IFU

Instructions for Use

COMPACT DRY™ PA

Cat. no. PA100	Compact Dry™ PA, 60x75mm Tray with 10x60mm Well	100 trays/box

INTENDED USE

Hardy Diagnostics Compact Dry™ PA is a ready-to-use test method recommended for the enumeration of *Pseudomonas aeruginosa* in food samples, feedstuffs, water, cosmetics, and beverages, or material samples from related industries.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Non-fermentative gram-negative bacteria (NFB) can exploit wet or soiled environments where they can contaminate sinks, faucets, and equipment, and taint finished products. *Pseudomonas aeruginosa* is typically found in soil and water, and can survive on plants and in damp environments. The organism rarely causes disease in healthy individuals, but it is an opportunistic pathogen capable of growth in very low nutrient concentrations. *P. aeruginosa* is characterized as a motile, gram-negative, strictly aerobic, rod-shaped bacterium, that is oxidase and catalase positive. *P. aeruginosa* can grow at elevated temperatures such as 42°C, and is known to form a biofilm--a matrix of extracellular polymeric substances that allows cells to attach to surfaces--especially in tubing or water pipes. It has been found in tap water, showers, toilets, medications, cosmetics, contact lens fluid, contaminated infusion solutions, and blood bottles, humidifiers, dialysis equipment, ventilators, and inhalers. Thus, it may be difficult to completely eradicate *P. aeruginosa* from the environment. Currently, methods for *P. aeruginosa* enumeration include selective culture media, the Most Probable Number (MPN) method, membrane filtration tests, or detection of fluorescein or pyocyanin production.[1-6]

Compact Dry™ PA is a ready-to-use selective chromogenic medium for performing *Pseudomonas aeruginosa* colony counts that contains dehydrated culture media and a cold water-soluble gelling agent in a non-woven cloth matrix. The medium is instantly hydrated when inoculated with a sample, and capillary action diffuses the sample evenly over the matrix to form a gel within seconds. Compact Dry™ PA contains a chromogenic substrate that yields a colored reaction when utilized by cells and permits the differentiation of *P. aeruginosa* from background flora.

Compact Dry™ PA has been validated and shown to perform as equivalent to reference method ISO 16266:2008 Water Quality - Detection and enumeration of *P. aeruginosa* by the membrane filtration method.[6] Compact Dry™ PA is also AOAC validated (AOAC no.100401) and the ready-to-use trays save space and greatly reduce the time needed to perform microbiological testing. Compared to other commonly used culture systems, Compact Dry™ has a longer shelf life, can be stored at room temperature, does not require manual sample spreading, is rigid, stackable and easy to label, and allows for direct colony picking for further subculture.

FORMULA

Compact Dry™ PA contains dehydrated culture media, a gelling agent, a selective agent, and a chromogenic substrate

to assist in differentiating *P. aeruginosa* from other organisms.

Final pH 7.0 +/- 0.2 at 25°C

STORAGE AND SHELF LIFE

Storage: Upon receipt, store at 1-30ºC. away from direct light. Media should not be used if there are any signs of deterioration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing. If foil pouch is opened and not all plates are used, return remaining plates to pouch and reseal until next use. Opened packages should be used as soon as possible.

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 quality control 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 blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory 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

Method of Use Direct Inoculation:

1. Remove the set of four trays from the foil pouch and separate each individual tray by gently bending along the connecting edge until each tray snaps free. Alternatively, if setting up a dilution series of the same sample, trays can be left connected to facilitate reading similar samples. Trays that are not used immediately should be resealed in the foil pouch. Refer to the "Storage and Shelf Life" section for proper storage of unused trays.

2. Remove the lid of the tray using two fingers to hold down one end of the lid and the thumb to lift the opposite end. Lids are easier to remove using a "peel back" method as opposed to a "pull off" method.

3. Inoculate by pipetting 1ml of sample directly to the center of a dry tray well, being careful not to touch the surface of the matrix with the pipet tip. Once dispensed, the sample will automatically diffuse across the surface by capillary action to form a gel; manual spreading of the inoculum is discouraged. Remember to account for the sample inoculum when calculating the dilution series.

4. Replace the lid and label the tray with appropriate information, including the sample dilution factor.

5. Invert the tray and incubate, upside down with the medium on top, at 35-37° C for 48 hours. NOTE: Use the

appropriate temperature/time designation according to the legal specification of the prescribed analysis regulation.

6. Count colonies using the Hardy Diagnostics Wizard™ Compact Dry™ plate reader (Cat. no. CDR1) designed exclusively for use with Compact Dry™. See the Wizard™ Compact Dry™ instruction manual. Alternatively, colonies can be counted when illuminated from the backside of the tray to calculate CFU/ml using the Scan® 100 colony counter (Cat. no. 435000) or comparable backlighting. If the colony count is high, use the 1cm x 1cm molded grid on the back of the tray to assist in colony counting. Use a sheet of white paper with gridded lines to diffuse the light if the molded grids in the tray are difficult to visualize with a light box.

