pH of the medium, thus not causing a change in its colour. In contrast, coliforms and some *Proteus* spp., are able to ferment sugars and produce acids as a result. This process effectively lowers the pH of the medium and changes its colour to yellow or orange. Acid fuchsin and bromothymol blue as pH indicators. Ferric ammonium citrate and sodium thiosulfate enable detection of hydrogen sulfide production. Hydrogen sulfide-producing bacteria grow as colonies with a black center.

3. Medium composition

In g/l distilled water:	
Enzymatic digest of animal tissues	16,5 g
Yeast extract	3,0 g
Salicin	2,0 g
Bile salts mixture	4,5 g
Agar	13,5 g
Lactose	12,0 g
Sucrose	12,0 g
Sodium chloride	5,0 g
Sodium thiosulfate	5,0 g
Ferric ammonium citrate	1,5 g
Acid fuchsin	0,1 g
Bromothymol blue	0,065 g

pH 7.6± 0.2 at 25° C.

Appearance of the medium - clear, dark green medium.

4. Medium preparation

The medium is ready to use. Bring the medium to room temperature immediately before use.

5. Equipment required, not provided

Standard laboratory equipment necessary to perform tests, including an incubator.

6. Precautions

- The product is intended for professional use only.
- Non-automated product.
- The medium contains components of animal origin, which may be associated with the presence of biological pathogens, therefore must be handled in accordance with the principles of handling potentially infectious biological material.
- Do not use plates if the medium shows signs of microbial contamination, discoloration, drying, cracking or other signs of deterioration.
- Do not use damaged plates.
- Do not use plates after the expiration date.
- Re-incubation of previously inoculated plates is not allowed.
- To ensure correct results, follow these instructions.
- If the handling of the medium differs from that described in this manual, the laboratory is obliged to validate the procedure adopted.

7. Storage

Store plates at 2-12°C until the expiration date. Store plates in their original packaging, in an inverted position (agar side up), away from direct light sources. To avoid freezing of agar, do not store plates close to the refrigerator walls. To avoid the appearance of water condensation on the plate lid, do not open the refrigerator more than necessary and do not store plates in an overfilled refrigerator.

8. Expiration date

The medium stored at 2-12°C retains its properties up to 3 months from the date of production.

9. Specimen type

Specimens of clinical materials of human origin: feces, rectal swabs, cultures of strains cultivated from specimens collected from the gastrointestinal tract.

Collect test specimens in accordance with current guidelines. Store test specimens until delivery to the laboratory in accordance with the laboratory's policy of specimens storage. Store stool specimens in a refrigerator at 4 °C (± 2 °C). Store specimens collected on swabs at room temperature, following the manufacturer's recommendations. Inoculate specimens as soon as possible after delivery to the laboratory.

10. Test procedure

1. Allow the medium to warm to room temperature before inoculation.
2. Inoculate the specimen by spreading it directly on the agar surface.
3. If the specimen is collected on a swab, gently rotate the tip of the swab on a small area of agar just around the edges of the plate, and then inoculate specimen using streak plate method with a sterile loop.
4. Incubate the inoculated plates in aerobic conditions at 35 ± 2 °C.
5. Examine for growth after 18-24 hours of incubation.

