One-Day (24-26 h) Standardized Laboratory Protocol for Molecular Subtyping of *Campylobacter jejuni* by Pulsed Field Gel Electrophoresis (PFGE)

BIOSAFETY WARNING: Please read all instructions carefully before starting protocol. Treat all plasticware, glassware, pipets, spatulas, etc. that come in contact with the cell suspensions or plugs as contaminated materials and dispose of, or disinfect according to the guidelines of your institution. Disinfect plug molds before they are washed. Contaminated items should be disinfected with 10% bleach for at least 30 minutes if they will be washed and reused.

Day 0

Streak an isolated colony from test cultures onto Trypticase Soy Agar with 5% defibrinated sheep blood (TSA-SB) plates (or comparable media) for confluent growth. It is recommended that a storage vial of each culture be created. To do this stab small screw cap tubes of TSA, HIA, or similar medium.with the same incolating loop used to streak the plate. This will ensure that the same colony can be retested if necessary. Incubate cultures at microaerobically 37ºC for 14-18 h.

Day 1

1. Turn on shaker water bath or incubator (54-55ºC), stationary water baths (55- 60ºC) and spectrophotometer (or equivalent instrument such as the Dade Microscan Turbidity meter or bioMérieux Vitek colorimeter).

2. Prepare **TE Buffer** (**10 mM Tris:1 mM EDTA, pH 8.0**) as follows:

   10 ml of 1 M Tris, pH 8.0
   2 ml of 0.5 M EDTA, pH 8.0
   Dilute to 1000 ml with sterile Ultrapure water (Clinical Laboratory Reagent Water (CLRW))

3. Prepare 1% SeaKem Gold agarose in **TE Buffer** (**10 mM Tris:1 mM EDTA, pH 8.0**) as follows:

   a. Weigh 0.50 g SeaKem Gold (SKG) into 250 ml screw-cap flask.
   b. Add 49.5 ml TE Buffer; swirl gently to disperse agarose.
   c. Remove cap, cover loosely with clear film, and microwave for 30-sec; mix gently and repeat for 10-sec intervals until agarose is completely dissolved.
   d. Place flask in a 55-60ºC water for 15 minutes or until ready to use.

Note: The TE Buffer used to make the plug agarose is also used to wash lysed PFGE plugs.

4. Label small tubes (Falcon 2054 tubes or equivalent) with culture numbers.

5. Transfer 2 ml of phosphate-buffered saline (PBS: 0.01 M, pH 7.2-7.4) or 0.85% NaCl to small labeled tubes (Falcon 2054 tubes). Use sterile polyester-fiber or cotton swab that has been moistened with sterile PBS to remove some of the growth from agar plate; suspend cells in PBS.

6. Adjust concentration of cell suspensions to:

    a. Dade Microscan Turbidity Meter: **0.35 - 0.45** (measured in Falcon 2054 tubes). For Falcon tubes 2057 adjust to **0.52 - 0.64**.

    b. Spectrophotometer: 610 nm wavelength, absorbance (Optical Density) of **0.680 (range of 0.570 to 0.820).**

    c. bioMérieux Vitek colorimeter: ≈ **20%** transmittance (measured in Falcon 2054 tubes)

Note: The values in Steps 7a, 7b and 7c give satisfactory results at CDC; each laboratory may need to establish the optimal concentration needed for satisfactory results

CASTING PLUGS

Label wells of PFGE plug molds with culture number. When reusable plug molds are used, put strip of tape on lower part of reusable plug mold before labeling wells.

Note 1: Unused plug agarose can be kept at room temperature and reused 1-2 times. Microwave on low-medium power for 10 -15 sec and mix; repeat for 5 -10 sec intervals until agarose is completely melted. This agarose melts rapidly!