7. If further biochemical testing is required for complete identification, validate the test method to ensure chromogens or selective agents in the medium do not affect the test results. If further testing requires a colorimetric reading, it is recommended the organism be subcultured to an appropriate nonselective solid medium, such as Tryptic Soy Agar (Cat. no. G60), to ensure chromogens utilized by the organism during growth on Compact Dry™ do not interfere with the test.

Method of Use Membrane Filtration:

1. Remove the lid on a Compact Dry™ plate and pipette 1.0ml of sterile purified water into the middle of the plate to activate the matrix just before use.

2. Using a membrane filtration set-up, filter 100-250ml of a water sample under reduced pressure through a sterile 0.45µm membrane filter.

3. Keep the set-up running and rinse the inside of the filter funnel using 20-30ml of sterile purified water. Repeat this step two to three times to ensure all of the sample has been filtered through the membrane.

4. Remove the membrane from the funnel using sterile forceps (e.g. Cat. no. 800000) and apply it filter side up (trap side away from the matrix) to the surface of the pre-moistened Compact Dry™ plate. Gently press the membrane onto the surface, making sure to remove bubbles so the membrane is completely flush and centered on the matrix.

5. Replace the lid and incubate the tray right-side-up using the information outlined above.

6. Count colonies and record results as outlined above.

INTERPRETATION OF RESULTS

After incubation, read trays using the Wizard™ Compact Dry™ plate reader (Cat. no. CDR1) or read colonies against a white or illuminated background such as with the Scan® 100 colony counter (Cat. no. 435000) or comparable back lighting.

Red colonies with green/yellow pigment are indicative of *P. aeruginosa*. Count the total number of red colonies with green/yellow pigment to obtain the total colony count. The growth area is 20cm^2. If the colony count is high, the total count can be obtained by multiplying the average number of colonies observed in one 1cm x 1cm square grid by 20.

Bacteria other than *Pseudomonas aeruginosa* may grow, but will not produce red colonies with green/yellow pigment and should not be included in the total count.

LIMITATIONS

During inoculation, do not touch the surface of medium and be careful to avoid any contamination by airborne microorganisms.

During incubation, keep cap tight on plates to avoid any possible dehydration.

A dilution may be needed when the sample has a dark color.

When the sample is viscous (thick), pipetting the sample on several points on a plate or an additional dilution may be

needed for an even suspension.

When the sample contains an enzyme, it may react with the enzyme substrate in the dry sheet and affect the color.

If the nature of sample does affect the reaction of the medium, inoculate only after the factor is eliminated by means of dilution and other techniques. (e.g. samples with high viscosity, colored, reactive with chromogenic substrate, and with a high or low pH).

It is recommended to use a stomacher and filter homogenized sample afterwards to eliminate carry over of tiny particles of foodstuff onto the surface of the medium.

Counting colonies may be difficult against a dark background. For best results, count colonies using the Hardy Diagnostics Wizard™ Compact Dry™ plate reader (Cat. no. CDR1) or with the tray held against a white or illuminated background such as with the Scan®100 colony counter (Cat. no. 435000).

If using a light box, the molded grid lines or colonies may be difficult to view due to excessive brightness. Diffuse the light using a sheet of white, gridded (1cm x 1cm) paper underneath the tray to facilitate colony counting.

Colonies are not distinguishable on trays if concentrations are above 100 CFU/ml, as high colony counts will result in the whole surface becoming colored. The sample should be diluted to a concentration of less than 100 CFU/ml for best use.

If further biochemical testing is performed on colonies subcultured directly from Compact Dry™, it is recommended the method be validated to ensure selective agents or chromogens in Compact Dry™ do not interfere with the test.

Some strains of *Pseudomonas* form colonies without the green/yellow pigment.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs such as EnviroTrans™, applicator sticks, scoops, dilution buffers such as Dilu-Lok™ II, other culture media, Wizard™ Compact Dry™ plate reader (Cat. no. CDR1), Scan® 100 colony counter (Cat. no. 435000), incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Pseudomonas aeruginosa ATCC® 9027	J	24-48hr	35-37°C	Aerobic	Growth; red colonies with green/yellow pigment
Klebsiella pneumoniae ATCC® 13883	J	24hr	35-37°C	Aerobic	Inhibited
Escherichia coli ATCC® 8739	J	24hr	35-37°C	Aerobic	Inhibited
Bacillus subtilis ATCC ® 6633	J	24hr	35-37°C	Aerobic	Inhibited
Staphylococcus aureus ATCC® 6538	J	24hr	35-37°C	Aerobic	Inhibited

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

USER QUALITY CONTROL

Check for signs of contamination and deterioration. Users of commercially prepared culture media may be required to perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction (where applicable). See the following reference for more specific information. [1-5]

PHYSICAL APPEARANCE

Compact Dry™ PA should appear dry, free of particles, and off white in color.

REFERENCES

1. Association of Official Analytical Communities. *Official Methods of Analysis* . AOAC, Washington, D.C.

2. American Public Health Association. *Standard Methods for the Examination of Dairy Products.* APHA, Washington, D.C.

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

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

5. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater.* APHA, Washington, D.C.

6. The International Organization for Standardization. *Water Quality - Detection and Enumeration of* Pseudomonas aeruginosa - *Part 2: Most Probable Number Method*. ISO 16266-2:2018(en).

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

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