11. Reading and interpretation

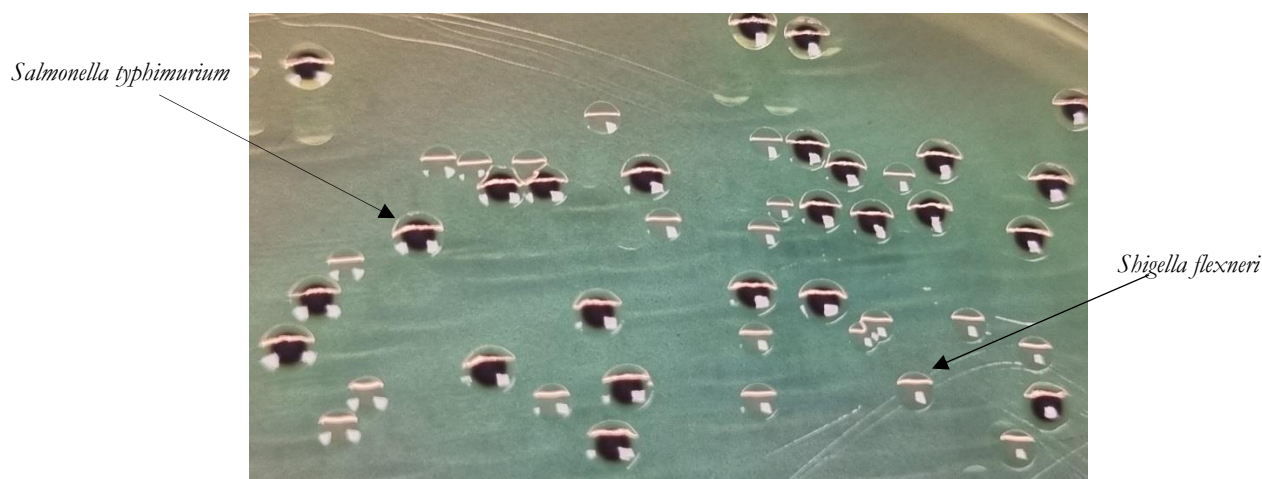
After incubation, observe:

- the presence of bacterial colony growth,
- colony morphology,
- colony colour

Typical morphology of microbial colonies grown on Hektoen Enteric Agar:

Microorganism	Typical colony morphology/staining
<i>Escherichia coli</i>	Colonies large, yellow to salmon- orange coloured; some strains exhibit no growth
<i>Klebsiella, Enterobacter</i>	Large, yellow to salmon-coloured colonies
<i>Proteus</i>	Blue-green to blue or salmon-coloured colonies, usually black-centered
<i>Salmonella</i>	Green to blue colonies, usually black-centered, possibly all black
<i>Shigella</i>	Green, convex colonies
<i>Pseudomonas</i>	Green to brown, irregular colonies
Gram-positive bacteria	No growth or very weak growth

For the final identification of cultured microorganisms, additional tests and/or confirmatory tests should be carried out using other methods used in the laboratory.



Colony morphology and growth pattern of *Salmonella* and *Shigella* bacteria on Hektoen Enteric Agar

12. Quality control

The nutritional properties and selectivity of the medium should be checked using reference strains giving the expected positive and negative reactions. The test should be performed using pure, 18-24 hour cultures of reference strains giving the desired reactions. Use the following reference strains to perform medium quality control:

Reference strain:	Growth intensity:	Colony morphology:
<i>Salmonella typhimurium</i> ATCC 14028	good growth	green with black center
<i>Shigella flexneri</i> ATCC 12022	good growth	green
<i>Escherichia coli</i> ATCC 25922	poor growth	yellow to salmon-orange
<i>Enterococcus faecalis</i> ATCC 29212	no growth	-

Other reference strains may be used in accordance with the laboratory's procedures and instructions. Quality control procedures should meet the requirements of applicable regulations and guidelines/recommendations.

13 Limitations of the method

- Due to variability in the nutritional value of the medium, some strains may grow poorly or not at all on Hektoen Enteric Agar.

- *Proteus* spp. may resemble *Salmonella* and *Shigella* bacteria in appearance, so additional confirmatory tests are recommended.

14. Characteristics of the method

Enterobacterales are a large, closely related group of bacteria, comprising more than forty genera, more than half of which are related to humans. Many enterobacteria inhabit the digestive tract of healthy people and animals. Others, such as *Salmonella*, *Shigella* and *Yersinia*, include obligate pathogens responsible for disease-specific course. Infection usually occurs when food contaminated with microorganisms is eaten. In the diagnosis of infections, it is important to isolate the etiological agent from the clinical material.

