

# HEKTÖEN ENTERIC AGAR

DETECTION OF *SALMONELLA* AND *SHIGELLA*

## 1 INTENDED USE

Hektoen Enteric Agar is a selective medium for the isolation and differentiation of pathogenic enterobacteria from biological samples of animal origin, water samples, dairy products and other food products. It is used in animal health in the context of *Salmonella* detection in mammals (NF U47-102). The medium is also recommended for the detection of *Shigella*, in food microbiology (NF EN ISO 21567).

Hektöen Enteric agar can also be used as the second media of choice in the standardized methods for the detection of *Salmonella*.

## 2 HISTORY

Hektoen Enteric Agar was formulated in 1967 by King and Metzger at the Hektoen Institute in order to increase the isolation frequency of *Shigella* and *Salmonella* species in comparison to that obtained with other selective isolation media. Although this medium enabled a wide variety of pathogenic enterobacteria to be isolated, it was less inhibitory towards non-pathogenic enteric bacteria. The current formulation differs from the original by the suppression of desoxycholate and the reduced concentration of bile salts. In parallel, the concentration of peptones was increased to offset the inhibitory effect of bile salts.

## 3 PRINCIPLES

Gram-positive flora are inhibited by bile salts which can also slightly inhibit the growth of a few Gram-negative microorganisms.

The medium contains three carbohydrates: lactose, sucrose and salicin. The high lactose concentration favors the visualization of enterobacteria by avoiding the problem of late fermentations. The other carbohydrates were added to insure a higher degree of differentiation and to reduce the toxicity caused by colored indicators so as to obtain excellent recovery of *Shigella*.

The principle of visualization is founded on the eventual fermentation of the 3 sugars present in the media. The microorganisms that ferment at least one of them form salmon-colored colonies, the others give rise to blue or green colonies. The color indicator system, composed of bromothymol blue and acid fuchsin, yields orange-yellow colonies for lactose-positive enterobacteria and blue-green colonies in the case of lactose-negative strains.

In the presence of sodium thiosulfate, H<sub>2</sub>S producing bacteria reduce ferric ammonium citrate and are detected by darkening due to the production of iron sulfide at the center of the colonies.

## 4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

- Peptic digest of meat.....	12,0 g
- Yeast extract .....	3,0 g
- Lactose.....	12,0 g
- Saccharose .....	12,0 g
- Salicin.....	2,0 g
- Bile salts.....	9,0 g
- Sodium chloride .....	5,0 g
- Sodium thiosulfate.....	5,0 g
- Ferric ammonium citrate .....	1,5 g
- Bromothymol blue .....	65 mg
- Acid fuchsin .....	0,1 g
- Bacteriological agar.....	13,5 g

pH of the ready-to-use media at 25 °C : 7,4-7,7.

## 5 PREPARATION

- Dissolve 75,1 g of dehydrated media (BK067) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Do not autoclave.
- Cool and maintain in a molten state at 44-47 °C.
- Pour into sterile Petri plates and let solidify on a cold, flat surface.
- Dry the plates in an incubator with the covers partially removed.

✓ **Reconstitution :**  
75,1 g/L

✓ **Sterilization :**  
Do not autoclave

## 6 INSTRUCTIONS FOR USE

- Inoculate by streaking onto the plates prepared as above, from enrichment media used for the detection of *Salmonella* or *Shigella*.
- Incubate at 37 ± 1 °C for 21 to 24 hours.

✓ **Inoculation :**  
Streak on surface

✓ **Incubation :**  
24 h at 37 °C

## 7 RESULTS

*Salmonella* present green or blue-green colonies, with a black center for the majority of strains.  
*Shigella* present green or bluish droplet-like colonies, without a black center.  
 Coliforms appear as yellow-salmon colored colonies.

