EO Labs
Microbiological Culture Media

UK CA IVD CE

Instructions for Use

REF - PP1520

Hektoen Enteric Agar

Pre-Poured Media

1. Intended Use

For *in vitro* diagnostic use. PP1520 Hektoen Enteric Agar is a moderately selective differential medium for the isolation of *Shigella* and *Salmonella* species from enteric pathology specimens, particularly from faecal (mixed flora) samples.

2. Composition*

Ingredient	g/L
Meat peptone	12.0
Sodium chloride	5.0
Yeast extract	3.0
Sodium thiosulphate	5.0
Lactose	12.0
Ammonium ferric citrate	1.5
Sucrose	12.0
Acid fuchsin	0.1
Salicin	2.0
Bromothymol blue	0.065
Bile salts	7.0
Agar	14.0
Sodium deoxycholate	2.4

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

This medium was originally developed by King and Metzger [1]. PP1520 Hektoen Enteric Agar is primarily used to isolate and discriminate between colonies of *Salmonella* and *Shigella* spp. with *Salmonella* spp. producing translucent colonies with black centres and *Shigella* spp. producing green colonies. Other faecal bacteria are inhibited or produce distinct colony colour and morphology. PP1520 Hektoen Enteric Agar meets the requirements of the APHA [2] and is recommended by ISO 21567:2004 [3] and ISO 6579 [4 5], in the Microbiology of Drinking Water [6], and by Public Health England's UK Standards for Microbiology Investigations [7 8] as a solid selective media for the enumeration of *Shigella* species.

4. Principle

Meat peptone and yeast extract supply the required nitrogen and other essential nutrients. Lactose, sucrose, and salicin are fermentable carbohydrates. Bromothymol blue is added as a pH indicator to identify carbohydrate fermenting organisms. Thiosulphate is reduced to hydrogen sulphide by *Salmonella* spp. but not by *Shigella* spp. The H_2S reacts with Fe^{3+} ions from ferric ammonium citrate to produce a black precipitate of iron sulphide allowing *Salmonella* spp., which appear as translucent colonies with black centres, to be distinguished from *Shigella* spp., which appear as green colonies. The bile salts and acid fuchsin inhibit Gram-positive organisms.

5. Physical Characteristics

	Medium
Appearance and Colour	Green firm gel
pH	7.5 ± 0.2

6. Materials Provided

PP1520 Hektoen Enteric Agar is supplied in packs of 10 x 90mm P090 Petri dishes (18-22ml fill) (product code: PP1520-P090); each plate is ink-jet printed with (abbreviated) product name, product code, lot number and expiry date.

7. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., autoclave, sterile loops or swabs, collection containers, incubators, and quality control organisms.

8. Specimens

PP1520 Hektoen Enteric Agar is suitable for the testing of the following specimens:

- Food and Environmental: products for human consumption, animal feed and environmental samples
- Clinical: rectal swabs and stool specimens

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

9. Test Procedures and Interpretation of results

Based on sample type and information provided, determine whether the specimen requires pre-enrichment prior to inoculation of the prepared medium.

Inoculate plated medium directly with the sample, or subculture onto plated medium after incubation in enrichment broth where required.

Food and Environmental Samples (according to ISO 6579 [4 5]):

Prepare a homogenised suspension using a ratio of 1:9 of sample to Buffered Peptone Water (E&O BM0082) and incubate aerobically for 16-20 hours at 37 ± 1°C. Subculture the required volumes to Rappaport-Vassiliadis Broth and Muller-Kauffmann tetrathionate novobiocin Broth (E&O BM0947) and incubate aerobically at 41.5°C and 37°C respectively.

Using a 10µl loop, inoculate one plate of Xylose Lysine Deoxycholate (XLD) Agar (E&O PP0320) and PP1520 Hektoen Enteric Agar from the Rappaport-Vassiliadis Broth. Repeat for the Muller-Kauffmann tetrathionate novobiocin Broth.

Incubate XLD aerobically at 37 ± 1°C for 21-27 hours, and PP1520 at 37 ± 1°C aerobically for 18-24 hours.

Clinical Specimens:

Directly inoculate rectal swab or a pea-sized portion (or several drops) of faecal material into the appropriate enrichment broth (*e.g.*, E&O PP0370 Selenite Mannitol Broth) and incubate at 37 ± 1°C aerobically for 12-18 hours.

After incubation, subculture 10µl from the top 1/3 of the enrichment broth, whilst avoiding agitation of the medium, onto PP1520 Hektoen Enteric Agar and incubate at 37 ± 1°C aerobically for 18-24 hours.

For a more detailed discussion on isolation and identification of enteric pathogens from clinical specimens, refer to the procedures described in appropriate references [9].

After incubation, examine agar for colonies (typical colony appearance outlined in Quality Control table below). Perform further biochemical, serological, molecular, or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant guidelines.

