

Instructions for Use

REF - PP0100

Chocolate Agar with 5% Horse Blood

Pre-Poured Media

1. Intended Use

For *in vitro* diagnostic use only. PP0100 Chocolate Agar with 5% Horse Blood is a highly nutritious medium which supports the growth of a wide range of pathogens including the most fastidious organisms and is particularly useful for the cultivation of *Haemophilus* and *Neisseria* species ^(1,2).

2. Composition*

<u>Ingredient</u>	<u>g/L</u>
Peptones	23.0
Starch	1.0
Sodium chloride	5.0
Agar	11.0
Defibrinated horse blood	50.0ml

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Chocolate Agar with 5% Horse Blood is based on the original Columbia Agar formulation described by Ellner *et al.* from Columbia University ⁽³⁾. Chocolate agar is the same as blood agar except that during preparation the red blood cells are lysed when added to molten agar base, which gives the medium a chocolate-brown colouration and from which the agar gets its name. As a result, cell lysis releases intracellular nutrients such as haemoglobin, haemin (X factor), and the coenzyme nicotinamide adenine dinucleotide (NAD or V factor) into the agar. These growth factors are available to fastidious organisms during growth. The most common bacterial pathogens that require this enriched medium for growth include *Neisseria gonorrhoeae* and *Haemophilus* species.

PP0100 is recommended as a non-selective primary isolation media by the UK Standards for Microbiology Investigations ^(1,2) and is formulated and tested in accordance with ISO 11133:2014 ⁽⁴⁾.

4. Principle

Peptone is the source of the required nitrogen, carbon, and vitamins. Starch increases growth of species and enhances the haemolytic reactions of some streptococci. The addition of chocolate (heated) horse blood allows for the incorporation of additional nutrients and growth factors into the agar medium to enhance recovery of some fastidious bacterial species.

5. Physical Characteristics

	Medium
Appearance and Colour	Brown firm gel
pH	7.3 ± 0.2

6. Materials Provided

PP0100 Chocolate Agar with 5% Horse Blood is supplied in packs of 10 x 90mm P090 Petri dishes (18-22ml fill) (product code: PP0100-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

PP0100 Chocolate Agar with 5% Horse Blood is suitable for the testing of clinical specimens. Samples may include swabs from the area of interest, biopsy material, pus, bone, and blood.

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations for the conservation of bacteria. 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.

Clinical Specimens ^(1,2)

Based on sample type and information provided, check to see if the clinical specimen needs to be pre-enriched prior to inoculation to PP0100. PP0100 may be used for direct inoculation with the sample or subculturing after incubation in enrichment broth.

- Process specimen as required by local guidelines.

- Inoculate specimen, or pre-enrichment sample, directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker.
- Incubate at $36 \pm 1^\circ\text{C}$ in 5% CO_2 for 24-48 hours.

After incubation, examine the agar for colonies (typical colony appearance is 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 local guidelines.

10. Quality Control

Organism	Incubation	Result (Specificity)
<i>S. pneumoniae</i> (NCTC 12977)	$37 \pm 1^\circ\text{C}$ in 5% CO_2 for 24-48 hours	Growth: Green colonies with α -haemolysis
<i>H. influenzae</i> (NCTC 12699)	$37 \pm 1^\circ\text{C}$ in 5% CO_2 for 24-48 hours	Growth: Grey colonies
<i>N. gonorrhoeae</i> (NCTC 8375)	$37 \pm 1^\circ\text{C}$ in 5% CO_2 for 24-48 hours	Growth: Grey colonies

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

To fully verify PP0100 Chocolate Agar with 5% Horse Blood performance, samples were tested to assess colony morphology and recovery level (where an acceptable range is $\geq 70\%$ and $\leq 120\%$) compared to a non-selective reference medium. Samples were inoculated with 30-150cfu and incubated at $37 \pm 1^\circ\text{C}$ in 5% CO_2 for 24-48 hours. All samples showed expected recovery and morphology of the required test organisms: *Streptococcus pneumoniae* (NCTC 12977), *Haemophilus influenzae* (NCTC 12699) and *Neisseria gonorrhoeae* (NCTC 8375). Therefore, it can be concluded that PP0100 Chocolate Agar with 5% Horse Blood, meets performance criteria when used according to the instructions outlined above. Trend analysis data are available on request.

12. Limitations of the Media

- It may be difficult to differentiate between target and non-target organisms using only chocolate agar since non-target organisms may overgrow organisms of interest. Subculture to selective agar may be required.
- Full confirmation of presumptively identified organisms may be obtained using biochemical, immunological, molecular, or mass spectrometric means.

13. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSSH 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 PP0100 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. UK Standards for Microbiology Investigations (2021). Identification of *Haemophilus* species and the HACEK group of organisms. ID 12, Issue 4.
2. UK Standards for Microbiology Investigations (2015). Identification of *Neisseria* species. ID 6, Issue 3.
3. Ellner, P., Stoessel, C., Drakeford, E. and Vasi, F. (1966) A New Culture Medium for Medical Bacteriology. Am J Clin Pathol, 45, 502-504.
4. 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.

Version History*

001 09/03/22 - New Document Created

*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	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place