

## UPUTSTVO ZA UPOTREBU

(SRB)

### Cetrimide Agar Plate

Podloga za selektivnu izolaciju *Pseudomonas aeruginosa* iz kliničkih uzoraka.

#### Sadržaj pakovanja:

Šifra artikla (pakovanja) REF	Opis	Šifra primarnog pakovanja:	Broj podloga
PRM024V20	Podloga izlivena u petri posudama od Ø90	PRM024	20
PRM024V60			60
PRM024V240			240
PRM024M40	Podloga izlivena u petri posudama od Ø50		40

#### Uputstva

Pod aseptičnim uslovima inkulirati ploču metodom površinskog zasejavanja. Nakon inkubacije posmatrati rast i boju kolonija, pigmentaciju podloge i fluorescenciju kolonija.

#### Princip i interpretacija

*Pseudomonas aeruginosa* dobro raste na svim normalnim laboratorijskim podlogama, ali specifčna izolacija organizma iz životne sredine ili ljudi, životinja i biljaka, najbolje se izvodi na podlozi koja sadrži selektivni agens, kao i sastojke za poboljšanje proizvodnje pigmenta. Selektivnost većine podloga zavisi od otpornosti vrste na različite antibakterijske agense. Cetrimid inhibira rast mnogih mikroorganizama dok omogućava da *Pseudomonas aeruginosa* razvije tipične kolonije.

Cetrimid je kvaterna amonijumova so koja deluje kao katjonski deterdžent koji smanjuje površinski napon u tački kontakta i ima precipitantne, kompleksirajuće i denaturišuće efekte na proteine bakterijske membrane. On pokazuje inhibitorne efekte na širok spektar raznovrsnih mikroorganizama uključujući i vrste *Pseudomonas*, osim *Pseudomonas aeruginosa*. King i sar. su razvili Medium A za poboljšanje proizvodnje piocijanina od strane *Pseudomonas* spp. (1). Cetrimid Agar razvijen od strane Lowbury (2) je modifikacija Tech Agar (Medium A) sa dodatkom 0,1% cetrimida za selektivnu izolaciju *P. aeruginosa*. Kasnije, zbog dostupnosti visoko prečišćenog cetrimida, njegova koncentracija u podlozi je smanjena (3). Inkubacija je bila izvedena na 37°C u vremenskom period od 18-24 sata (4).

*P. aeruginosa* se može identifikovati na osnovu svojih karakteristika: proizvodnja piocijanina, plava boja, rastvorljivost u vodi, nefluorescentnost fenazinskog pigmenta kupovan sa njihovom kolonijalnom morfolologijom i karakterističan miris nalik na grožđe od amino-acetofenona (5). *P. aeruginosa* je jedina vrsta *Pseudomonasa* ili Gram-negativnih robova za koje je poznato da izlučuju piocijanin. Ova podloga je stoga, važna u identifikaciji *P. aeruginosa*. Ova podloga se koristi za ispitivanje kozmetike (6) i kliničkih uzoraka (5,7) na prisustvo *P. aeruginosa*, kao i za procenu efikasnosti dezinfekcionih sredstava protiv ovog organizma (8).

Pankreasni hidrolizat želatin obezbeđuje neophodne hranljive materije za *P. aeruginosa*. Natrijum hlorid održava osmotsku ravnotežu u podlozi. Magnezijum hlorid i kalijum sulfat stimulišu proizvodnju piocijanina (9). Za izolaciju *P. aeruginosa*, ploče Cetrimid Agar treba inkulirati iz neselektivne podloge kao što je Brain Heart Infusion ili Soyabean Casein Digest Medium. Ako je broj veliki, Cetrimide Agar se može direktno inkulirati uzorkom. Kolonije *P. aeruginosa* mogu izgledati pigmentisano plavo, plavo zeleno ili nepigmentisano. Kolonije koje pokazuju fluorescenciju na 250 nm i plavo zelenu pigmentaciju se smatraju verovatno pozitivnim.

