NOTE

GENEPATH

GenePath® Group 1 Reagent Kit

Catalog# 310-0111

Vancomycin-resistant enterococci (VRE): *Enterococcus faecium* and *Enterococcus faecalis*

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

The Bio-Rad *GenePath Reagent Kits* are intended for qualitative 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 to the typing of *Enterococcus faecium* and *Enterococcus faecalis* isolates.

PFGE results shown in this Technote demonstrate that both *E. Faecium* and *E. faecalis* isolates can be typed using the GenePath Group 1 kit components and an additional lytic enzyme, mutanolysin. The necessary modifications to the GenePath kit 1 are described in this Technote. Please refer to the GenePath Group 1 Instruction Manual for additional assay information.

2. Sample Preparation and Sample Loading Using GenePath Group 1 Reagent Kit

The GenePath Group 1 Instruction Manual Sections should be used with the following modifications.

Section 1.4 : Required Items not Available from Bio-Rad

Mutanolysin solution

Lyophilized mutanolysin powder can be purchased from Sigma (Catalog number: M4782; 5000 units per vial). To prepare a 5 units/µl stock solution, 1 ml of sterile distilled water should be added to the vial. Store unused mutanolysin in –20 ^{0}C freezer.

Section 2 Sample Preparation

The protocol in the GenePath Group 1 Instruction manual Sections 2.1 through 3.1 was used with the following changes.

For Organisms grown in broth culture

1. Section 2.2 Steps 4-5:

An overnight culture can be used directly or diluted to a specific cell density for plug preparation. Transfer 1.2 ml of overnight culture of *E. faecium* or *E. faecalis* 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.

Alternatively, use an overnight culture that is standardized to a specific cell turbidity or optical density value.

To standardize by turbidity reading using the HACH DR100 turbidity meter, measure adjusted overnight cultures till the transmittance value is 50%. Transfer 1.2 ml of the 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 and 3mm using diluted cultures. The turbidity method was used to obtain the results shown in this Technote.

To standardize by optical density using a spectrophotometer, measure the optical density at 610 nm, and adjust culture with growth medium to obtain a reading of 0.45-0.55. Transfer 1.2 ml 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 and 3mm using diluted cultures.

2. Section 2.2 Step 7

In this step each sample should be done individually. To the 150 µl cell suspension, add 3 µl Lysozyme/Lysostaphin and 3 µl mutanolysin and 150 µl Embedding agarose.

3. Section 2.2 Step 9

For each sample, add 10 µl of mutanolysin solution and 10 µl of Lysozyme/Lysostaphin to the 500 µl of Lysis Buffer 1.

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 the 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

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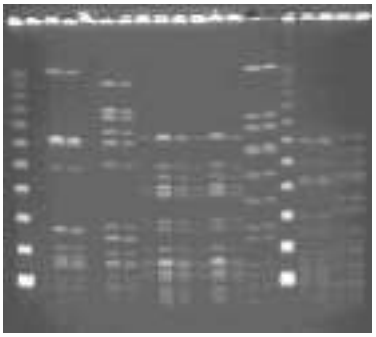
4. Gel staining and photography

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 water). Destain for 30-60 minutes with shaking in 500 ml water. Destaining longer can help reduce background staining. Pictures can be taken using the GenePath System Gel Doc 2000 with the Molecular Analyst software (Bio-Rad products) or with a Polaroid camera.

5. PFGE Gel Results

Figure 1. PFGE of *Enterococcus faecium* and *Enterococcus faecalis* - Effect of different lysis enzymes used in sample preparation.



Lane	Isolate[a]	Diameter pellet (mm)	Lytic enzyme[b]
1	Lambda ladder	---	---
2	*E.faecalis* #1	3	L/L
3	*E.faecalis* #1	3	L/l and Mut
4	*E.faecalis* #1	3	Mut
5	*E.faecalis* #2	3	L/L
6	*E.faecalis* #2	3	L/l and Mut
7	*E.faecalis* #2	3	Mut
8	*E. faecium* #1	3	L/L
9	*E. faecium* #1	3	L/l and Mut
10	*E. faecium* #1	3	Mut
11	*E. faecium* #2	3	L/L
12	*E. faecium* #2	3	L/l and Mut
13	*E. faecium* #2	3	Mut
14	*S.aureus* Culture Control	2	---
15	*S.aureus* Control Plug	---	---
16	Lambda ladder	---	---
17	*E. faecium* #3	2	L/L and Mut
18	*E. faecium* #3	3	L/L and Mut
19	*E. faecium* #4		L/L and Mut
20	*E. faecium* #4		L/L and Mut

a *E. faecium* isolates #3 and #4 were obtained from Dr. Richard Goering at Creighton University. The rest of VRE isolates used were obtained from Dr. YiWei Tang at Vanderbilt University.

b L/L: Lysozyme/Lysostaphin (a component of the GenePath kit 1); Mut: mutanolysin (purchased from Sigma Corp. and diluted to 5 units/µl with sterile water).

Bio-Rad Phone Numbers:

United States 1-800-227-1600 • **Australia** 61-2-9914-2800 • **Austria** 43-1-877-8901 • **Belgium** 32-9-385-5511 • **Canada** 905-712-2771 • **France** 33-1-4960-6834 • **Germany** 49-89-31884-0 • **Netherlands** 31-318-540666 • **Hong Kong** 852-2-789-3300 • **Italy** 39-2-216091 • **Japan** 81-3-5811-6266 • **New Zealand** 64-9-443-3099 • **Spain** 34-1-661-7085 • **Sweden** 46-8-627-5000 • **Switzerland** 41-1-809-5555 • **United Kingdom** 44-1-442-232552