Instructions For Use

ENGLISH

Cetrimide Agar

Medium for isolation and differentiation of *Pseudomonas aeruginosa*.

INTENDED PURPOSE

Selective medium for detection of *Pseudomonas aeruginosa* in various types of material, according to USP/EP/JP. This medium is intended as an aid in the diagnosis, requiring further tests to complete the diagnostic results.

DESCRIPTION

Cetrimide Agar is a selective medium for the isolation and identification of *Pseudomonas aeruginosa* from samples of various origins, such as clinical specimens and pharmaceutical products. Bacterial detection on this medium is based on an enhanced production of a number of water-soluble iron chelators, including pyocyanin and pyoverdin.

Its formula complies with the performance requirements in the harmonized chapters of the European, United States, and Japanese Pharmacopoeias.

TYPICAL FORMULA*	(g/litre)
Pancreatic Digest of Gelatin	20.0
Magnesium Chloride	1.4
Dipotassium sulfate	10.0
Cetrimide	0.3
Agar	13.6
Glycerol	10.0 ml
Final pH 7.2 ± 0.2 at 25°C	

*Adjusted and/or supplemented as required to meet performance specifications.

METHOD PRINCIPLE

Pancreatic digest of gelatin provides amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Magnesium chloride and potassium sulfate enhance the production of water-soluble pigments, pyoverdine (fluorescein) and pyocyanin, which combine to yield the bright green color characteristic of *P. aeruginosa*. Cetrimide (cetyltrimethylammonium bromide) is the selective agent with bactericidal activity against a broad range of Gram-positive organisms and some Gram-negative bacteria. Agar is the solidifying agent. Glycerol is a source of carbon.

PREPARATION

Dehydrated medium

Suspend 46.7 g of the powder in 990 ml of distilled or deionized water. Add 10 ml of Glycerol Supplement. Mix well. Heat to boil until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes.

Medium in bottles

Melt the content of the bottle in a water bath at 100°C (losing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as: Autoclave, water bath, sterile Petri plates, test tubes, inoculating loops, swabs, incubator, quality control organisms.

SPECIMENS

Clinical specimens should be sampled at the acute stage, before antimicrobial therapy (where possible) and examined as soon as possible after collection.
Good laboratory practices for collection, transport and storage of the clinical specimens should be applied. Refer to specific guidelines for more information about specimen collection and preparation.

TEST PROCEDURE

Ensure there is no visible moisture on the plates before use.

For use in medical microbiology

Inoculate the plates by directly streaking the specimen on the agar surface or spread the sample from an enrichment culture to obtain well-isolated colonies.

Incubate aerobically at 32.5 ± 2.5°C for 18-72 h.

Note: Incubation time and temperature vary according to the type of specimen and the microorganisms being tested for. The user is responsible for choosing the appropriate parameters for the intended use, in accordance with current standards.

For use in industrial bacteriology:

Following the harmonized USP/EP/JP method for microbiological examination of non-sterile products, inoculate the sample in Tryptic Soy Broth, then subculture on a Cetrimide Agar plate and incubate at 30-35°C for 18-72 hours.

For more details, consult appropriate guidance.

INTERPRETING RESULTS

Pseudomonas aeruginosa cultivates with yellow-green to blue-green colonies which fluoresce under UV light.

Pigment production along with a positive oxidase reaction typically identify *P. aeruginosa*.

However, some strains of *P. aeruginosa*, particularly the mucoid ones, may not produce pyocyanin, as well as displaying a slow oxidase reaction and may therefore require further tests to confirm identification.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in bottles: 2 years.
Ready-to-use plates: 6 months.

QUALITY CONTROL

Appearance of Glycerol Supplement: Dense colorless substance of oily appearance.

Appearance of Dehydrated Medium: Free-flowing, homogeneous, light-beige.

Appearance of Prepared Medium: Slightly opalescent, light amber with precipitate.

