NOTE

GENE PATH

GenePath® Group 6 Reagent Kit
Catalog# 310-0116

Klebsiella pneumoniae

Not FDA approved for *in-vitro* diagnostic use

Please familiarize yourself with the contents of this insert and the Group 6 Instruction Manual before using the product for the first time.

1. Intended Use

The Bio-Rad *GenePath Reagent Kits* are intended for the qualitative determination of genetic strain typing based on restriction enzyme digestion of DNA.

This GenePath Note provides verification and protocol modification information on the application of the PFGE technique on the typing of *Klebsiella pneumoniae*, including mucoid isolates of *K. pneumoniae*. Please refer to the GenePath Group 6 Instruction Manual for additional assay information.

2. Sample Preparation Using GenePath Group 6 Reagent Kit

For Isolates grown in broth media.
The protocol in the GenePath Group 6 Instruction Manual Sections 2.1 through 3.1 was used with the following changes.

1. Section 2.2 Step 4: Transfer 60 µl of growth to a 1.5 ml microcentrifuge tube. Centrifuged at 13K rpm in a microcentrifuge for 2 minutes. Measure pellet size: pellet size should range from 2.2-2.5 mm., which corresponds to the left tube in Figure 2.1 of the instruction manual. Do not use larger size pellets. Adjust the pellet size as indicated in Section 2.2 Step 5.
2. Follow the Group 6 Instruction Manual as written for the remaining protocol, until Section 3.2 Sample Loading. Cut a very narrow slice from the plug (1-1.5 mm x 5 mm) to load on the gel, and use the Alternative sample loading method given on page 15.

For Isolates grown on solid media.
This may save a day if you start from pure isolate growing on solid agar media. Follow the protocol in the GenePath Group 6 Instruction Manual from Section 2.2 page 12, "Organisms Grown on Solid Media", through Section 3.1 with the following changes.

1. Using sterile swab, suspend growth of a pure isolate on into 2-3 ml of sterile saline, sterile PBS, sterile GenePath 1X Wash Buffer, or broth media.
2. Aliquot 150 µl into a 1.5 ml microcentrifuge tube. Centrifuge at 13K rpm in a microcentrifuge for 2 minutes. Measure pellet sizes: pellet size should range from 2.2-2.5mm., which corresponds to the left tube in Figure 2.1 of the instruction manual. Do not use larger size pellets. Adjust the pellet size as indicated in Section 2.2 Step 5.
3. Follow the Group 6 Instruction Manual as written for the remaining protocol, until Section 3.2 Sample Loading. Cut a very narrow slice from the plug (1-1.5 mm x 5 mm) to load on the gel, and use the Alternative sample loading method given on page 15.

3. Electrophoresis Conditions to Separate the DNA.

- Select the "157" program on GenePath Instrument.
- Specific electrophoresis parameters:

Run time	22 hours
Initial Switch	2.2 seconds
Final Switch	54.2 seconds
Ramp	Linear
Voltage Gradient	6 V/cm
Angle	120

4. Staining Gel

Allow the gel to stain for 15 minutes in the ethidium bromide solution (5 drops of 1 mg/ml ethidium bromide in 300 ml deinonized water). Using a gel scoop or gloves, remove the gel from the ethidium bromide solution and place it in a dish containing at least 500 ml dH_2O. Destain for 30 minutes in the water. Change to fresh deionized water after 30 minutes. Destain for another 30 minutes in the water. Destaining longer can help reduce background staining.

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5. PFGE Gel Results

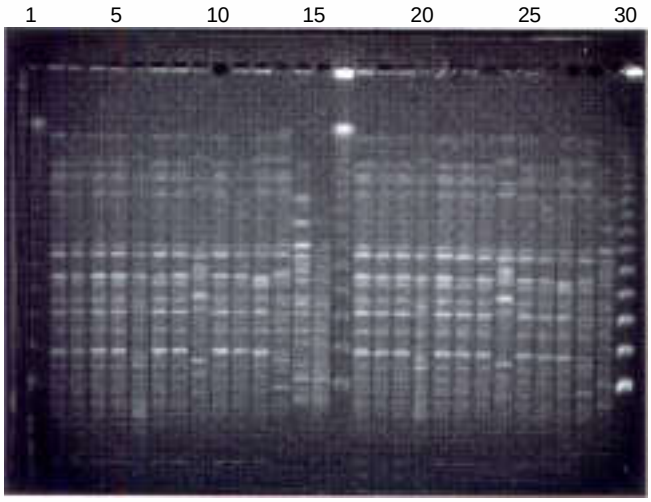

Figure 1. PFGE of *K. pneumoniae* isolates
Lanes 1, 16 and 30, Lambda Ladder; lanes 14 and 29, Group 6 Culture Control; lane 15, Group 6 Control Plug. Lanes 2-13, samples from clinical isolates prepared from broth media. Lanes 17-28, samples from clinical isolates prepared from solid media.

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