Instructions for Use

REF - KM0068 Sabouraud Dextrose Agar with Chloramphenicol (50mg/L)

Dehydrated Culture Media

1. Intended Use

For *in vitro* diagnostic use. KM0068 Sabouraud Dextrose Agar with Chloramphenicol (50mg/L) is a selective medium for the isolation of yeasts and fungi suitable for use in all areas of mycology.

2. Composition*

Ingredient	g/L
Tryptone	5.0
Meat peptone	5.0
Glucose	40.0
Agar	15.0
Chloramphenicol	0.05

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Sabouraud Dextrose Agar with Chloramphenicol (50mg/L) is a modification of a medium originally described by Sabouraud [1] and is used in qualitative procedures for cultivation of dermatophytes and for the isolation of yeasts and fungi. Sabouraud Dextrose Agar with Chloramphenicol (50mg/L) can be used for isolation of yeast and fungi from clinical specimens, environmental and cosmetic samples. Fungi produce typical cultural appearance on the agar and may be readily identified according to standard macroscopic characteristics.

4. Principle

The peptone supplies the required nitrogen and other essential nutrients. The high concentration of glucose provides an energy source for yeasts and fungi and, along with the acidic pH (5.4), helps inhibit bacterial growth. Chloramphenicol is a broad-spectrum antimicrobial that is included to increase the selectivity of the medium inhibiting a wide range of Gram-positive and Gram-negative bacteria.

5. Preparation Instructions

Suspend 65.0g of dehydrated culture medium in 1 litre of deionised/purified water and mix for 10 minutes.
Autoclave to sterilise at 121°C for 15 minutes before cooling in a water bath to 45-50°C.
Aseptically dispense the specified volume into appropriate sterile containers and allow to cool.

Avoid overheating this medium as the combination of heat and acidic pH may result in a softer agar.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Straw fine powder	Straw firm gel
pH	N/A	5.6 ± 0.2

7. Materials Provided

KM0068 is available in the formats detailed below. Each tub is ink-jet printed with (abbreviated) product name, product code, lot number and expiry date.

Product Code	Product Format
KM0068-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0068-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0068-10KG-10000	1 x 10kg Dehydrated Culture Media Tub

8. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., sterile loops or swabs, collection containers, incubators, and quality control organisms.

9. Specimens

KM0068 is suitable for testing many specimen types including the following:

- Clinical: Skin swabs taken from infected locations, skin flakes or scrapings, hair, or nail samples [2].
- Cosmetics: Finished product, raw materials, environmental testing of surfaces and equipment [3,4].
- Food: products for human consumption and animal feed [5].

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.

10. Test Procedures and Interpretation of results

Allow media to come to room temperature.

Follow procedure recommended for sample type (see Section 9).

Inoculate agar surface directly with the sample or after fragmentation and or dilution. To inoculate, thoroughly streak the surface of the medium using a swab or sterile loop. Refer to local or national protocols and guidelines based on sample type as the sample preparation and inoculation procedure may vary with the specimen.

Incubate between 25-30°C aerobically or as applicable to the type of sample and suspected fungal species.

Examine the plates at 24 and 48 hours for yeast and every 2 to 3 days for fungi.

Confirm the identity of isolates by performing appropriate identification tests as required.

11. Quality Control

Organism	Incubation	Result (Specificity)
*T. rubrum (*NCPF 419)	Yeasts and *E. coli*: Incubate aerobically at 25 ± 1°C for 48 hours Fungi: Incubate aerobically at 25 ± 1°C for 5-10 days	Growth: White fluffy colonies with dark red/brown underside
A. brasiliensis (NCPF 2275)		Growth: White colonies with black pigmentation, pale yellow underside
C. albicans (NCPF 3179)		Growth: White colonies
A. fumigatus (DSMZ 819)		Growth: White colonies with blue/green pigmentation, pale yellow underside
S. cerevisiae (NCTC 3178)		Growth: White colonies
E. coli (NCTC 12241)		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).

12. Performance

To fully verify KM0068 Sabouraud Dextrose Agar with Chloramphenicol (50mg/L) performance, dehydrated culture media samples were used to prepare Sabouraud Dextrose Agar with Chloramphenicol (50gm/L) and tested to assess colony morphology and recovery level compared to a reference medium. Samples were inoculated with target organisms and non-target organisms then plates were incubated at 25 ± 1°C aerobically for 48 hours for yeasts and *E. coli* and at 25 ± 1°C aerobically for 5-10 days for fungi. All samples showed expected recovery and morphology of the required test organisms: *Trichophyton rubrum (*NCPF 419), *Aspergillus brasiliensis* (NCPF 2275), *Candida albicans* (NCPF 3179), *Aspergillus fumigatus* (DSMZ 819) and *Saccharomyces cerevisiae* (NCTC 3178) and no recovery of the non-target test organism: *Escherichia coli* (NCTC 12241). Therefore, it can be concluded that KM0068 Sabouraud Dextrose Agar with Chloramphenicol (50mg/L), meets performance criteria when used according to the instructions outlined above. Trend analysis data is available upon request.

13. Limitations of the Media

- Due to variation in nutritional requirements, some strains may grow poorly on this medium.
- KM0068 does not promote the conidiation of filamentous fungi.
- Chloramphenicol added to the medium to inhibit bacteria may also inhibit certain pathogenic fungi.
- Additional biochemical, mass spectrometry or molecular tests may be required to confirm identity of isolated organisms.

14. Precautions and Warnings

This product is considered hazardous under CLP regulations. Refer to KM0068 Material Safety Data Sheet. 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.

15. Storage conditions and Shelf life

Store product in the original container with the lid tightly closed at between 10 and 30°C in low humidity conditions away from direct sunlight. Kept under these conditions, the product may be used up to the date of expiry shown on the product label.

Do not use if the product is not free-flowing or displays any sign of colour change, formation of large lumps or hardening of the powder. Additionally, do not use medium if it has been stored inappropriately, the packaging has been damaged or has passed the expiry date.

Dehydrated culture media does not need to be used all at once; replace the cap and ensure that the container is tightly closed and stored according to labelled instructions.

Dispose of in accordance with local and national authority requirements.

16. References

1. Sabouraud, R. (1892) Contribution a l'etude de la trichophytie humaine. Etude clinique, microscopique et bacterioloqique sur la pluralité des trichophytons de l'homme. Annals of Dermatology and Syphiligraphy, 3, pp.1061-1087.

2. UK Standards for Microbiology Investigations (2016). Investigation of dermatological specimens for superficial mycoses. SMI B39 Issue 3.1.

3. Curry, A. S., J. G. Graf, and G. N. McEwen, Jr. (eds.). (1993). CTFA Microbiology Guidelines. The Cosmetic, Toiletry, and Fragrance Association, Washington, D.C.

4. ISO 16212:2017. Cosmetics – Microbiology – Enumeration of yeasts and moulds. Geneva; ISO.

5. APHA Technical Committee on Microbiological Methods for Foods. Compendium of Methods for the Microbiological Examination of Foods, APHA, Washington, D.C.

Version History*

001 07/08/23 - New Document Created
002 04/10/23 - Updates made to section 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	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place