remel Gram-Sure

1. INTENDED USE

Remel Gram-Sure is a reagent-impregnated disk recommended for use in qualitative procedures to differentiate aerobic, Gram-negative and Gram-positive rods or *coccobacilli*.

2. SUMMARY AND EXPLANATION

The Gram stain is generally the first step performed in the identification of bacteria and often determines the subsequent identification procedures. The tendency of some Gram-positive rods to appear Gram-variable or Gram-negative, especially members of the genera *Bacillus*, *Erysipelothrix, Lactobacillus,* and *Listeria,* may result in misclassification. In 1976, Cerney reported using L-alanine-4-nitroanilide to detect cell-wall aminopeptidase, which correlated with Gram negativity.¹In 1990, Manafi and Kneifel evaluated chromogenic and fluorogenic substrates for the ability to differentiate Gram-positive and Gram-negative bacteria.² They preferred the fluorogenic compound, L alanine-7-amido-4-methylcoumarin, for a rapid test due to the pronounced blue fluorescence of a positive reaction and increased test sensitivity.

3. PRINCIPLE

The cell walls of Gram-negative organisms contain aminopeptidase, which is detected through hydrolysis of L-alanine-7-amido-4-methylcoumarin from a nonfluorescent substrate to a fluorescent compound.

4. REAGENTS (CLASSICAL FORMULA)*

Reactive Ingredient: L-alanine-7-amido-4-methylcoumarin

5. PRECAUTIONS

This product is for *in vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully.

STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Minimize exposure to light and avoid temperature extremes which may damage the product.

7. PRODUCT DETERIORATION

This product should not be used if (1) the color has changed from white, (2) the expiration date has passed, (3) the desiccant has changed from blue to pink, or (4) there are other signs of deterioration.

Protect disks from moisture by removing from the vial only those disks necessary for testing. Promptly replace the cap and return the vial to 2-8°C.

8. SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected and handled following recommended guidelines.^{3,4}

9. MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Demineralized water, (7) 10 x 75 mm test tube, (8) Longwave ultraviolet light.

10. PROCEDURE

- 1. Add 0.25 ml of demineralized water to a 10 x 75 mm test tube.
- 2. Inoculate heavily using a pure culture of the test isolate.
- 3. Add one Gram-Sure disk to the inoculated tube.
- 4. Incubate at room temperature (RT) for 5-10 minutes.
- 5. Observe under longwave ultraviolet light for the appearance of blue fluorescence.

11. INTERPRETATION

Positive Test (Gram-negative) - Blue fluorescence

Negative Test (Gram-positive) - No fluorescence

12. EXPECTED VALUES

Aerobic, Gram-negative rods and coccobacilli demonstrate a bright blue fluorescence, whereas Gram-positive rods and coccobacilli demonstrate no fluorescence. This test is designed to serve as an adjunct to (not a replacement of) the traditional Gram stain method.

13. QUALITY CONTROL

All lot numbers of Gram-Sure have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

| CONTROL | INCUBATION | RESULTS |
|--|----------------|----------|
| <i>Escherichia coli</i> ATCC [®] 25922 | 5 minutes @ RT | Positive |
| Staphylococcus aureus ATCC [®] 25923 | 5 minutes @ RT | Negative |

14. LIMITATIONS

- Obligate anaerobic organisms may fail to produce the expected results and should not be tested with this product.
- Gram-Sure is only part of the overall scheme for identification of aerobic bacteria. Further testing is required for definitive identification. Consult appropriate references for further instructions.^{3,4}

15. BIBLIOGRAPHY

- 1. Cerney, G. 1976. Eur. J. Appl. Microbiol. 33:223-225.
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- Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry, and M.A. Pfaller. 2007. Manual of Clinical Microbiology. 9th ed. ASM Press, Washington, D.C.
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16. PACKAGING

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17. SYMBOL LEGEND

| REF | Catalogue Number |
|------------|---|
| IVD | In Vitro Diagnostic Medical Device |
| i | Consult Instructions for Use (IFU) |
| 1 | Temperature Limitations (Storage temp.) |
| LAB | For Laboratory Use Only |
| LOT | Batch Code (Lot Number) |
| Σ | Use By (Expiration Date) |
| *** | Manufactured by |

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