IFU

Instructions for Use

PSEUDOMONAS AGAR F AND P (TECH AGAR)

Cat. no. G198	Pseudomonas Agar F, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. G201	Pseudomonas Agar P, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. L96	Tech Agar, 16x100mm Tube, 5.5ml Slant	20 tubes/box

INTENDED USE

Hardy Diagnostics Pseudomonas Agar F is used for the detection and differentiation of *Pseudomonas aeruginosa* by enhancement of fluorescein production. Pseudomonas Agar P, also known as Tech Agar, is recommended for enhancement of pyocyanin production by *Pseudomonas aeruginosa.*

SUMMARY

Pseudomonas aeruginosa is an environmental isolate commonly found in soil, water, food, and in many man-made products. The organism can utilize an array of nutrients and will thrive in a variety of environments, including medical equipment such as catheters, infusion fluids, disinfectants, and cosmetics. *P. aeruginosa* can also readily produce a biofilm, an aggregate of cells that can adhere to many surfaces. Because its cells can grow on most surfaces, *P. aeruginosa* is considered an opportunistic pathogen, particularly in immunocompromised persons, and can cause ocular, burn wound, and respiratory tract infections; it is the most common non-fermentative, gram-negative rod isolated by clinical microbiologists.[5,6]

Most strains of *P. aeruginosa* secrete a variety of pigments, such as pyoverdin, a yellow, water-soluble, fluorescent pigment, and pyocyanin, a blue-green, water- and chloroform-soluble, non-fluorescent pigment. Pyoverdin is most often produced by *P. aeruginosa* and other pseudomonads commonly isolated from humans.[2] However, when present, pyoverdin can suppress pyocyanin production. In contrast, *P. aeruginosa* is the only *Pseudomonas* spp. known to produce pyocyanin, a pigment that diffuses into the surrounding medium: yet, some strains are apyocyanogenic.[2]

The detection of pigment generating strains was first described by King et al. who developed two different media to detect pigment producing pseudomonads.[4] King et al. described Medium A, also known as Pseudomonas Agar P or Tech Agar, for the detection of pyocyanin and Medium B, also known as Pseudomonas Agar F or Flo Agar, for the detection of pyoverdin. The media were later modified according to recommendations made by the U.S. Pharmacopoeia and are also listed in the FDA Bacteriological Analytical Manual (BAM) for the differentiation of *Pseudomonas aeruginosa*.[8, 9]

Pseudomonas Agar F incorporates equal concentrations of peptones, which contain phosphorous to stimulate fluorescein production. Dipotassium phosphate added to the medium increases the phosphorous concentration and further enhances pigment production. Essential ions are provided by magnesium sulfate. Pseudomonas Agar P contains digest of gelatin to provide essential amino acids and other nitrogenous compounds to promote bacterial growth. Digest of gelatin is low in phosphorous, which helps inhibit the production of pyoverdin, a pigment that suppresses pyocyanin production.[2] Moreover, magnesium, potassium and sulfate ions promote the production of pyocyanin. Both media contain agar as the solidifying agent and glycerol as the energy source.

FORMULA

Ingredients per liter of deionized water:*

Pseudomonas Agar F:	
Pancreatic Digest of Casein	10.0gm
Proteose Peptone No. 3	10.0gm
Glycerol	10.0gm
Dipotassium Phosphate	1.5gm
Magnesium Sulfate	1.5gm
Agar	15.0gm

Final pH 7.0 +/- 0.2 at 25ºC.

Pseudomonas Agar P and Tech Agar:	
Pancreatic Digest of Gelatin	20.0gm
Potassium Sulfate	10.0gm
Glycerol	10.0gm
Magnesium Chloride	1.4gm
Agar	15.0gm

Final pH 7.0 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store Cat. no. G198 and G201 at 2-8ºC. and Cat. no. L42 and L96 at 2-30ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation

Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Consult listed reference for more information on specimen collection.[2,3,5,9] Specimens must be isolated from a pure culture on a suitable medium. Colony morphology and Gram stain characteristics should be confirmed as appropriate for *Pseudomonas* spp. prior to use.

Method of Use:

1. Using a sterile inoculating loop, streak the sample over the surface of the agar.

2. Incubate the specimen at 35 to 37ºC. for 18 to 24 hours. If the isolate fails to grow, reincubate the sample at 25 to 30ºC. for 1 to 2 days.

3. Observe cultures for growth and pigment production. If examining Pseudomonas Agar F, a long wavelength UV light (Cat. no. UVL56) is needed.

INTERPRETATION OF RESULTS

Colonies growing on Pseudomonas Agar F should be observed using a long wavelength UV lamp (Cat. no. UVL56) for fluorescein pigmentation. Fluorescein production should appear as a greenish-yellow fluorescent pigment in the colonies and surrounding medium.

Colonies growing on Pseudomonas Agar P and Tech Agar should display a blue to blue-green pigment in the colony as well as the surrounding medium, indicating pyocyanin production.[2] Confirm the presence of pyocyanin by performing a chloroform ($CHCl_3$) extraction and observe for a blue pigment solubilized in the chloroform solution.[9]

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Some strains of *Pseudomonas* will yield only small amounts of pigment in the medium. If this occurs, a yellow-green color will be evident on Pseudomonas Agar F or a blue-green pigment will be visible on Pseudomonas Agar P and Tech Agar. If the blue-green coloration is observed on Pseudomonas Agar P or Tech Agar, confirm the presence of pyocyanin by performing extraction with chloroform.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, UV Lamp (Cat. no. UVL56), other culture media, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control

microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Pseudomonas Agar F:					
Pseudomonas aeruginosa ATCC® 27853	A	18-24hr	35°C	Aerobic	Growth; greenish-yellow fluorescent pigment
Burkholderia (*Pseudomonas*) *cepacia* ATCC® 25416	A	18-24hr	35°C	Aerobic	Growth; no pigment, no fluorescence

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Pseudomonas Agar P and Tech Agar:**					
Pseudomonas aeruginosa ATCC® 27853**	E	18-24hr	35°C	Aerobic	Growth; blue to blue-green pigment
Burkholderia (*Pseudomonas*) *cepacia* ATCC® 25416	E	18-24hr	35°C	Aerobic	Growth; no pigment

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Pseudomonas Agar F and Pseudomonas Agar P should appear clear, slightly opalescent, and light to medium amber in color.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

3. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

4. King, E.O., M.K. Ward and D.E. Raney. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. *J. Lab. Clin. Med.*; 44: 301.

5. Jorgensen., et al. *Manual of Clinical Microbiology,* American Society for Microbiology, Washington, D.C.

6. Reyes, E.A.P., M.J. Bales, W.H. Cannon, and J.M. Matsen. 1981. Identification of *Pseudomonas aeruginosa* by Pyocyanin Production on Tech Agar. *J. Clin. Microbio.*; 13(3):456-458.

7. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

8. Wentworth, B. 1987. *Diagnostic Procedures for Bacterial Infection*, 7th ed. APHA, Washington, D.C.

9. U.S. Food and Drug Administration. *Bacteriological Analytical Manual.* AOAC, Arlington, VA. www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm

IFU-10693[A]

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]