

Instructions for Use

REF - PP1080

Columbia Agar with 7% Horse Blood and CNA Supplement

Pre-Poured Media

1. Intended Use

For *in vitro* diagnostic use only. Columbia Agar with 7% Horse Blood and CNA Supplement is a selective medium for the isolation of *Staphylococcus* spp. and *Streptococcus* spp. from clinical specimens.

2. Composition*

<u>Ingredient</u>	<u>g/L</u>
Peptones	23.0
Starch	1.0
Colistin	0.01
Sodium chloride	5.0
Nalidixic acid	0.01
Agar	11.0
Defibrinated horse blood	70.0ml

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Columbia Agar with 7% Horse Blood and CNA Supplement is based on the original Columbia Agar formulation described by Ellner *et al.* from Columbia University ⁽¹⁾. Ellner *et al.* described Columbia CNA Agar as a variation of Columbia Blood Agar that is selective for Gram-positive cocci. The product is highly nutritious to support growth of fastidious organisms, and the presence of intact red blood cells allows determination of type and extent of haemolysis ⁽²⁾.

PP1080 is recommended as a selective primary isolation media by the UK Standards for Microbiology Investigations ^(2,3).

4. Principle

Supplementation of the base medium with defibrinated horse blood supplies additional growth factors for fastidious microorganisms, and aids in determining haemolytic reactions. Peptone is the source of the required nitrogen, carbon, and vitamins. Starch increases growth of species and enhances the haemolytic reactions of some *streptococci*. This medium is relatively free of reducing sugars which have been reported to adversely influence the haemolytic reactions of β -haemolytic streptococci ⁽⁴⁾. Colistin and nalidixic acid are added to be inhibitory to *Micrococcus* species as well as Gram-positive rods and Gram-negative bacteria. It suppresses growth of *Proteus*, *Klebsiella* and *Pseudomonas* species while permitting unrestricted growth of *S. aureus*, haemolytic streptococci, and enterococci.

5. Physical Characteristics

	Medium
Appearance and Colour	Cherry red firm gel
pH	7.3 \pm 0.2

6. Materials Provided

PP1080 Columbia Agar with 7% Horse Blood and CNA Supplement is supplied in packs of 10 x 90mm P090 Petri dishes (18-22ml fill) (product code: PP1080-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

PP1080 Columbia Agar with 7% Horse Blood and CNA Supplement is suitable for the testing of the following specimens:

- Clinical: swabs of the throat or other infected areas, blood, urine, skin, infected material, or nasal secretions

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, check to see if the specimen needs to be pre-enriched prior to inoculation to the prepared medium.

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

Clinical Specimens:

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 at $37 \pm 1^\circ\text{C}$ aerobically for 18-24 hours.

After incubation, examine agar for colonies, taking note of morphology and haemolysis (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>S. aureus</i> (NCTC 12981)	$37 \pm 1^\circ\text{C}$ aerobically for 18-24 hours	Growth: White colonies
<i>S. pyogenes</i> (NCTC 12696)	$37 \pm 1^\circ\text{C}$ aerobically for 18-24 hours	Growth: White colonies with β -haemolysis
<i>S. pneumoniae</i> (NCTC 12977)	$37 \pm 1^\circ\text{C}$ aerobically for 18-24 hours	Growth: Grey/green colonies with α -haemolysis
<i>S. epidermidis</i> (NCTC 13360)	$37 \pm 1^\circ\text{C}$ aerobically for 18-24 hours	Growth: White colonies
<i>P. mirabilis</i> (NCTC 10975)	$37 \pm 1^\circ\text{C}$ aerobically for 18-24 hours	Inhibited
<i>P. aeruginosa</i> (NCTC 12903)	$37 \pm 1^\circ\text{C}$ 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 PP1080 Columbia Agar with 7% Horse Blood and CNA Supplement performance, 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 and 10^4 - 10^5 cfu for the non-target organisms and incubated at $37 \pm 1^\circ\text{C}$ aerobically for 18-24 hours. All samples grew and showed good recovery and the correct morphology of the required test organisms: *Staphylococcus aureus* (NCTC 12981), *Streptococcus pyogenes* (NCTC 12696), *Streptococcus pneumoniae* (NCTC 12977) and *Staphylococcus epidermidis* (NCTC 13360), and inhibition of the non-target organisms: *Proteus mirabilis* (NCTC 10975) and *Pseudomonas aeruginosa* (NCTC 12903). Therefore, it can be concluded that PP1080 Columbia Agar with 7% Horse Blood and CNA Supplement, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

12. Limitations of the Media

- Additional biochemical, serological, or molecular tests should be performed on sub-culture isolates to confirm identity.
- 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 PP1080 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. Ellner, P., Stoessel, C., Drakeford, E. and Vasi, F. (1966) A New Culture Medium for Medical Bacteriology. Am J Clin Pathol, 45, 502-504.
2. Public Health England (2021) Introduction to the preliminary identification of medically important bacteria and fungi from culture. UK Standards for Microbiology Investigations. Bacteriology – Identification, ID 1(3). London: Standards Unit, Microbiology Services.
3. Public Health England (2021) Identification of *Streptococcus* species, *Streptococcus* species and morphologically similar organisms. UK Standards for Microbiology Investigations. Bacteriology – Identification, ID 4(4). London: Standards Unit, Microbiology Services.

4. Casman, E. (1947) A Noninfusion Blood Agar Base for *Neisseriae*, *Pneumococci* and *Streptococci*. Am J Clin Pathol, 17(4), 281-289.

Version History*

- 001 08/02/2023 - New Document Created
- 002 27/02/2025 - Updated to current standards, corrected typographical errors. *Escherichia coli* removed and *S. epidermidis* (NCTC 13360) added to Quality Control panel
- 003 31/03/2025 - Update based on new formatting and device file.











*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/PP1080 REV. 003

TABLE OF APPLICABLE SYMBOLS

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