Expected Cultural Response:

Control strain		Inoculum	Incubation	Criteria	Specification
Pseudomonas aeruginosa	WDCM 00026 (ATCC® 9027; NCTC 12924)	50-100 CFU	18-72 h / 32.5 ± 2.5°C	Good growth ($P_R \geq 0.5$)	Yellow green to blue-green colonies
Pseudomonas aeruginosa	WDCM 00024 (ATCC® 10145; NCTC 10332)				Yellow green to blue-green colonies
Pseudomonas aeruginosa	WDCM 00025 (ATCC® 27853; NCTC 12903)				Yellow green to blue-green colonies
Escherichia coli	WDCM 00012 (ATCC® 8739; NCTC 12923)	10^4-10^6 CFU	72 h / 32.5 ± 2.5°C	Inhibition	—-
Escherichia coli	WDCM 00013 (ATCC® 25922; NCTC 12241)			Inhibition	—-
Staphylococcus aureus	WDCM 00193 (ATCC® 6538; NCTC 10788)			Inhibition	—-
Staphylococcus aureus	WDCM 00034 (ATCC® 25923; NCTC 12981)			Inhibition	—-

A recovery rate (P_R) of 0.5 is equivalent to a recovery rate of 50%.

Please refer to the actual batch related Certificate of Analysis (CoA).

PERFORMANCE CHARACTERISTICS

Performance testing of Cetrimide Agar was carried out using the QC strains listed above. The results obtained met the established criteria.

LIMITATIONS

Invalid results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology. The operator should understand the principles of the procedures, including its performance limitations, in advance of operation to avoid potential mistakes.

Growth depends on the requirements of each individual microorganism. It is therefore possible that certain strains which have specific requirements (substrate, temperature, incubation conditions, etc.) may not develop.

Occasionally some enteric organisms (e.g *Klebsiella*, *Enterobacter*, *Citrobacter*, *Proteus*) will exhibit a slight yellowing of the medium; however, this coloration is easily distinguished from fluorescein production because this yellowing does not fluoresce.

Additional testing such as biochemical tests and serological procedures should be performed to confirm the findings and a diagnosis of *P. aeruginosa*.

Cetrimide Agar is intended as an aid in the diagnosis of infectious diseases, requiring further tests to complete the diagnostic results.

WARNING AND PRECAUTIONS

1) **For *in vitro* diagnostic use (IVD).**
2) **For laboratory professional use only.**
3) Operators must be trained and have certain experience. Please read the instructions carefully before using the product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.
4) Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.
5) Do not use if the product or packaging appears to be damaged.
6) Follow standard precautions. All patient specimens should be considered potentially infectious and handled accordingly.
7) Handle all specimens as if infectious using safe laboratory procedures. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
8) Avoid cross-contamination of samples by using disposable tips and changing them after each sample.
9) Do not mix reagents of different batches. Please use the product within the validity period.
10) Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled
11) Results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms, and epidemiological risk factors.
12) Ensure laboratory equipment is calibrated and maintained in accordance with the laboratory's procedure.
13) When test results are transmitted from the laboratory to an informatics centre, attention has to be done to avoid erroneous data transfer.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

See ordering info on the next page. There may be additional product ref. numbers as well. For an updated listing of available products, visit **liofilchem.com**

Product	Format	Packaging	Ref.
Cetrimide Agar	Plate 90 mm	20 plates	10033
	Bottle	6 x 100 ml	402270
		6 x 200 ml	412270
	Dehydrated media	100 g	620041
		500 g	610041
		5 kg	6100415
Glycerol Supplement	Bottle	4 x 50 ml	80021

Revision History

Revision	Release Date	Change Summary
0	2024-06-07	Updated layout and content in compliance with IVDR 2017/746, version reset to revision 0

In case of malfunctions or defects, contact immediately Liofilchem (*) or the local representative.

In case of incident associated with the device, notify immediately Liofilchem (*) or its local representative and the National Competent Authority.

*Please login to https://www.liofilchemstore.it/login.php (user ID and password required) and click on Complaint.

This IFU document and the SDS are available from the online Support Center:

liofilchem.com/ifu-sds