EO Labs
Microbiological Culture Media

UK CA IVD CE

Instructions for Use

REF - PP3038

Colorex™ STEC

Pre-Poured Media Plates

1. Intended Use

For *in vitro* use only. Colorex™ STEC is a selective chromogenic medium [1] for the detection of Shiga Toxin producing *Escherichia coli* (STEC - including serotypes O26, O111 & O157).

2. Composition*

Ingredient	g/L
Peptones and yeast extract	8.0
Salts	5.2
Chromogenic mix	2.6
Agar	15.0
Selective Supplement	10.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Over the past few years there has been an increase in the number of food poisoning outbreaks where non-O157 Shiga toxin producing *E. coli* (STEC) have been shown to be the etiological agent. Illness triggered by Shiga toxin-producing *E. coli* (STEC) ranges from self-limiting diarrhoea to haemorrhagic colitis and haemolytic uremic syndrome (HUS). Colorex™ STEC is a chromogenic medium that allows for the efficient detection & presumptive identification of STEC in clinical specimens, foodstuffs, and environmental samples.

4. Principle

Positive STEC colonies utilise one of the chromogenic substrates to exhibit a mauve colouration enabling easy interpretation. Gram-negative bacteria will either be inhibited or exhibit colourless or blue colonies due to utilisation of a secondary chromogenic substrate. The medium contains several selective agents to reduce the level of background flora from the sample and Gram-positive bacteria will be inhibited.

5. Physical Characteristics

	Medium
Appearance and Colour	Straw firm gel
pH	6.9 ± 0.2

6. Materials Provided

PP3038 Colorex™ STEC is supplied in packs of 10 x 90mm P090 Petri dishes (18-22ml fill) (product code: PP3038-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™ STEC can be used with the following specimens:

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

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

9. Test Procedures and Interpretation of results

If the Colorex™ STEC agar plates have been refrigerated, allow to warm to room temperature before inoculation. Protect plates from direct light at all times.

Clinical specimens – Rectal swabs or stool specimens [2]

Based on clinical information, check to see if the specimen needs to be pre-enriched prior to inoculation to a selective agar medium.

- If required, add approximately 1g (pea-sized pellet) of faecal material to 10ml of modified Tryptone Soya Broth (mTSB – E&O Ref – BM2130) and incubate at 35-37°C for 18-24 hours.

- Inoculate specimen, or pre-enriched sample, directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker.
- Incubate in aerobic conditions at 35-37°C for 18-24 hours.
- Identify presumptive positive isolates by examining any growth for mauve coloured colonies (See table below).
- Perform further biochemical / serological testing to confirm identity of presumptive positive isolates. Serological agglutination tests can be performed directly from the colonies cultured on Colorex™ STEC. Refer to relevant local guidelines.
- Extraction for PCR testing to confirm presence of toxin genes can also be performed on colonies directly from the medium.

Food & environmental samples [3]

- Add a test portion of the sample, at a ratio of 1/10 (mass to volume), to pre-warmed modified Tryptone Soya Broth (mTSB).
- Incubate mTSB at 41.5°C ± 1°C for 6 hours then perform immunomagnetic separation (IMS). Re-incubate the mTSB for a further 12-18 hours if necessary.
- Inoculate 50µl of the washed and re-suspended IMS particles to cefixime tellurite sorbitol MacConkey (CT-SMAC - E&O Ref – PP1940) and Colorex™ STEC agar plates. Streak across the agar medium surface using a sterile loop or automated plate streaker.
- Incubate Colorex™ STEC in aerobic conditions at 35-37°C for 18-24 hours. Refer to standard methodology for CT-SMAC agar incubation conditions.
- Identify presumptive positive isolates by examining any growth for mauve coloured colonies on Colorex™ STEC (see table below).
- Perform further biochemical / serological testing to confirm identity of presumptive positive isolates. Serological agglutination tests can be performed directly from the colonies cultured on Colorex™ STEC. Refer to standard method or relevant local guidelines.

Microorganism	Typical Colony Appearance
STEC	Mauve
Other Enterobacteriaceae	Colourless, blue, or inhibited
Gram positive bacteria	Inhibited

Fluorescence under UV lamp (365nm)	
STEC O157	Non fluorescent
STEC non O157	+/- fluorescent

10. Quality Control

Organism	Incubation	Result (Specificity)
E. coli O157 (NCTC 12900)	37 ± 1°C aerobically for 18-24 hours	Growth: Mauve colonies
E. coli (NCTC 12241)	37 ± 1°C aerobically for 18-24 hours	Inhibited
S. aureus (NCTC 12981)	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 relevant local guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

11. Performance

Colorex™ is a trademark of CHROMagar. Scientific publications regarding the performance of the STEC medium with clinical specimens and food samples can be found at https://www.chromagar.com/en/product/chromagar-stec/

A clinical study evaluated the performance of the STEC medium to detect STEC in 329 stool samples. Sensitivity and specificity values were 91.4% and 86.7% respectively [4].

Analytical performance from a study examining performance versus 142 diarrheagenic *E. coli* isolates across 7 serotypes is shown in the table below [5]:

Serotype	Sensitivity - Analytical data	Serotype	Sensitivity - Analytical data
O157	96%	O121	80%
O26	100%	O145	100%
O45	86%		
O103	50%	**Specificity**	81%
O111	92%		

12. Limitations of the Media

- Some STEC could have a poor or no growth on this media.
- Some strains of non-STEC could appear as mauve colonies without fluorescence.
- Rare O157 are fluorescent positive; therefore, final confirmation as STEC must be done by appropriate methods.

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 PP3038 Colorex™ STEC in the original packaging between 2 - 8°C in the dark. Kept under these conditions, it may be used up to date of expiry shown on product label. A fine haze or precipitate may be present within the agar medium, but this does not affect the product performance.

Dispose of in accordance with local and national authority requirements. Do not use media if it is contaminated, discoloured, out of date or cracked. Additionally, do not use media if it has been stored inappropriately or the packaging has been damaged. A light haze or precipitate may be present, but this will not affect the performance of PP3038.

15. References

1. Tzschoppe, M., Martin, A. and Beutin, L. (2012) A rapid procedure for the detection and isolation of enterohaemorrhagic *Escherichia coli* (EHEC) serogroup O26, O103, O111, O118, O121, O145 and O157 strains and the aggregative EHEC O104:H4 strain from ready-to-eat vegetables. Int. J. Food. Microbiol. 152(1-2), p.19-30.
2. Public Health England (2020). Gastroenteritis. UK Standards for Microbiology Investigations (UK SMI) S 7 Issue 2.
3. ISO 16654:2001(en) Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Escherichia coli* O157.
4. Gouali *et al.* (2013) Evaluation of CHROMagar STEC and STEC O104 Chromogenic Agar Media for detection of Shiga Toxin-producing *Escherichia coli* in stool specimens. J Clin Micro. Vol. 51 (3), p. 894-900.
5. Lubeskie *et al.* (2016) Performance comparison of CHROMagar™ STEC and the Shiga Toxin QUIK CHEK™ assay using a panel of Shiga toxin *Escherichia coli* isolates. ECCMID Poster

Version History*

001 25/10/22 - New Document Created
002 12/11/24 - Update to Sections 6 and 14

*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

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

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