PFGE Typing Protocol Recommended by ARPAC for *Acinetobacter baumannii*

Several different procedures are available for the preparation of intact chromosomal DNA from *A. baumannii* in agarose plugs. The following protocol was used to construct the database on this site and is based on that described by Bannerman *et al.* (1995) *J Clin Microbiol* 33, 551-555.

PLUG PRODUCTION

Do not shake anything!

1. Grow the isolate to be typed on blood agar plates overnight (18 h at 35°C). Subculture at least once to ensure purity. Make a suspension directly from an overnight subcultured blood agar plate into 2 - 3 ml of **TE buffer** (see below).

2. Read optical density on a spectrophotometer at a wavelength of 540 nm. Desired O.D. is 1.8 - 2.0. Dilute if necessary with **TE buffer**.

3. Transfer 200 – 500 µl (depending on how many plugs are being produced; 200 µl is enough for two plugs) of suspension to a sterile 1.5 ml Eppendorf tube.

4. Prepare 2.0% w/v low melting point agarose in **TE buffer** for making plugs. Once melted keep warm in 50 – 60°C water bath until ready to use.

5. Prepare one plug at a time! Mix an equal amount of melted agarose with cell suspension. Mix quickly and dispense approx. 150 µl into the plug mold. Let plugs set at 4°C for 20 min to harden.

6. **Additional lysis steps not required**

7. Add 3 ml of **ES solution** (see below) containing **1 mg/ml Proteinase K** to a 20 ml plastic Universal bottle. Transfer each plug into the Universal bottle, making sure the plug is covered by the buffer and is not hanging on side of the bottle. Incubate in a 55°C water bath for 3 h.

8. Wash the plugs at least four times with 5 ml of **TE buffer**, each time for at least 3 h (at least one wash should be overnight). Store the plugs in the Universal bottle in 3 ml **TE buffer** at 4°C overnight and then proceed with restriction digestion.

RESTRICTION DIGESTION

9. Cut a slice of the plug to fit into the well of the gel (2 mm). Transfer the slice to a sterile 1.5 ml Eppendorf tube and wash with 1ml **TE buffer** for 15 min. Return the rest of the plug to the Universal bottle and cover with 3 ml of fresh **TE buffer** for storage at 4°C (usually for a maximum of 4 weeks).

10. Aspirate the **TE buffer** from the plug slices. Wash the plugs twice with x 1 *Apa*I buffer (supplied by enzyme manufacturer), for 20 min each time. Add 200 µl fresh *Apa*I buffer and ***Apa*I** restriction enzyme (20 units per plug). Incubate plugs at 30°C overnight.

11. The next day, make a 1.5% w/v agarose (NOT low melting temperature agarose) gel in **0.5 x TBE**. Pour into mold, add comb, but reserve a few ml of molten agarose (keep at 50 - 60°C) for subsequent sealing-in of gel slices.

12. Use 1.5 - 2 L of **0.5 x TBE** in the CHEF DRII (or equivalent) electrophoresis chamber, sufficient to just cover the gel; chill the buffer to 14°C before starting the run.

13. Aspirate restriction enzyme mixture. Rinse slices with 200 µl of **cold (14°C) 0.5 x TBE.**

14. On each gel, use an *Apa*I digest of strain RUH 2034 as a standard for gel normalisation purposes. Strain RUH 2034 can be requested from the Workpackage Manager (ktowner@trent.phls.nhs.uk). This standard should be run in the left and right-hand lanes, and at least once every six lanes. Additionally, a lambda ladder can be run for molecular sizing purposes.

15. Add slices to wells in gel, making sure the plug sits at the bottom and to the front of the well. Seal with molten agarose. Allow to harden for 10 min before loading the gel into the PFGE chamber.

16. **Standardised electrophoresis conditions are mandatory for comparison with the database**.

    <u>Settings for CHEF DRII apparatus</u>:

    ramped pulse: initial 5 sec and final 13 sec;
    200 v for 20 h at 14°C.

17. On completion of electrophoresis, stain with ethidium bromide solution for 45 minutes. De-stain in distilled water for several hours or up to overnight to obtain good contrast.

18. Photograph and/or produce an electronic TIFF file for comparison with the database.

<u>Reagents needed for procedure</u>

TE Buffer

10 mM Tris-HCl
5 mM EDTA

to produce 100 ml of buffer: 1 ml 1M Tris-HCl pH 8
1 ml 0.5 M EDTA pH 8
QS to 100 ml with sterile distilled H_2O

Adjust to pH 7.5 and autoclave or filter sterilize. Store at room temperature for up to 6 months.

10 x TBE Buffer

1.0 M Tris base
0.9 M Boric acid
10 mM EDTA

Check that pH is 8.2-8.4. Commercially available buffer can be used.

ES Buffer (Deproteination Buffer)

0.5 M EDTA
10% Sarkosyl
10 g sodium lauroyl sarcosine (Sarkosyl), QS to 100 ml with 0.5 M EDTA pH 8. Filter sterilise. Before using add 1mg/ml Proteinase K.