Department of Microbiology
Unusual Gram-Negative Rods Identification Procedure

I. Purpose

Gram-negative organisms that are unidentifiable by routine methods may be subjected to a variety of differential media and spot tests if the isolate warrants full identification. All organisms that cannot be identified by routine methods must be brought up for review during Rounds.

II. Specimen Information

Organisms must be isolated in pure culture prior to performing any of conventional tests. Slow growing organisms, such as those frequently encountered in cystic fibrosis cultures, should be given sufficient time to grow so that colony morphologies may be distinguished.

III. Reagents & Equipment

- MacConkey Agar
- TSA Agar with 5% Sheep Blood
- Oxidase Reagent
- Indole Reagent
- Catalase Reagent
- OF Media: Base, Dextrose, Lactose, Maltose, Mannitol, Sucrose, and Xylose
- Mineral Oil
- TSI Slant
- Nitrate Broth
- Urea Agar Slant
- Simmons Citrate Agar Slant
- Tech (Pseudomonas P) Agar
- Sodium Acetate Agar Slant (if *Moraxella* spp. is suspected)
- Gelatin Strips (if *Moraxella* spp. is suspected)
- Inoculation loop and needle
- Wooden applicator sticks
- Glass slides
- Cover slips
- Test tube rack
- Aerobic incubator set at 35 ± 2°C

IV. Procedure

The following tests should be performed and documented in the computer. Refer to individual test procedures for specific instructions on inoculation, interpretation, and Quality Control testing.

A. Gram Stain
B. Motility
C. Oxidase
D. Indole production
E. Catalase
F. Subculture to TSA blood agar

G. Growth on MacConkey agar
H. Carbohydrate utilization: OF Base, OF Dextrose - oxidation, OF Dextrose-fermentation (oil overlay), OF Xylose, OF Mannitol, OF Lactose, OF Maltose, OF Sucrose
I. Nitrate reduction
J. Urease
K. Citrate utilization
L. Growth at 42°C
M. Pyocyanin pigment production
N. Fluorescent pigment production
O. Acetate utilization (if *Moraxella* spp. is suspected)
P. Gelatinase (if *Moraxella* spp. is suspected)

V. Interpretation and Reporting

A. Reactions for some of the biochemical tests may require several days of incubation. Tubes should be held for a minimum of 4 days before considered negative unless the isolate is identified sooner with Rounds consultation.
B. Technical staff should refer to identification charts and, based on the biochemical reactions of the isolate, develop a list of possible identifications. Staff should be prepared to review test results during Rounds.
C. All identification should be reviewed during Rounds.

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Updates and Revisions: 03/2011 Additional tests added to battery and deleted NF ID system (Remel). 07/21/2011 Added TSI slant.