Characteristics	Microorganisms
Yellow-salmon colonies	<i>Escherichia coli</i> , <i>Citrobacter</i> , <i>Klebsiella</i> , <i>Enterobacter</i> , <i>Arizona</i> , <i>Serratia</i>
Yellow-salmon colonies with a black center	<i>Proteus vulgaris</i>
Green colonies with a black center	<i>Proteus mirabilis</i> , <i>Salmonella</i>
Green or blue-green colonies	<i>Shigella</i> , <i>Salmonella</i> , <i>Providencia</i> , <i>Proteus</i> <i>morganii</i> , <i>Proteus rettgeri</i>
Small bluish to brownish colonies	<i>Pseudomonas</i> (oxidase positive)

See ANNEX 1 : PHOTO SUPPORT.

## 8 QUALITY CONTROL

**Dehydrated media :** beige powder, free-flowing and homogeneous.

**Prepared media :** dark green agar.

Typical culture response after 24 hours of incubation at 37 °C

Microorganisms	Growth	Characteristic colonies
<i>Salmonella</i> Typhimurium WDCM 00031	Good, score 2	Green with black center
<i>Salmonella</i> Enteritidis WDCM 00030	Good, score 2	Green with black center
<i>Shigella sonnei</i> WDCM 00127	Good, score 2	Green
<i>Shigella flexneri</i> WDCM 00125	Good, score 2	Green
<i>Escherichia coli</i> WDCM 00013	Partially inhibited, score 0-1	Orange-salmon
<i>Enterococcus faecalis</i> WDCM 00087	Inhibited, score 0	-
<i>Staphylococcus aureus</i> WDCM 00034	Inhibited, score 0	-

## 9 STORAGE / SHELF LIFE

**Dehydrated media :** 2-30 °C.

The expiration date is indicated on the label.

**Prepared media in plates (\*) :** 30 days at 2-8 °C.

**Prepared media in vials (\*) :** Not recommended.

(\*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

## 10 PACKAGING

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### Dehydrated media :

500 g bottle ..... BK067HA

## 11 BIBLIOGRAPHY

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NF U47-102. Janvier 2008. Méthodes d'analyse en santé animale. Isolement et identification de tout sérovar ou de sérovar(s) spécifié(s) de salmonelles chez les mammifères.

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## 12 ADDITIONAL INFORMATION

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The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

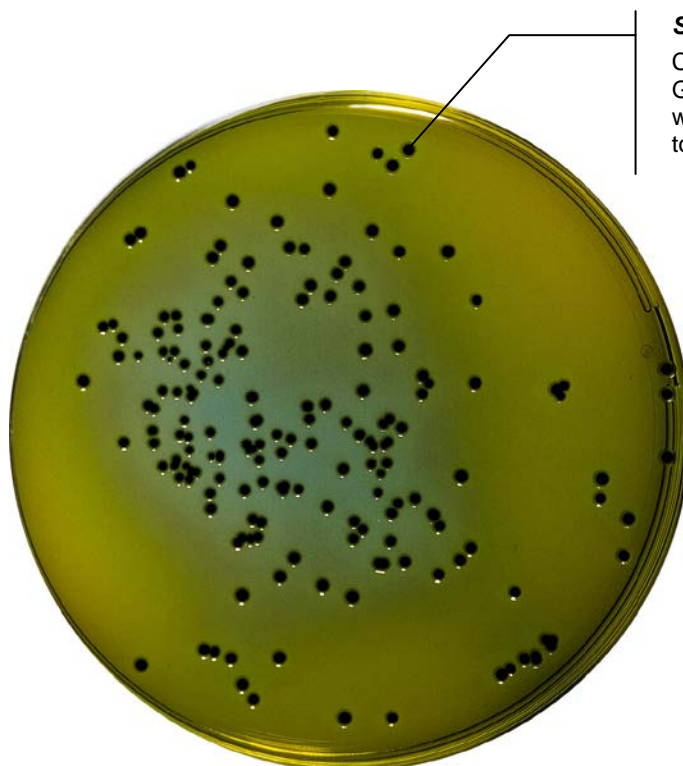
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## Hektöen Enteric Agar

Detection of pathogenic enterobacteria

### Results :

Growth obtained after 24 hours of incubation at 37°C.



### ***Salmonella Typhimurium***

Characteristic colony :  
Green color with a black center,  
which becomes entirely black  
towards the end of incubation.