*P. aeruginosa* može izgubiti svoju fluorescenciju pod UV zračenjem ako se kulture ostave na sobnoj temperaturi u kratkom vremenskom periodu. Fluorescencija se ponovo pojavljuje nakon što se ploče ponovo inkubiraju (4). Tip peptona koji se koristi u podlozi može takođe uticati na proizvodnju pigmenta (4,10). Određeni sojevi *P. aeruginosa* mogu da ne proizvode piocijanin. Druge vrste roda *Pseudomonas* ne proizvode piocijanin, ali fluoresciraju pod UV svetlošću. Većina ne-pseudomonas vrsta su inhibirane na Cetrimide Agar, kao i neke *Pseudomonas* vrste. Neki ne-fermentativni organizmi i neke aerobne sporogene bakterije mogu pokazati vodorastvornu tamnu do braon pigmentaciju na ovoj podlozi.

Vrste roda Serratia mogu pokazati ružičastu pigmentaciju (3). Biohemijski testovi i serološke procedure je potrebno sprovesti kako bi se potvrdili nalazi.

#### Kontrola kvaliteta

Podaci i rezultati kontrole kvaliteta dati su u sertifikatu analize za svaku seriju.

#### Skladištenje i rok upotrebe

Čuvati između 15-25°C. Nakon prvog otvaranja čuvati na 2-8°C. Upotrebiti pre isteka datuma označenog na nalepnici.

#### Mere predostrožnosti

Ovaj proizvod ne sadrži hazardne supstance u koncentracijama koje su iznad propisanih limita određenih važećim zakonskim regulativama i zato nije klasifikovan kao opasan. Ipak, preporučeno je slediti smernice iz bezbednosnog lista za pravilnu upotrebu. Ovaj proizvod je namenjen isključivo za upotrebu u laboratorijskim uslovima, od strane profesionalno obučene osobe.

Proizvod ne upotrebljavati ukoliko je primarno pakovanje oštećeno ili proizvod ne odgovara navedenim karakteristikama.

#### Odlaganje otpada

Odlaganje otpada mora biti u skladu sa nacionalnim i lokalnim regulativama koje su na snazi. Svaka laboratorija je odgovorna za rukovanje i odlaganje otpada koji nastaje u toku rada.

#### Upotrebljeni simboli

<b>CE</b>	Evropski znak usaglašenosti		Držati uspravno
<b>IVD</b>	In vitro dijagnostičko medicinsko sredstvo	<b>REF</b>	Kataloški broj
	Ne izlagati direktno sunčevim zracima	<b>LOT</b>	Lot broj
	Konsultovati uputstvo za upotrebu		Rok upotebe
	Ne koristiti više puta		Temperatura čuvanja
	Veličina pakovanja		Proizvođač
<b>EC REP</b>	Ovlašćeni predstavnik u Evropskoj uniji		
<b>EC REP</b>	Salus Cons kft. 6722 Szeged, Bécsi krt 23, HUNGARY e-mail: office@saluscons.com		

#### Literatura

- King, Ward and Raney, 1954, J. Lab. Clin. Med., 44:301.
- Lowbury, 1951, J. Clin. Pathol., 4:66.
- Lowbury and Collins, 1955, J. Clin. Pathol., 8:47
- Brown and Lowbury, 1965, J. Clin. Pathol., 18:752.
- Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H. (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
- USFDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.
- Forbes B. A., Sahm A. S. and Weissfeld D. F., Bailey & Scotts Diagnostic Microbiology, 10th Ed., 1998, Mosby, Inc., St. Louis, Mo.
- Williams, (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC, Washington, D.C.
- MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification - Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
- Goto and Enomoto, 1970, Jpn. J. Microbiol., 14:65..

Broj rešenja o registraciji: 515-02-02534-22-003

## INSTRUCTION FOR USE

(EN)

### Cetrimide Agar Plate

Medium is used for the selective isolation of *Pseudomonas aeruginosa* from various specimens.

#### Package contents:

Item code (packaging) REF	Description	Primary packaging code:	Number of products
PRM024V20	Substrate poured into petri dishes of Ø90	PRM024	20
PRM024V60			60
PRM024V240			240
PRM024M40			40

#### Directions

Surface spread the test inoculum aseptically on the plate. After incubation, observe growth, color of colonies and fluorescence of colonies under UV light.

