NOTE

GENE PATH

GenePath® Group 1 Reagent Kit

Catalog# 310-0111

Coagulase Negative Staphylococci (CNS): Staphylococcus epidermidis and Staphylococcus haemolyticus

Not FDA approved for *in-vitro* diagnostic use

Please familiarize yourself with the contents of this insert and the Group 1 Instruction Manual before using the product for the first time.

1. Intended Use

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 for typing of *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* isolates. Please refer to the GenePath Group 1 Instruction Manual for additional assay information.

2. Sample Preparation Using GenePath Group 1 Reagent Kit

For isolates grown in broth media.
The protocol in the GenePath Group 1 Instruction Manual Sections 2.1 through 3.2 can be used with the following modifications.

Section 2.2 Step 4:

CNS Cell pelleting using overnight culture:

An overnight culture can be used directly or diluted to a specific cell density for plug preparation. For plug preparation using overnight culture, transfer 120 µl of overnight CNS culture to a 1.5 ml microcentrifuge tube. Centrifuge at 13K rpm in a microcentrifuge for 2 minutes. Measure pellet diameter using a transparent ruler. Adjust the pellet size to between 2mm to 3mm (between the two sizes shown in Figure 2.1 in Group 1 Instruction Manual) using overnight culture as indicated in Section 2.2 Step 5.

To standardize cell amounts for plug preparation, the cell density of overnight cultures can be adjusted to a specified optical transmittance value by using turbidity measurements. For results shown in this note, overnight cultures were adjusted with fresh growth medium till the transmittance value at 450 nm read 20% transmittance by using the HACH turbidity meter (DR100 Colorimeter). Transfer 400 µl of the adjusted culture to a 1.5 ml microcentrifuge tube. Centrifuge at 13K rpm in a microcentrifuge for 2 minutes. Adjust the pellet size to between 2mm to 3mm (between the two sizes shown in Figure 2.1 in Group 1 Instruction Manual) using the standardized culture as indicated in Section 2.2 Step 5.

Follow the Group 1 Instruction Manual Section 2.2 Step 6 through 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 "StA" program on GenePath Instrument.
- Specific electrophoresis parameters:

Run time	20 hr.
Initial switch	5.3 seconds
Final switch	34.9 seconds
Ramp	linear
Voltage gradient	6 V/cm
Angle	120^0

4. Staining Gel

Allow the gel to stain for 15 minutes with shaking in the ethidium bromide solution (300 µl of 1 mg/ml ethidium bromide in 300 ml deionized water). Destain for 30-60 minutes with shaking in 500 ml water. Destaining longer can help reduce background staining. Capture and image using GenePath System Gel Doc 2000 with the Molecular Analyst software or with a Polaroid camera.

5. PFGE Gel Results

Correlation between pellet diameter and PFGE band intensity

The results presented demonstrate that samples prepared using the GenePath Group 1 Reagent Kit yield good PFGE band patterns when cell pellet diameters range between 2mm and 3 mm. Within this pellet size range, the PFGE band intensity is proportional to the pellet diameter.

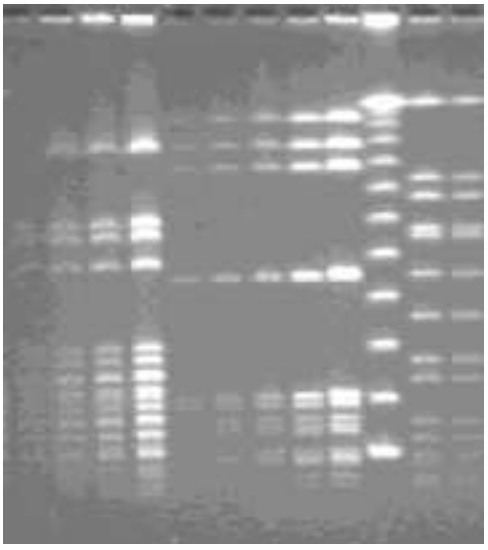

Figure 1. PFGE of Coagulase Negative *Staphylococcus*: Compare pellets with diameters between 1mm to 3.5 mm.

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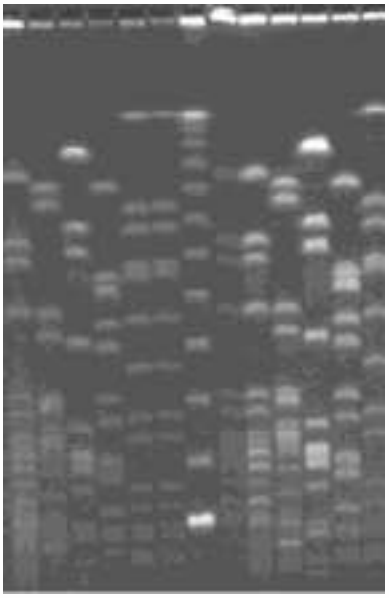
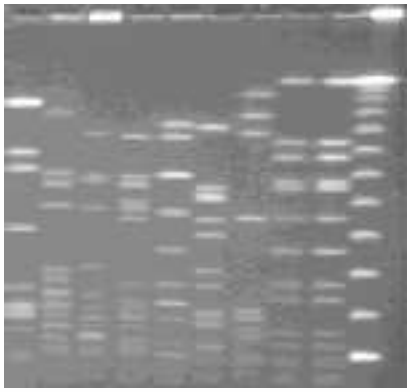
Lanes	Contents of Gel Lanes
1 to 4	*S. epidermidis* (Cell pellet diameters : 1, 2, 3, and 3.5 mm, respectively)
5 to 9	*S. haemolyticus* (Cell pellet diameters: 1, 1.3, 2, 2.3 and 3 mm, respectively.)
10	Lambda ladder
11	Group 1 *S. aureus* Control plug
12	Group 1 *S. aureus* Culture Control

Figure 2. PFGE of Coagulase Negative *Staphylococcus* : Comparison of pellet diameters of 2mm and 3mm.



Lanes	Content of gel lanes	Pellet diameter (mm)
1, 2, 3 and 8	*S. epidermidis*	2
4	*S. haemolyticus*	2
5	Group 1 *S. aureus* Culture Control	2
9,10, and 11	*S. epidermidis*	3
13	Group 1 *S. aureus* Culture Control	3
6	Group 1 *S. aureus* Control Plug	3
7	Lambda Ladder	

Figure 3. PFGE of Coagulase Negative *Staphylococcus* : pellet diameter of 2 mm.



Lanes	Contents of gel lanes
1 to 5	*S. epidermidis* isolates 1-5
6 to 7	*S. haemolyticus* isolate 1 and 2.
8	Lambda ladder
9	Group 1 *S. aureus* Culture Control
10	Group 1 *S. aureus* Control Plug.

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