Instructions for Use

REF - PP7021 **Colorex™ Listeria (ISO)**

Pre-Poured Media

1. Intended Use

Colorex™ Listeria (ISO) is a selective chromogenic medium for the isolation and presumptive identification of *Listeria monocytogenes* from food and environmental samples.

2. Composition*

Ingredient	g/L
Enzymatic digest of animal tissues	18.0
Enzymatic digest of casein	6.0
Yeast extract	10.0
Sodium pyruvate	2.0
Glucose	2.0
Magnesium glycerophosphate	1.0
Magnesium sulphate	0.5
Sodium chloride	5.0
Lithium chloride	10.0
Disodium hydrogen phosphate	2.5
5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside	0.05
Agar	15.0
Phosphatidylinositol	2.0
Nalidixic acid	0.02
Ceftazidime	0.02
Polymyxin B	76700 IU
Amphotericin B	0.01

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Listeria monocytogenes is commonly found in soil, sewage, or faeces. It is difficult to eradicate, and can cause serious food poisoning; therefore, *L. monocytogenes* is frequently tested in food processing facilities to avoid contamination. Contamination can occur at all steps of the food manufacturing chain from raw materials to place of consumption.

Colorex™Listeria (ISO) is a rapid method for the detection and presumptive identification of *L. monocytogenes*. Based on the method of Ottaviani *et al*. [1 2], this medium can allow detection and enumeration of *Listeria* spp. as early as 24 hours.

PP7021 Colorex™Listeria (ISO) is recommended by ISO 11290-1:2017 [3] and ISO 11290-2:2017 [4] and tested in accordance with ISO 11133:2014 [5].

4. Principle

Colorex™Listeria (ISO) is made selective by the inclusion of lithium chloride, ceftazidime, polymyxin B and nalidixic acid to suppress other bacteria and amphotericin B (to suppress yeasts and fungi). The combination of chromogenic substrate hydrolysis by β-D-glucosidase and phosphatidylinositol hydrolysis by phospholipase C allows the differentiation of *L. monocytogenes* from other *Listeria* species.

5. Physical Characteristics

	Medium
Appearance and Colour	Pale cream firm gel
pH	7.2 ± 0.2

6. Materials Provided

PP7021 Colorex™Listeria (ISO) is supplied in packs of 10 x 90 mm P090 Petri dishes (18-22ml fill) (product code PP7021-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., sterile loops or swabs, collection containers, incubators, and quality control organisms.

8. Specimens

Colorex™ Listeria (ISO) can be used with the following specimens:

- Food industry: Products for human consumption, animal feed and environmental samples.

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

9. Test Procedures and Interpretation of results

Allow the plates to come to room temperature.

Food Specimens [3 4]

Detection method: According to ISO 11290-1:2017 [3], this process requires two selective enrichment steps in order to detect stressed *Listeria* spp. and inhibit contaminating flora respectively.

- Process the food specimen as required by local guidelines.
- Prepare a homogenised suspension using a ratio of 1:9 of sample to a primary enrichment medium such as E&O BM0647 Fraser Broth (Half Strength) and incubate at 30°C for 25 ± 1 hour.
- After incubation with primary enrichment medium, transfer a small sample of the specimen to a secondary enrichment medium such as E&O BM0640 Fraser Broth and incubate at 37°C for 24 ± 2 hours.
- After incubation with both primary and secondary enrichment medium, inoculate specimen directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker.
- Incubate the plates at 37 ± 1°C for 24 hours (further incubation up to 48 ± 2 hours may be required if colonies are not fully distinguished at 24 hours).

Enumeration method: According to ISO 11290-2:2017 [4], the initial suspension may require a series of dilutions as described in ISO 6887 (all parts) [6].

- Process the food specimen as required by local guidelines
- Prepare a homogenised suspension with an appropriate diluent according to the sample type *e.g.* E&O BM0082 Buffered Peptone Water (ISO).
- Inoculate 0.1ml of the suspension, and a decimal dilution series if required, onto the medium and streak across the agar surface using a sterile spreader or automated plate streaker.
- Incubate the plates at 37 ± 1°C for 24 ± 2 hours (further incubation up to 48 ± 2 hours may be required if colonies are not fully distinguished at 24 hours).