Note 2: Proteinase K solutions (20 mg/ml) are available commercially. Alternatively, a stock solution of Proteinase K can be prepared from the powder in sterile Ultrapure water (CLRW). For best results, aliquot in 300-500 µl into small tubes and store in a freezer (-20 ºC) until ready to use. Just before use, thaw appropriate number of vials needed for the samples; keep Proteinase K solutions on ice. If the Proteinase K stock solution was prepared from powder, discard any thawed solution at the end of work day. Store commercially prepared Proteinase K solutions according to directions provided by the supplier.

1. Add 20 µl of Proteinase K (20 mg/ml stock) to labeled 1.5-ml microcentrifuge tubes (200 µl is needed for 10 cell suspensions.).

2. Transfer 400 µl (0.4 ml) of adjusted cell suspensions to each labeled tube and mix gently with pipet tip. The use of a 1000 µl pipet (P-1000) and tip is recommended for this step; the use of a smaller pipet and tip might cause DNA shearing.

3. Add 400 µl (0.4 ml) melted 1% SeaKem Gold agarose to the 400 µl cell suspension and mix gently by pipeting up and down two to three times. **Over-pipeting could cause DNA shearing**. Maintain temperature of melted agarose by keeping flask in beaker of warm water (55-60ºC).

4. Immediately, dispense part of mixture into appropriate well(s) of disposable plug mold. Do not allow bubbles to form. Two plugs of each sample can be made from these amounts of cell suspension and agarose and are useful if repeat testing is required. Allow plugs to solidify at room temperature for 10-15 min. They can also be placed in the refrigerator (4ºC) for 5 minutes.

Note: The generation of cell suspension and the subsequent casting of the plugs should be performed as rapidly as possible in order to minimize premature cell lysis. If large numbers of samples are being prepared, it is recommended that they be processed in batches of ~10 samples at a time. Once the first batch of isolates are in the cell lysis incubation, then start preparing the cells suspensions the next group samples, and so on. All batches can be lysed and washed together, since additional lysis time will not affect the initial batches.

LYSIS OF CELLS IN AGAROSE PLUGS

Note: When 50-ml tubes are used for lysis, two plugs (reusable plug molds) or three plugs (disposable plug molds) of the same strain can be lysed in the same tube.

1. Label 50-ml polypropylene screw-cap or 50-ml Oak Ridge tubes with culture numbers.

2. Prepare **Cell Lysis Buffer** (**50 mM Tris:50 mM EDTA, pH 8.0 + 1% Sarcosyl**) as follows:
   25 ml of 1 M Tris, pH 8.0
   50 ml of 0.5 M EDTA, pH 8.0
   50 ml of 10 % Sarcosyl (N-Lauroylsarcosine, Sodium salt)[1]
   Dilute to 500 ml with sterile Ultrapure water (CLRW)

3. Calculate the total volume of **Cell Lysis**/**Proteinase K Buffer** needed as follows:

   a. 5 ml Cell Lysis Buffer (50 mM Tris:50 mM EDTA, pH 8.0 + 1% Sarcosyl) is needed per tube (e. g., 5 ml x 10 tubes = 50 ml).

   b. 25 µl Proteinase K stock solution (20 mg/ml) is needed per tube of the cell lysis buffer (e. g., 25 µl x 10 tubes = 250 µl). The final concentration of Proteinase K in lysis buffer is 0.1 mg/ml.

   c. Prepare the master mix by measuring the correct volume of Cell Lysis Buffer and Proteinase K into appropriate size test tube or flask and mix well.

Note: Discard any thawed Proteinase K stock solution that was prepared from powder by the user at end of work day. Store commercially prepared Proteinase K solutions according to directions of the supplier.

4. Add 5 ml of Proteinase K/Cell Lysis Buffer to each labeled 50 ml tube.

5. If a flat edge is wanted on the plugs, trim excess agarose from top of plugs with scalpel or

[1]The N-Lauroylsarcosine, Sodium salt can be added directly to the other ingredients and allowed to dissolve. See page 13 of this document or Section 5a of the PulseNet PFGE Manual.

razor blade. Open mold and transfer plugs from mold with a 6-mm wide spatula to appropriately labeled tube. Be sure plugs are under buffer and not on side of tube.