10. Quality Control

Organism	Incubation	Result (Specificity)
S. typhimurium (NCTC 12023)	37 ± 1°C aerobically for 18-24 hours	Growth: Translucent colonies with black centre
S. sonnei (NCTC 8574)	37 ± 1°C aerobically for 18-24 hours	Growth: Green colonies
S. flexneri (NCTC 12698)	37 ± 1°C aerobically for 18-24 hours	Growth: Green colonies
E. coli (NCTC 12241)	37 ± 1°C aerobically for 18-24 hours	Partial inhibition: May grow as salmon pink coloured colonies.
E. faecalis (NCTC 12697)	37 ± 1°C aerobically for 18-24 hours	Inhibited

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

11. Performance

To fully verify the performance of PP1520 Hektoen Enteric Agar, samples were tested to assess colony morphology and recovery level (where an acceptable range is ≥50% and ≤120%) compared to a non-selective reference medium. Samples were inoculated with 30-150cfu for the target organisms and 10^4-10^5 cfu for the non-target organisms then incubated at 37 ± 1°C aerobically for 18-24 hours. All samples showed expected recovery and morphology of the required test organisms: *Salmonella typhimurium* (NCTC 12023), *Shigella sonnei* (NCTC 8574) and *Shigella flexneri* (NCTC 12698) and partial to no recovery of the non-target test organisms *Escherichia coli* (NCTC 12241) and *Enterococcus faecalis* (NCTC 12697). Therefore, it can be concluded that PP1520 Hektoen Enteric Agar, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

12. Limitations of the Medium

- *Proteus* spp. may resemble *Salmonella* or *Shigella*. Further testing should be conducted to confirm the presumptive identification or organisms isolated on this medium.
- Due to natural variation, some strains may grow poorly on this medium.
- Medium should not be autoclaved as components may denature.
- Some sulphate reducing bacteria other than salmonellae may be present in the colon. Most of these are inhibited by the bile in Hektoen Enteric Agar but some non-salmonella organisms producing black-centred colonies are possible. These may be distinguished by differential sugar fermentation and the production of coloured colonies with black centres. Further testing should be undertaken.
- Rare strains of *Salmonella* are capable of lactose fermentation [10] and may form red/yellow colonies with black centres.

13. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

Refer to E&O Poured Plate Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store PP1520 in the original packaging between 2 and 8°C in the dark. Kept under these conditions it may be used up to the date of expiry shown on product label.

Dispose of in accordance with local and national authority requirements. Do not use the medium if it is contaminated, discoloured, out of date, cracked or a precipitate is present. Additionally, do not use medium if it has been stored inappropriately or the packaging has been damaged.

15. References

1. King, S. and Metzger, W. (1968) A new plating medium for the isolation of enteric pathogens. Applied Microbiology, 16, pp.577-578.
2. APHA (1992) Compendium of Methods for the Microbiological Examination of Foods. 3rd ed. Washington DC: APHA Inc.
3. International Organization for Standardization (2004) 21567:2004 Microbiology of food and animal feeding stuffs - Horizontal method for the detection of Shigella spp. Geneva, ISO.
4. ISO (2017) 6579-1:2017 Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella*. Geneva: ISO.
5. ISO (2020) 6579-1:2017/AMD 1:2020 Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* - Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC. Geneva: ISO.
6. The Microbiology of Drinking Water (2006) Part 9 – Methods for the isolation and enumeration of *Salmonella* and *Shigella* by selective enrichment, membrane filtration and multiple tube-most probable number techniques. Standing Committee of Analysts, Environment Agency, Nottingham, UK.
7. Public Health England (2022) Identification of *Shigella* species. UK Standards for Microbiology Investigations. Bacteriology – Identification, ID 20(4). London: Standards Unit, Microbiology Services.
8. Public Health England (2021) Identification of *Salmonella* species. UK Standards for Microbiology Investigations. Bacteriology – Identification, ID 24(4). London: Standards Unit, Microbiology Services.
9. Bopp, C., Brenner, F., Fields, P., Wells, J., and Strockbine, N. (2003) *Escherichia*, *Shigella*, and *Salmonella*. In: Murray, P., Baron, E., Jorgensen, J., Pfaller, M. and Yolken, R. (ed.) Manual of Clinical Microbiology, 8th ed. Washington DC: American Society for Microbiology.
10. McDonough, P., Shin, S. and Lein, D. (2000) Diagnostic and public health dilemma of lactose-fermenting *Salmonella enterica* serotype Typhimurium in cattle in the Northeastern United States. J Clin Microbiol, 38(3), pp.1221-1226.

Version History*

001 28/02/2024 - New document created
002 24/02/2026 - Updated references and appropriate test procedures

*Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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TABLE OF APPLICABLE SYMBOLS

REF o REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Σ Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place