There are many media available on the market that support the diagnosis of these microorganisms. The choice of one of them depends on the specificity of the laboratory and often the preferences of employees. One of the media used in the diagnosis of enteric infections is Hektoen Enteric Agar.

Wartburton D.W. et al. in Int. J. Food Microbiol. compared six different media used to isolate *Salmonella*. These included Brilliant Green Sulpha Agar, Byzmuth Sulphite Agar, Hektoen Enteric Agar, Xylose Lysine Deoxycholate Agar, EF-18 Agar and Rambach Agar. 123 strains of *Salmonella* and 28 foods inoculated with *Salmonella* were used in the study. In quantitative tests, all tested media were comparable. In qualitative tests, the best medium was EF-18 Agar and Hektoen Enteric Agar. The other media were comparable.

15. Disposal of used material

Used and unused materials should be disposed of in accordance with current medical waste handling regulations and laboratory procedures for the disposal of infectious and potentially infectious materials.

16. Reporting of adverse events

According to current regulations, adverse events and incidents that can be directly linked to the described medium must be reported to the manufacturer and to the competent authorities.

17. References

1. King, S., and W. I. Metzger. 1968. A new plating medium for the isolation of enteric pathogens. Appl. Microbiol. 16:577-578.
2. King, S., and W. I. Metzger. 1968. a new plating medium for the isolation of enteric pathogens. II. Comparison of Hektoen Enteric Agar with S and EMB Agar. Appl Microbiol. 16:579-581.
3. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
4. Vanderzant, C., and D. F. Splittstoesser (eds.). 2015. compendium of methods for the microbiological examination of foods, 4 th ed. American Public Health Association, Washington, D.C..
5. Association of Official Analytic Chemists. 2016 Official methods of analysis of AOAC International, 20th ed. AOAC International, Arlington, VA.
6. Warburton D.W., Bowen B., Konkle A., Crawford C., Durzi S., Foster R., Fox C., Gour L., Krohn G., LaCasse P., A comparison of six different plating media used in the isolation of Salmonella, Int. J. Food Microbiol., Jun 1994, 277-89

History of document changes

Date of change	Section	Description of the change
2023/04/07	Entire document	Adaptation to the requirements of EU Regulation 2017/746

NOTE

The revision history of the document does not include editorial changes.

SYMBOL	NAME OF SYMBOL	DESCRIPTION	REF.
	Manufacturer	Indicates the medical device manufacturer.	5.1.1
	Date of manufacture	Indicates the date after which the medical device is not to be used.	5.1.3
	Catalogue number	Indicates the manufacturer's catalogue number so that the medical device can be used..	5.1.6
	Batch code	Indicates the manufacturer's batch code so that the batch or lot can be identified.	5.1.5
	In vitro diagnostic medical device	Indicates a medical device that is intended to be used as an invitro diagnostic medical device.	5.5.1
	Do not re-use	Indicates a medical device that is intended for one single use only.	5.4.2
	Contains sufficient for <n> tests	Indicates the total number of tests that can be performed with the medical device.	5.5.5
	Use -by date	Indicates the date after which the medical device is not to be used	5.1.4
	Temperature limit	Indicates the temperature limits of temperature shall be indicates adjacent to the upper and lower horizontal lines.	5.3.7
	Safety symbol (Compliance with EU requirements)	The CE marking on a product is a manufacturer's declaration that the product complies with the essential requirements of the relevant European Union health, safety and environmental regulations.	nd.

	Consult instructions for use or consult electronic instructions for use	Indicates the need for the user to consult the instructions for use.	5.4.3
	Sterilized using aseptic processing techniques	Indicates a medical device that has been manufactured using accepted aseptic techniques.	5.2.2
	Do not use if package is damaged and consult instructions for use	Indicates that a medical device that should not be used if the package has been damaged or opened and that the user should consult the instructions for use for additional information.	5.2.8
	Contains biological material of animal origin	Indicates a medical device that contains biological tissue, cells, or their derivatives, of animal origin	5.4.8



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