Note: The excess agarose, scalpel, spatula, tape, etc. are contaminated. Dispose of or disinfect them appropriately.

6. **Remove tape from reusable mold.** Place both sections of plug mold, spatulas, and scalpel in 70% isopropanol (IPA) or other suitable disinfectant. **Soak them for 15 minutes before washing them.** Discard disposable plug molds or disinfect them in 10% bleach for 30-60 minutes if they will be washed and reused.

7. Place tubes in rack and incubate in a 54-55ºC shaker water bath for 15-30 min with constant and vigorous agitation (175-200 rpm). Be sure water level in water bath is above level of lysis buffer in tubes.

8. Pre-heat enough sterile Ultrapure water (CLRW) to 54-55ºC so that plugs can be washed two times with 10-15 ml water (200-250 ml for 10 tubes).

WASHING OF AGAROSE PLUGS AFTER CELL LYSIS

Note: Most laboratories will find that their plugs are sufficiently stable to perform the following washing steps at 54-55ºC. However, if you notice that your plugs are nicked along the edges or breaking it will be necessary for your laboratory to lower the water bath or incubator to 50ºC for the following washing steps.

1. Remove tubes from water bath, and carefully pour off lysis buffer. Plugs can be held in tubes with a screened cap (Bio-Rad) or spatula.

Note: Be sure to remove all of the liquid during this and subsequent wash steps by touching lip of tube onto an absorbent paper towel.

2. Add at least 10-15 ml sterile Ultrapure water (CLRW) to each tube. Discard water. This acts as a quick wash to remove lysis buffer left behind from the previous step. Add 10-15 ml of sterile Ultrapure water (CLRW) that has been pre-heated to 54-55°C to each tube and shake the tubes vigorously in a 54-55°C water bath for 10-15 min.

    a. Pre-heat enough sterile **TE Buffer** (**10 mM Tris:1 mM EDTA, pH 8.0**) in a 54-55°C water bath so that plugs can be washed **three** times with 10-15 ml TE (300-350 ml for 10 tubes) after beginning last water wash.

3. Pour off water, add at least 10 -15 ml pre-heated (54-55°C) sterile TE Buffer, and shake the tubes vigorously in 55°C water bath for 10-15 min.

4. Pour off TE and repeat wash step with pre-heated TE two more times.

5. Decant last wash and add 5 ml sterile TE (room temperature). Store plugs in 5 ml sterile TE buffer at 4°C until ready to do the restriction digestion. Plugs can be transferred to smaller tubes (17-mm x 100-mm, 12-mm x 75-mm, etc.) for storage. Plugs made with disposable plug molds can be stored in 2 ml round bottom tubes containing 1.5 to 1.7 ml of TE.

RESTRICTION DIGESTION OF DNA IN AGAROSE PLUGS WITH *Sma*I

Note: A small slice of the plug or the entire plug can be digested with the primary restriction enzyme; ***Sma*I**. Restriction digestion of a small slice of the plug is recommended because less enzyme is required and other slices of the plug can be subjected to restriction analysis with other enzymes. *Kpn*I is recommended as the secondary enzyme for analysis of *Campylobacter jejuni* isolates. The use of a secondary enzyme is useful in situations where the PFGE patterns obtained with the primary enzyme from two or more isolates are indistinguishable