Identify presumptive positive isolates by examining any growth for coloured colonies (See table below):

Organism		Expected Colour
L. monocytogenes	→	Blue with halo
L. innocua	→	Blue without halo
E. faecalis	→	Inhibited
E. coli	→	Inhibited

Perform further biochemical, serological, or mass spectroscopy and antibiotic sensitivity testing to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

10. Quality Control

Organism	Incubation	Result (Specificity)
L. monocytogenes (NCTC 11994)	37 ± 1°C for 24 ± 2 hours	Growth: Blue/green colonies with halo
L. innocua (NCTC 11288)	37 ± 1°C for 24 ± 2 hours	Growth: Blue/green colonies with NO halo
E. coli (NCTC 12241)	37 ± 1°C for 24 ± 2 hours	Inhibited
E. faecalis (NCTC 12697)	37 ± 1°C for 24 ± 2 hours	Inhibited
S. cerevisiae (NCPF 3178)	37 ± 1°C for 24 ± 2 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

Colorex™ is a trademark of CHROMagar.

To fully verify PP7021 Colorex™Listeria (ISO) performance, 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 for 24 ± 2 hours. All samples showed expected recovery and morphology of the required test organism: *L. monocytogenes* (NCTC 11994) and *L. innocua* (NCTC 11288) and no recovery of the non-target test organisms: *Escherichia coli* (NCTC 12241), *E. faecalis* (NCTC 12697)

and *S. cerevisiae* (NCPF 3178). Therefore, it can be concluded that PP7021 Colorex™Listeria (ISO), meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

12. Limitations of the Medium

- Rare strains of *L. monocytogenes* may exhibit weak phosphatidyl inositol phospholipase C (PIPLC) activity so may produce a weak halo, or no halo, during the recommended incubation window (24-48 hours). Refer to confirmation methods in ISO 11290 [3 4] for further information.
- Some strains of *L. monocytogenes* exposed to stress conditions, particularly acid stress, may produce a very weak halo or no halo. Refer to confirmation methods in ISO 11290 [3 4] for further information.
- Some strains of *Listeria ivanovii* may also form blue colonies with white halo due to lecithinase activity, however, *L. ivanovii* is rarely found in food.
- Some strains of *Bacillus cereus* can also grow as blue-green colonies with a white halo. These can easily be distinguished from colonies of *L. monocytogenes* as they are much larger with an irregular edge to the colony and very large white halo.
- Biochemical and, if indicated, serological testing using pure cultures may be necessary for complete identification; these tests should be performed from a non-selective agar.
- 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 the E&O Poured Plate Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store PP7021 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 or if cracks in the agar medium are present. Additionally, do not use media if it has been stored inappropriately or the packaging has been damaged.

15. References

1. Ottaviani, F., Ottaviani, M., and Agosti M. (1997) Differential agar medium for *Listeria monocytogenes*. Quimper Froid Symposium Proceedings, P6 ADRIA Quimper, France, 16-18 June.
2. Ottaviani, F., Ottaviani, M.G., and Agosti, M. (1997). Esperienze su un agar selettivo e differenziale per *Listeria monocytogenes*. Industrie Alimentari, 36: 888-889.
3. International Organization for Standardization (2017) 11290-1:2017 Microbiology of the food chain – Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. – Part 1: Detection method. Geneva, ISO.
4. International Organization for Standardization (2017) 11290-2:2017 Microbiology of the food chain – Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. – Part 2: Enumeration method. Geneva, ISO.
5. 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.
6. International Organization for Standardization (2017) 6887:2017 Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – (All parts). Geneva, ISO.

Version History*

001 29/05/0023 - New Document Created
002 20/01/2024 – Updated Section 12: Limitations of the Medium

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

EC REP
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TABLE OF APPLICABLE SYMBOLS

REF o 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