#### Principle and interpretation

*Pseudomonas aeruginosa* grows well on all normal laboratory media but specific isolation of the organism, from environmental sites or from human, animal or plant sources, is best carried out on a medium, which contains a selective agent and also constituents to enhance pigment production. Most selective media depend upon the intrinsic resistance of the species to various antibacterial agents. Cetrimide inhibits the growth of many microorganisms whilst allowing *Pseudomonas aeruginosa* to develop typical colonies.

Cetrimide is a quaternary ammonium salt, which acts as a cationic detergent that reduces surface tension in the point of contact and has precipitant, complexing and denaturing effects on bacterial membrane proteins. It exhibits inhibitory actions on a wide variety of microorganisms including *Pseudomonas* species other than *Pseudomonas aeruginosa*. King et al developed Medium A for the enhancement of pyocyanin production by *Pseudomonas* (1). Cetrimide Agar developed by Lowbury (2) is a modification of Tech Agar (Medium A) with addition of 0,1 % cetrimide for selective isolation of *P. aeruginosa*. Later, due to the availability of the highly purified cetrimide, its concentration in the medium was decreased (3). The incubation was carried out at 37°C for a period of 18-24 hours (4). *P. aeruginosa* can be identified due to their characteristic production of pyocyanin, a blue, water-soluble, non-fluorescent phenazine pigment coupled with their colonial morphology and the characteristic grape-like odor of aminoacetophenone (5). *P. aeruginosa* is the only species of *Pseudomonas* or Gram-negative rod known to excrete pyocyanin. These media are therefore, important in the identification of *P. aeruginosa*. These media are used for the examination of cosmetics (6) and clinical specimens (5, 7) for the presence of *P. aeruginosa*, as well as for evaluating the efficacy of disinfectants against this organism (8).

Pancreatic digest of gelatin provide necessary nutrients for *P. aeruginosa*. Sodium chloride maintains osmotic equilibrium in the medium. Magnesium chloride and potassium sulphate stimulates pyocyanin production (9).

For the isolation of *P. aeruginosa*, plates of Cetrimide Agar should be inoculated from non-selective medium such as Brain Heart Infusion Broth or Soyabean Casein Digest Medium. If the count is high, the test sample can be directly inoculated onto Cetrimide Agar. *P. aeruginosa* colonies may appear pigmented blue, blue-green or non-pigmented. Colonies exhibiting fluorescence at 250nm and a blue green pigmentation are considered as presumptive positive.

*P. aeruginosa* may lose its fluorescence under UV if the cultured are left at room temperature for a short time. Fluorescence reappears after the plates are re-incubated (4). Type of peptone used in the base may also affect pigment production (4, 10).

Certain strains of *P. aeruginosa* may not produce pyocyanin. Other species of *Pseudomonas* do not produce pyocyanin but fluoresce under UV light.

Most non-*Pseudomonas* species are inhibited on Cetrimide Agar, and some species of *Pseudomonas* may also be inhibited. Some non-fermenters and some aerobic spore formers may exhibit a water-soluble tan to brown pigmentation on this medium. *Serratia* may exhibit pink pigmentation (3). Biochemical tests and serological procedures should be performed to confirm the findings.

#### Quality control

The data and results of quality control are given in the certificate of analysis for each lot.

#### Storage and shelf life

Storage between 15-25°C. After opening storage between 2-8°C. Use before expiry date on the label.

#### Warning and precautions

In vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

#### Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

#### Symbols used on labels

	European Conformity mark		This side up
	is an in vitro diagnostic medical device (IVD)		Catalogue number
	Do not expose directly to sunlight		Batch code
	Consult instructions for use		Use-by date
	Do not re-use		Temperature limit
	Pack size		Manufacturer
	European Authorized Representative (Authorised Representative)		

		Salus Cons kft. 6722 Szeged, Bécsi krt 23, HUNGARY e-mail: office@saluscons.com
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#### Reference

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2. Lowbury, 1951, J. Clin. Pathol., 4:66.
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