Note: All PulseNet PFGE protocols use a strain of *Salmonella choleraesuis* ss. *choleraesuis* serotype Braenderup H9812 (ATCC BAA-664) **as the molecular size standard**. PFGE plugs (or blocks) of the *Salmonella* strain H9812 are made according the "PulseNet One-Day (24-28 h) Standardized Laboratory Protocol for Molecular Subtyping of *E. coli* O157:H7, *Salmonella* serotypes, and *Shigella sonnei* by PFGE" as described in the training manual (www.cdc.gov/pulsenet). This strain is used as a size standard for the normalization and analysis of PFGE patterns for all the organisms tracked by PulseNet, including *E. coli* O157:H7, *Salmonella*, *Shigella*, *Listeria monocytogenes*, *Vibrio cholerae* and *Campylobacter jejuni*. After plugs of the size standard are made, approximately 2-mm slices are cut and restricted with 40-50 Units of *Xba*I enzyme for 2 hours at 37°C. The plug slices are loaded on the electrophoresis gel in lanes 1, 5, 10 (10-well gel), 1, 5, 10, 15 (15-well gel), or 1, 5, 10, 15, 20 (20-well gel. New lots of *S*. Braenderup H9812 PFGE plugs should be tested with "old" lots to confirm that the pattern and band intensity is the same and that no observable genetic changes have occurred.

*Sma*I Restriction Reactions (*Kpn*I conditions appear in parenthesis)

1. Label 1.5-ml microcentrifuge tubes with culture numbers; label 3 (10-well gel) or 4 (15-well gel) tubes for the size standard plug slice.

Note: The appropriate restriction buffer will vary between vendors and may differ between enzymes from the same vendor. Always use the restriction buffer recommended by the vendor for the particular restriction enzyme.

a. **Optional Pre-Restriction Incubation Step**: Prepare a master mix by diluting the appropriate 10X restriction buffer (Roche Applied Science or equivalent) 1:10 with sterile Ultrapure water (CLRW) according to the following table:

Reagent	µl/Plug Slice	µl/10 Plug Slices	µl/15 Plug Slices
Sterile Clinical Laboratory Reagent Water	180 µl	1800 µl	2700 µl
Restriction Buffer	20 µl	200 µl	300 µl
Total Volume	200 µl	2000 µl	3000 µl

c. Add 200 µl diluted 1X restriction buffer to labeled 1.5-ml microcentrifuge tubes.

d. Carefully remove plug from TE with spatula and place in a sterile disposable Petri dish or on large glass slide.

e. Cut a ~2.0 mm-wide slice from each test samples and the appropriate number of *S.* ser. Braenderup H9812 standards with a scalpel (or single edge razor blade, cover slip, etc.) and transfer to tube containing restriction buffer. Be sure plug slice is under buffer. Replace rest of plug in original tube that contains 5 ml TE buffer and store at 4°C.

Note: The shape and size of the plug slice that is cut will depend on the size of the comb teeth that are used for casting the gel. Gel wells that are cast with combs that have 10-mm-wide teeth will require a different size plug slice than those cast with combs that have smaller teeth (5.5-mm) teeth. The number of slices that can be cut from the plugs will also depend on the skill and experience of the operator, integrity of the plug, and whether the slices are cut vertically or horizontally (plugs made in disposable molds).

f. Incubate plug slices in a 25°C water bath for 5-10 min or at room temperature for 10-15 min.

g. After incubation, remove buffer from plug slice using a pipet fitted with 200-250 µl tip all the way to bottom of tube and aspirate buffer. Be careful not to cut plug slice with pipet tip and that plug slice is not discarded with pipet tip.

2. Prepare the restriction enzyme master mix by diluting 10X restriction buffer 1:10 with sterile Ultrapure water (CLRW) and adding *Sma*I restriction enzyme (40 U/sample) according to the following table. Mix in the same tube that was used for the diluted restriction buffer:

Note: Keep vial of restriction enzyme on ice or in insulated storage box (-20°C) at all times.

