

Instructions for Use

REF - PP0260

Slanetz and Bartley Medium

Pre-Poured Media

1. Intended Use

PP0260 Slanetz and Bartley Medium is used for the enumeration of enterococci from water samples.

2. Composition*

<u>Ingredient</u>	<u>g/L</u>
Tryptose	20.0
Yeast extract	5.0
Glucose	2.0
Dipotassium hydrogen phosphate	4.0
Sodium azide	0.4
Tetrazolium chloride	0.1
Agar	12.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

PP0260 Slanetz and Bartley Medium was originally described by Slanetz and Bartley ⁽¹⁾ for the enumeration of enterococci from water samples using a membrane filtration technique, but it may also be used as a spread plate for the examination of other sample types. Enterococci reduce tetrazolium chloride to the insoluble red dye formazan. Resulting colonies are dark red or maroon on the surface of a membrane or agar. Other organisms may reduce tetrazolium chloride, so identification is presumptive. Colonies may be confirmed as enterococci using aesculin hydrolysis in a medium such as Kanamycin Aesculin Azide Agar (E&O PP0850).

PP0260 is recommended by the International Organization for Standardization ⁽²⁾, Standing Committee of Analysts ⁽³⁾ and tested according to ISO 11133:2014 ⁽⁴⁾.

4. Principle

Tryptose and yeast extract supply the required nitrogen, and other essential nutrients. Glucose is a fermentable sugar and dipotassium hydrogen phosphate acts as a pH buffer. Selectivity is achieved through the addition of sodium azide to suppress the growth of Gram-negative organisms. Tetrazolium chloride is reduced by enterococci to formazan resulting in dark red colonies of enterococci on the agar.

5. Physical Characteristics

	Medium
Appearance and Colour	Rose firm gel
pH	7.2 ± 0.2

6. Materials Provided

PP0260 Slanetz and Bartley Medium is supplied in packs of 10 x 90mm P090 Petri dishes (18-22ml fill) (product code: PP0260-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

PP0260 Slanetz and Bartley Medium is suitable for the testing of the following specimens:

- Food and Environmental: products for human consumption, animal feed and environmental samples
- Water Samples: Potable waters, water from heating systems, cooling towers, air conditioning systems and drinking water, ground/river/sea water and effluents.

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

Follow local, national, or international guidelines.

Allow plates to come to room temperature.

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

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

Food and Environmental Specimens:

For solid samples, prepare a homogenised suspension using a ratio of 1:9 of sample to Maximum Recovery Diluent (E&O BM0760). With homogenised and liquid specimens, the medium should be inoculated with the specimen and incubate at $37 \pm 1^\circ\text{C}$ aerobically for 24-48 hours.

Water Specimens ^(2 3)

According to ISO 7899-2:2000 ⁽²⁾, it is recommended to process samples within 6 hours of taking the sample.

- Process the water specimen as required by local guidelines.
- For filtration method: filter the required volume of water through a $\leq 0.45\mu\text{m}$ filter and place the filter directly onto the agar surface avoiding bubbles or inclusions.
- For spread plate method: Inoculate sample directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker.
- Incubate at $36 \pm 2^\circ\text{C}$ aerobically for 44 ± 4 hours (incubation at 44°C will improve selectivity but may affect recovery of enterococci ⁽³⁾).

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

10. Quality Control

Organism	Incubation	Result (Specificity)
<i>E. faecalis</i> (NCTC 12697)	$37 \pm 1^\circ\text{C}$ aerobically for 24-48 hours	Growth: Maroon colonies
<i>E. faecalis</i> (NCTC 775)	$37 \pm 1^\circ\text{C}$ aerobically for 24-48 hours	Growth: Maroon colonies
<i>E. faecium</i> (DSM 2146)	$37 \pm 1^\circ\text{C}$ aerobically for 24-48 hours	Growth: Maroon colonies
<i>E. coli</i> (NCTC 12241)	$37 \pm 1^\circ\text{C}$ aerobically for 24-48 hours	Inhibited
<i>S. aureus</i> (NCTC 12981)	$37 \pm 1^\circ\text{C}$ aerobically for 24-48 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 PP0260 Slanetz and Bartley Medium, samples were tested to assess colony morphology and recovery level (where an acceptable range is $\geq 50\%$ and $\leq 120\%$) 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 $37 \pm 1^\circ\text{C}$ aerobically for 24-48 hours. All samples showed expected recovery and morphology of the required test organisms: *Enterococcus faecalis* (NCTC 12697), *Enterococcus faecalis* (NCTC 775) and *Enterococcus faecium* (DSM 2146) and no recovery of the non-target test organisms: *Escherichia coli* (NCTC 12241) and *Staphylococcus aureus* (NCTC 12981). Therefore, it can be concluded that PP0260 Slanetz and Bartley Medium, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

12. Limitations of the Media

- Count all red, maroon, or pink colonies as presumptive enterococci. Not all species reduce tetrazolium chloride therefore pale colonies should not be ignored.
- Although the selective properties of this medium are very good it is advisable to regard the colony count as a presumptive or unconfirmed count. Further identification may be required depending on the scope of the examination.
- Due to natural variation, some strains may grow poorly on this medium.

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 PP0260 in the original packaging between 2 and 8°C in the dark. Kept under these conditions it may be used up to date of expiry shown on product label.

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

15. References

1. Slanetz, L. and Bartley, C. (1957) Numbers of enterococci in water, sewage, and feces determined by the membrane filter technique with an improved medium. J Bacteriol, 74(5), 591-595.
2. International Organization for Standardization (2014) 11133:2014 Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media. Geneva: ISO.
3. International Organization for Standardization (2000) 7899-2:2000 Water quality - Detection and enumeration of intestinal enterococci - Part 2: Membrane filtration method. Geneva: ISO.

Version History*

- 001 24/10/2023 - New Document Created
002 03/03/2025 - *E. faecalis* (NCTC 775) added to Quality Control panel

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



E&O Laboratories Ltd, Burnhouse, Bonnybridge, Scotland, FK4 2HH, Tel: +44 (0) 1324 840 404, info@eolabs.com, www.eolabs.com



Syntec Scientific Ltd., Unit 2 The Business Centre, Northwest logistics Park, Ballycoolin, Dublin 15, Ireland D1SPY00

IFU/PP0260 REV. 002

TABLE OF APPLICABLE SYMBOLS

 REF Catalogue number	 LOT Batch code		 Manufacturer	 Use by
 Temperature limitation	 Contents sufficient for <n> tests	 Consult Instructions for Use	 Keep away from direct light	 Store in a dry place