IFU

Instructions for Use

LANAGRAM™

Cat. no. Z49	LanaGram™	15 tests/kit

INTENDED USE

Hardy Diagnostics LanaGram™ is used in confirming the gram stain reaction of gram-negative and gram-positive aerobic or facultatively anaerobic rods or coccobacilli. This test is not recommended for use with gram-positive cocci, microaerophilic or obligate anaerobic organisms (see "Limitations" below).

SUMMARY

Many organisms including *Bacillus* , *Erysipelothrix* , *Lactobacillus* and *Listeria* are classified in part on the basis of their positive reaction in gram stain. Occasionally, however, isolates are encountered in the clinical laboratory that erroneously appear to be gram-variable or gram-negative.[2] Some of these organisms will demonstrate a gram-positive stain with young cultures or exhibit some other distinguishing trait, such as spore formation which is compatible with *Bacillus* spp. With others, the erroneous staining reaction may lead to misidentification or the inability to generate a compatible biochemical profile.

Alternately, some organisms that are gram-negative may, at times, appear to be gram-positive, since they have been known to resist the alcohol-acetone decolorization step in the gram stain. Among these problem organisms are members of the Neisseriaceae family, which include *Moraxella* (*Branhamella*) *catarrhalis* , *Neisseria* spp., *Kingella* and *Acinetobacter* spp. Erroneous gram stain reactions represent one of the most frequent causes of misidentification, which can result in a delay of appropriate therapy.[6]

Additionally, older, less viable cultures of gram-positive organisms that are unable to repair their cell walls, or whose cell wall formation is compromised by beta-lactam antibiotics, may stain gram negative. Slow growing gram-positive organisms may also stain gram-negative when the cell wall becomes thinner and more permeable.[7]

Cerny described a method for distinguishing gram-negative bacteria from gram-positive.[5] Using L-alanine-p-nitroanilide (LANA) reagent, he found complete correlation between the presence of aminopeptidase and gram-negativity in aerobic and facultative organisms. The presence of cell wall aminopeptidase is detected by the hydrolysis of L-alanine-p-nitroanilide indicating a gram-negative reaction. In his comparison study of LANA and the 3% KOH method, Carlone, et al. yielded more correct gram stain determinations than with the 3% KOH method when tested against aerobic and facultative organisms.[4]

REAGENT FORMULA

LanaGram™ disks are impregnated with L-alanine-p-nitroanilide in TRIS buffer.

STORAGE AND SHELF LIFE

Storage: Upon receipt, store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration, 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

For best results use fresh cultures less than 48 hours old.

Disks should appear white to light yellow prior to the addition of water.

1. Place 2-3 drops of distilled or deionized water (pH 6.8+/- 0.2) in the small test tube containing the LanaGram™ disk.

2. Add sufficient organism from 2-3 well isolated identical colonies to produce a milky suspension.

3. Incubate at 35 +/- 1.0ºC. for 5-20 minutes.

INTERPRETATION OF RESULTS

The development of a pale yellow to bright yellow color is a positive test for aminopeptidase and evidence of a gram-negative organism. No color change indicates a negative aminopeptidase reaction and that the organism is gram-positive. Negative test reactions (no color change) should be held for the full 20 minutes. See "Limitations" section below.

LIMITATIONS

LanaGram™ will not give predictable reactions for some gram-positive cocci, microaerophilic or obligate anaerobic organisms. Such genera as *Campylobacter* , *Bacteroides* and *Streptococcus* are likely to produce false results.

Do not perform LanaGram™ test on colonies grown on media containing large amounts of dye such as EMB or MacConkey Agar. Dye carry-over may mask the yellow color change, resulting in false-negative reactions.

Tests performed on yellow pigmented colonies may appear positive immediately, but this is due to the bacterial pigmentation and should not be recorded as positive. In this case, a saline control (with no disk) should be inoculated

and the test should be observed for a deepening of the yellow color to indicate a positive reaction.

Gardnerella vaginalis may require up to 60 minutes to yield a positive aminopeptidase reaction. An extended incubation of the test may be required for bacteria isolated from vaginal specimens.

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, culture media, sterile swabs, incinerators, incubators, and distilled water with a neutral pH, etc., 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	Reaction
Escherichia coli ATCC® 25922	Positive aminopeptidase reaction, yellow color change observed; gram-negative organism.
Bacillus subtilis ATCC® 6633	Negative aminopeptidase reaction, no color change to yellow observed; gram-positive organism.

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

LanaGram™ disks should appear white to light yellow in color.

Showing positive aminopeptidase reaction.

Showing negative aminopeptidase reaction.

Escherichia coli (ATCC® 25922) was suspended in 3 drops of deionized water with a LanaGram™ Disk (Cat. no. Z49) and incubated aerobically for 5 minutes at 35ºC. The yellow color change was indicative of a gram-negative organism.

Bacillus subtilis (ATCC® 6633) was suspended in 3 drops of deionized water with a LanaGram™ Disk (Cat. no. Z49) and incubated aerobically for 5 minutes at 35ºC. No color change was indicative of a gram-positive organism.

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. Versalovic, J., et al. *Manual of Clinical Microbiology.* American Society for Microbiology, Washington, D.C.

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

4. Carlone, G.M., M.J. Valdez and M.J. Pickett. 1983. Method for distinguishing gram-positive from gram-negative bacteria. *J. Clin. Microbiol.*; 16:1157-1159.

5. Cerny, G. 1974. Method for the distinction of gram-negative from gram-positive bacteria. *Eur. J. Appl. Microbiol.*; 3:223-225.

6. Cowan, S.T. and J. Liston. 1974. The mechanism of identification. In *Bergey's Manual of Determinative Bacteriology*, 8th ed. Williams & Wilkins, Baltimore, MD; p. 10-13.

7. Donnelly, J.P. 1996. The Secrets of Gram Stain, *Infect. Dis. Alert.*; p. 109-112.

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

HDQA 2207F [D]