Reagent	µl/Plug Slice	µl/10 Plug Slices	µl/15 Plug Slices
Sterile Clinical Laboratory Reagent Water	179 µl	1790 µl	2685 µl
Restriction Buffer	20 µl	200 µl	300 µl
Enzyme (40 U/µl)	1 µl	10 µl	15 µl
Total Volume	200 µl	2000 µl	3000 µl

Note: Keep vial of restriction enzyme on ice or in insulated storage box (-20˚C) at all times.

a. Optional Addition of Bovine Serum Albumulin (BSA): Several restriction enzyme vendors specifically recommend the addition of 1X BSA to enzyme restriction mixtures. However, BSA can be added to all enzyme restriction mixtures and may assist in reducing the incidence of incomplete restriction. If BSA is added to the enzyme reaction mixture, the volume of BSA added should be deducted from the volume of water to maintain the total volume of 200 µl per slice. For example, if (NEB BSA; 2µl of 100X per plug slice) the amount of water is 173 µl instead of 175 µl for each plug slice.

3. Add 200 µl restriction enzyme mixture to each tube. Close tube and mix by tapping gently; be sure plug slices are under enzyme mixture.

4. Incubate sample and control plug slices at 25°C (room temp or water bath/chiller or incubator) for 4 h. **For *Kpn*I, incubate for 4-6 hours at 37°C**.

5. If plug slices will be loaded into the wells (Option B, page 9), continue with Steps 1-4 of the next section (**CASTING AGAROSE GEL**) approximately 1 h before restriction digest reaction is finished so the gel can solidify for at least 30 minutes before loading the restricted PFGE plugs.

CASTING AGAROSE GEL

A. Loading Restricted Plug Slices on the Comb:

1. **Confirm that water bath is equilibrated to 55-60°C.**

2. Make volume of 0.5X Tris-Borate EDTA Buffer (TBE) that is needed for both the gel and electrophoresis running buffer according to one of the following tables.

5X TBE:

Reagent	Volume in milliliters (ml)					
5X TBE	200	210	220	230	240	250
Reagent Grade Water[2]	1800	1890	1980	2070	2160	2250
Total Volume of 0.5X TBE	2000	2100	2200	2300	2400	2500

10X TBE:

Reagent	Volume in milliliters (ml)					
10X TBE	100	105	110	115	120	125
sterile Ultrapure water (CLRW)	1900	1995	2090	2185	2280	2375
Total Volume of 0.5X TBE	2000	2100	2200	2300	2400	2500

3. Make 1% SeaKem Gold (SKG) Agarose in 0.5X TBE as follows:

   a. Weigh appropriate amount of SKG into 500 ml screw-cap flask. Add appropriate amount of 0.5X TBE; swirl gently to disperse agarose.
   b. Remove cap, cover loosely with clear film, and microwave for 60-sec; mix gently and repeat for 15-sec intervals until agarose is completely dissolved.
   c. Recap flask and place in 55-60°C water bath.

   Mix 1.0 g agarose with 100 ml 0.5X TBE for 14-cm-wide gel form (10 or 15 wells)
   Mix 1.5 g agarose with 150 ml 0.5X TBE for 21-cm-wide gel form (15 wells)

SAFETY WARNING: Use heat-resistant gloves when handling hot flasks after microwaving.

[2] De-ionized water (does not need to be sterilized).

4. A small volume (2-5 ml) of melted 1% SKG agarose may be needed to fill wells after plugs are loaded. Prepare 50 ml by melting 0.5 g agarose with 50 ml 0.5X TBE in 250 ml screw-cap flask as described above. Unused SKG agarose can be kept at room temperature, melted, and reused several times. Microwave for 15-20 sec and mix; repeat for 10-sec intervals until agarose is completely melted. Place in 55-60°C water bath until ready to use. Alternatively, save approximately 5 ml of the melted agarose used to cast the gel in a pre-heated (55-60°C) 50 ml flask and place in 55-60°C water bath until used.

Note: Confirm that gel form is level on leveling table, that **front** of comb holder and teeth face the bottom of gel, and that the comb teeth touch the gel platform.

