

Lysozyme Procedure for *Nocardia*

I. Principle:

Growth in lysozyme is one of the most valuable tests for differentiating between nocardiae and other aerobic actinomycetes. The growth of the test organism in the lysozyme-supplemented glycerol control broth is compared with the growth of unsupplemented glycerol broth. Growth in both the Lysozyme broth and the Lysozyme control broth is indicative of a potential *Nocardia* species and further testing should be performed.

II. Reagents:

Lysozyme broth (Remel)
Lysozyme control broth-glycerol (Remel)

III. Materials and Storage:

This product is ready for use. Refrigerate in original container at 2-8° C.

IV. Procedure:

- A. Make a light culture suspension (0.5 McFarland standard) by placing organism into 1 mL of 0.85% sterile saline.
- B. Using a sterile Pasteur pipette inoculate each of the following tubes with 1 drop of suspension.
 1. Lysozyme Broth
 2. Lysozyme Glycerol Control Broth
- C. Incubate aerobically at 30° C and observe twice a week for four weeks looking for turbidity.

V. Interpretation:

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| A. Growth in Lysozyme Broth <i>and</i>
Growth in Glycerol Control Broth | Possible <i>Nocardia</i> sp. |
| B. No growth in Lysozyme Broth
Growth in Glycerol Control Broth | NOT <i>Nocardia</i> sp. |

VI. Limitations:

- A. Growth in Lysozyme should be used in conjunction with key characteristics
 1. Partially acid fast by the modified Kinyoun procedure using 0.5% sulfuric acid as the decolorizing agent.

2. Microscopic morphology: short to extensively branched rods often with vegetative hyphae <1um in diameter. They may fragment into bacillary or coccoid nonmotile forms.
 3. Colony morphology: Variable, smooth to the more common rough form. Colors vary from pink, orange, or tan.
- B. Other organisms that can be weakly acid fast and may grow in the presence of lysozyme are *Gordonia*, *Rhodococcus*, *Saccaropolyspora*, *Thermoactinomyces*, and *Tsukamurella*

VII. References:

- A. Conville, PS. And Witebsky, F.G. Current Issues Pertaining to the *Nocardia* species. *Clinical Microbiology Newsletter*. Vol 26, (8):pp 57-62. 2004.
- B. Koneman, E.W., Allen, S.D. et al. 1997. Diagnostic Microbiology 5th ed. Lippincott.
- C. Murray, P.R., Baron, E.J., Jorgensen, J.H., et al. 2003. Manual of Clinical Microbiology. 8th ed. ASM Press.
- D. Remel. Lysozyme Broth and Lysozyme Control Broth media insert. Revised August 26, 2003.

VIII. Document Control

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Medical Director Approval: Reviewed by Dr. Schappert 3/10/2010.

Microbiology Director Approval: Dr. Ann Robinson 05/07/2004

Microbiology Supervisor Reviews: Jerry Claridge 07/2005, 06/2006, 06/2007, 05/2008, 07/2009, 04/01/2011, 03/2013, Jason Ammons 07/2015