AG – CETRIMIDE AGAR - INSTRUCTIONS FOR USE

(Ready Plated Media)

- **INTENDED USE:**

For the isolation and enumeration of *Pseudomonas* species in water, food products, and environmental samples.

- **PRINCIPLE:**

Pseudomonas auroginosa: bright yellow green colour

Pseudomonas spp. are aerobic, rod-shaped bacteria that do not form spores. These Gram-negative organisms are commonly found in water, soil, vegetation, and humid environments. *Pseudomonas aeruginosa* is particularly versatile, able to grow in distilled water, survive in disinfectants, contaminate cosmetics, and persist in food products.

Pseudomonas Selective Agar is specifically designed for isolating and identifying *P. aeruginosa*. This medium builds on the work of King, Ward, and Raney in 1954, who developed formulations to enhance the production of key pigments, pyocyanin and fluorescein. The addition of cetrimide, refined by Lowbury and Collins, helps inhibit other microorganisms, ensuring the selective growth of *Pseudomonas*.

This agar meets international standards, including those set by the European, United States, and Japanese Pharmacopeias, as well as ISO 22717 and FDA-BAM, making it suitable for detecting *P. aeruginosa* in cosmetics, non-sterile pharmaceuticals, water, and food samples.

Key ingredients include:
- Pancreatic digest of gelatin: Provides essential nutrients for bacterial growth and pigment production.
- Cetrimide: A detergent that disrupts bacterial membranes, effectively inhibiting non-*Pseudomonas* species.
- Magnesium chloride and potassium sulfate: Stimulate the production of the pigments fluorescein and pyocyanin.
- Glycerol: Acts as a carbon source and promotes pigment synthesis.

This medium allows easy identification of *P. aeruginosa* based on its characteristic pigment production and colony appearance, making it a dependable choice for microbiological testing across various industries.

- **MATERIALS PROVIDED:**

PRODUCT	TYPE	REF	PACK
AG – CEA Agar - 90mm	Ready Plated Media	AG/CEA/22/01	10 plates in a pack

- **MATERIALS REQUIRED BUT NOT PROVIDED:**

Sterile loops, incubator, and laboratory equipment as required.

- **SPECIMENS:**

Pseudomonas Selective Agar can be used to directly test clinical samples from non-sterile sites, such as respiratory secretions, wounds, ear swabs, eye samples, and urine. For accurate results, collect samples before starting antibiotics and handle them properly during collection, transport, and storage. It's also ideal for testing non-clinical samples like cosmetics and non-sterile pharmaceutical products. Follow established guidelines for sample preparation and handling.

- **TEST PROCEDURE:**

  - Allow plates to reach room temperature.
  - Ensure the agar surface is smooth and moist but not excessively wet.
  - For loop inoculation: Streak the specimen across four quadrants of the plate to achieve isolated colonies, avoiding overlap between sections 1 and 4.

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- For swab inoculation: Roll the swab over a small edge area of the plate, then streak from this inoculated area using a loop.
- Incubate plates aerobically at 35–37°C.
- Record results after 18–24 hours. If no typical colonies are observed, re-incubate for an additional 24–36 hours (up to 72 hours total).
- For cystic fibrosis samples, incubate solid media at 35–37°C for up to 5 days, as some strains grow slowly.

Detection of P. aeruginosa in Non-Sterile Pharmaceutical Products (European Pharmacopoeia Method):

- Prepare a 1:10 dilution of at least 1 g of the sample in Tryptic Soy Broth (TSB).
- Use 10 mL or the quantity corresponding to 1 g/mL of the product.
- Mix and incubate at 30–35°C for 18–24 hours.
- Subculture on Pseudomonas Selective Agar and incubate at 30–35°C for 18–72 hours.
- The presence of colonies suggests potential P. aeruginosa, which must be confirmed through identification tests.
- Detection in Cosmetics:
- Follow specific guidelines and consult references for sample preparation and analysis.

• OBSERVATION AND IDENTIFICATION OF *PSEUDOMONAS* SPECIES

a- Observe and document colony morphology and color.
b- Growth indicates possible *Pseudomonas* species.

2. **Fluorescence Test (UV Light, 254 nm)**:
   Positive: Bright yellow-green fluorescence in agar around the colonies (*P. aeruginosa*, *P. fluorescens*, *P. putida*).
3. **Pigment Production (Normal Light)**:
   a- **Pyocyanin**: Blue or greenish-blue colonies.
   b- **Fluorescein**: Yellow-green colonies.
   c- **Pyorubin**: Light pink, red, or dark brown colonies (may co-occur with pyocyanin and/or fluorescein).
   d- Pyocyanin and fluorescein combine to produce the characteristic bright green of *P. aeruginosa*.
4. **Key Features of *P. aeruginosa***:
   a- Bright green pigmentation.
   b- Distinct colony morphology.
   c- Grape-like odor due to aminoacetophenone production.

• USER QUALITY CONTROL:

All manufactured lots of the products are released for sale after Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for quality control.

CONTROL STRAINS	INCUBATION	EXPECTED RESULTS
P.aeruginosa ATCC 27853	36 ± 2˚C, 18-24 hrs	Bright yellow-green fluorescence in agar around the colonies
Escherichia coli ATCC 25922	36 ± 2˚C, 18-24 hrs	Inhibited Growth

Key: ATCC is a trademark of American Type Culture Collection

• LIMITATIONS OF THE METHOD:

a- Fluorescence Loss: P. aeruginosa fluorescence under UV light may fade at room temperature but can reappear with re-incubation.
b- Delayed Reactions: Mucoid *P. aeruginosa* strains may show delayed oxidase positivity, requiring further confirmation.
c- Interpretation Dependence: Results must be evaluated in the context of clinical history, sample origin, and other diagnostic findings.

• PRECAUTIONS AND WARNINGS:

- This product is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.

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- Sterilize all biohazard waste before disposal. Dispose of the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product are available with AstraGene and can be provided on request.

- **STORAGE CONDITIONS AND SHELF LIFE:**
  - Upon receipt, store at +2 - 8°C away from direct light in a cool, dry place. The user is responsible for the storage method (temperature) of the medium.
  - If properly stored, the product may be used up to the expiration date. Do not use it beyond the mentioned expired date.

- **SYMBOLS:**

	Date of manufacture		Use-by-date		Do not use if package is damaged		Manufacturer
	Batch Code		Refer to the instructions		ISO		GMP
	*In-Vitro* diagnostic Medical devices		Mark of conformity				

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