5. Remove enzyme/buffer mixture and add 200 µl 0.5X TBE. Incubate at room temperature for 5 min.

6. Remove plug slices from tubes; put comb on bench top and load plug slices as close to the bottom edge of the teeth as possible. Load the plug slices in the following order:

   a. Load *Salmonella* serotype Branderup H9812 standards in lanes (teeth) 1, 5, 10 (10-well gel) or in lanes 1, 5, 10, 15 (15-well gel).

   b. Load samples on remaining teeth of the comb.

7. Remove excess buffer with tissue. Allow plug slices to air dry on the comb for 5-10 minutes or seal them to the comb with 1% SKG agarose (55-60°C).

8. Position comb in gel form and confirm that the plugs slices are correctly aligned on the bottom of the comb teeth, that the lower edge of the plug slice is flush against the black platform, and there are no bubbles.

9. Carefully pour the agarose (cooled to 55-60°C) into the gel form.

10. Put black gel frame in electrophoresis chamber. Add 2-2.2 L freshly prepared 0.5X TBE. Close cover of unit. (The amount of buffer needed depends on whether residual buffer was left in tubing or if unit was flushed with water after the last gel was run.)

11. Turn on cooling module (14°C), power supply, and pump (setting of 70 for a flow of 1 liter/minute).

12. Remove comb after gel solidifies for 30-45 minutes.

13. Fill in wells of gel with melted and cooled (55- 60°C) 1% SKG Agarose (optional). Unscrew and remove end gates from gel form; remove excess agarose from sides and bottom of casting platform with a tissue. Keep gel on casting platform and carefully place gel inside black gel frame in electrophoresis chamber. Close cover of chamber.

B. Loading Restricted Plug Slices into the Wells:

1. Follow steps 1-4 in "Option A" above (**Loading Restricted Plug Slices on the Comb**).

2. Cool melted SKG agarose in 55-60°C water bath for 15-20 min; carefully pour agarose into gel form (casting stand) fitted with comb. Be sure there are no bubbles.

Note: Confirm that gel form is level on gel-leveling table before pouring gel, that front of comb holder and teeth face bottom of gel, and the bottom of the comb is 2-mm above the surface of the gel platform.

3. Put black gel frame in electrophoresis chamber. Add 2-2.2 L freshly prepared 0.5X TBE. Close cover of unit. (The amount of buffer depends on whether residual buffer was left in tubing, or if unit was flushed with water after the last gel was run.)

4. Turn on cooling module (14°C), power supply, and pump (setting of 70 for a flow of 1 liter/minute) approximately 30 min before gel is to be run.

5. Remove enzyme/buffer mixture; add 200 µl 0.5X TBE. Incubate at room temperature for 5 min.

6. Remove comb after gel solidifies for at least 30 minutes.

7. Remove restricted plug slices from tubes with tapered end of spatula and load into appropriate wells. Gently push plugs to bottom and front of wells with wide end of spatula. Manipulate position with spatula and be sure that are no bubbles.

   a. Load *Salmonella* serotype Branderup H9812 standards in lanes 1, 5, 10 (10-well gel) or in lanes 1, 5, 10, 15 (15-well gel).

   b. Load samples in remaining wells.

Note: Loading the plug slices can be tedious; each person has to develop his/her own technique for consistently placing the plug slices in the wells so the lanes will be straight and the bands sharp.

8. Fill in wells of gel with melted 1% SKG Agarose (equilibrated to 55- 60°C). Allow to harden for 3-5 min. Unscrew and remove end gates from gel form; remove excess agarose from sides and bottom of casting platform with a tissue. Keep gel on casting platform and carefully place gel inside black gel frame in electrophoresis chamber. Close cover of chamber.

ELECTROPHORESIS CONDITIONS

A. ***Sma*I**: Select following conditions on Chef Mapper for *Campylobacter jejuni.*

   Auto Algorithm
   50 kb - low MW
   400 kb - high MW
   Select default values except where noted by pressing "enter".
   Change run time to 18 hours
   (Default values: Initial switch time = 6.76 s; Final switch time = 35.38 s).

B. ***Kpn*I:** Select following conditions on Chef Mapper for *Campylobacter jejuni.*

Two State
Gradient – 6.0V
Change run time to 18 hours
Included Angle – 120
Initial switch time =5.2 s
Final Switch time =42.3 s
Select default values except where noted by pressing "enter".

Note: The electrophoresis running times recommended above are based on the equipment and reagents used at the CDC. Running times in your laboratory may vary (faster or slower) and should be determined empirically.

Note: Make note of the initial milliamp (mAmp) reading on the instrument. The initial mAmps should be between 110-170 mAmps. A reading outside of this range may indicate that the 0.5X TBE buffer was prepared improperly and the buffer should be remade.

Day 2

STAINING AND DOCUMENTATION OF PFGE AGAROSE GEL

1. When electrophoresis run is over, turn off equipment; remove and stain gel with ethidium bromide by diluting 40 µl of ethidium bromide stock solution (10 mg/ml) with 400 ml of reagent grade water (this volume is for a staining box that is approximately 14-cm x 24-cm; a larger container may require a larger amount of staining solution). Stain gel for 20 - 30 min in covered container.

Note: Ethidium bromide is toxic and a mutagen; the solution can be kept in dark bottle and reused 5 - 6 times before discarding according to your institution's guidelines for hazardous waste or use the destaining bags recommended for disposal of ethidium bromide (**Section 10**).

2. Destain gel in approximately 500 ml reagent grade water for 60 - 90 min; change water every 20 minutes. Capture image on Gel Doc 1000, Gel Doc 2000, or equivalent documentation system. If background interferes with resolution, destain for an additional 30-60 min.

Note: If both a digital image and conventional photograph are wanted, photograph gel first before capturing digital image.

3. Follow directions given with the imaging equipment to save gel image as an ***.img** or ***.1sc** file; convert this file to ***.tif** file for analysis with BioNumerics software program The gel image should fill the entire window of the imaging equipment (computer) screen (without cutting off wells or lower bands). Ensure that the image is in focus and that there is little to no staturation (over-exposure) in the bands. Additional instructions are provided in PNL07 of the PulseNet QA/QC manual.

4. Drain buffer from electrophoresis chamber and discard. Rinse chamber with 2 L reagent grade water or, if unit is not going to be used for several days, flush lines with water by letting pump run for 5-10 min before draining water from chamber.

5. If the lowest band in the H9812 standard does not migrate within 1 -1.5 cm of the bottom of the gel, the run time will need to be determined empirically for the conditions in each laboratory.

Please note the following if PFGE results do not have to be available within 24 hours:

1. Plugs can be lysed for longer periods of time (up to 2 hours).

2. The washing steps with TE to remove the lysis buffer from the PFGE plugs can be done for longer periods of time (15-30 min) and at lower temperatures (37°C or room temperature). They can be started on Day 1 and finished the morning of Day 2 after overnight refrigeration of the plugs in TE.

3. The restriction digestion can be done for longer periods of time (4-16 hours). Restrict for at least 4 hours when using 20 units of *Sma*I.

Use of trade names and commercial sources is for identification purposes only and does not imply endorsement by CDC or the U.S. Department of Health and Human Services.

NOTE: CLIA LABORATORY PROCEDURE MANUAL REQUIREMENTS

Efforts have been made to assure that the procedures described in this protocol have been written in accordance with the 1988 Clinical Laboratory Improvement Amendments (CLIA) requirements for a procedure manual (42 CFR 493.1211). However, due to the format required for training, the procedures will require some modifications and additions to customize them for your particular laboratory operation.

Any questions regarding the CLIA requirements for a procedure manual, quality control, quality assurance, etc., should be directed to the agency or accreditation organization responsible for performing your laboratory's CLIA inspection. In addition, some states and accreditation organizations may have more stringent requirements that will need to be addressed.