KCMC Biotechnology Laboratory, Microbiology	STANDARD OPERATING PROCEDURE	Effective Date 10 March 2007	SOP-Number MIC.038.01
		Page 1 of 16	Date 10 March 2007

Title: GenProbe LUMINOMETER OPERATION AND MAINTENANCE

Author/Date: Susan Morpeth, Anne Morrissey, November 2006

Approvals/Date:

_____

John A. Crump, MB, ChB, Microbiology Laboratory Director

_____

Anne Morrissey, MS, MT(ASCP)SM, Microbiology Lab Supervisor

This SOP has been read and understood by:

Name	Date
Signatures on original copy.	
1.	
2.	
3.	
4.	
5.	
6.	
7.	
8.	
9.	
10.	

Annual Review	
By	**Date**

Title: GenProbe LUMINOMETER OPERATION AND MAINTENANCE

Document History:

Version Number	Reason for Changes	Date
MIC.038.01	Change of Optics Check from weekly to monthly	9 March 2007
	Extension of tritium standard decay calendar	
	Explanation of Pump test printout for Reagent Dispensing Check	

Copies distributed to:

Name	Date

Title: GenProbe LUMINOMETER OPERATION AND MAINTENANCE

PURPOSE

Procedures for the operation and maintenance of the GenProbe Luminometer Leader 50i instrument used for detection of mycobacterial ribosomal RNA using the ACCUPROBE® MYCOBACTERIUM TUBERCULOSIS COMPLEX, or MYCOBACTERIUM AVIUM COMPLEX CULTURE IDENTIFICATION TESTS

PRINCIPLE:

Nucleic acid hybridization tests are based on the ability of complementary nucleic acid strands to specifically align and associate to form stable double-stranded complexes. The ACCUPROBE SYSTEM uses a single-stranded DNA probe with a chemiluminescent label that is complementary to the ribosomal RNA of the target organism. After the ribosomal RNA is released from the organism during a lysing/sonication process, the labeled DNA probe combines with the target organism's ribosomal RNA to form a stable DNA:RNA hybrid. The Selection Reagent allows for the differentiation of non-hybridized and hybridized probe. The labeled DNA:RNA hybrids are measured in the GEN-PROBE Luminometer and the amount of chemiluminescence is expressed in Relative Light Units (RLU) A positive result is a reading equal to or greater than the cut-off value below this cut-off is a negative result.

SCOPE

This Standard Operating Procedure applies to the operation and maintenance of GEN-PROBE® Luminometer Leader 50i instrument by technical staff of the microbiology laboratory who are trained and are competent in performing this test.

MAINTENANCE PROCEDURES

SUMMARY

Frequency	Maintenance		
Each use	Wash cycle	Reagent volume check	
Weekly	Warm water flush	Quality control of reagent dispensing system	
Monthly	Optics check	Cleaning optical chamber	Software quality control
As needed	Paper replacement	Fuse replacement	

"EACH USE" MAINTENANCE:

A. Wash Cycle
B. Reagent Volume Check

A. Wash Cycle

MATERIALS:

- GEN-PROBE Luminometer Leader 50 instrument
- Clean empty tubes to contain ends of inlet lines
- Clean empty GEN-PROBE polystyrene tubes
- Detection Reagent 1 (store at 2-25°C)
- Detection Reagent 2 (store at 2-25°C)
- Volume "Combo" QC tube

1. Turn GEN-PROBE Luminometer Leader 50 instrument ON 20 minutes prior to use.
2. Remove the inlet lines from the distilled water; place each line into a separate clean empty tube.
3. Do three wash cycles to clear the lines of water as follows:
4. Press WASH.
5. Follow screen directions to perform wash cycle procedure:
6. Insert empty clean GEN-PROBE polystyrene tube into optical chamber and close cover.
7. Screen displays "Priming Injectors"
8. Lid opens automatically
9. At the end of the cycle remove the used tube.
10. Repeat cycle (steps 3-9) with a clean, empty polystyrene tube each time for a total of 3 cycles.
11. Place the inlet tubing into and tighten caps on the appropriate Detection Reagent bottles.

AFTER THE DETECTION REAGENTS ARE CONNECTED TO THE INSTRUMENT THE WASH CYCLE SHOULD BE RUN THREE TIMES BEFORE ANY SAMPLE ANALYSIS IS PERFORMED. ALSO, IF THE INSTRUMENT HAS BEEN IN OPERATION BUT HAS BEEN IDLE FOR MORE THAN 30 MINUTES, ONE WASH CYCLE SHOULD BE PERFORMED

12. Do three wash cycles (steps 3-9) to prime the inlet lines with the Detection Reagents.

13. NOTE: You must use a new tube for each wash cycle to prevent spilling reagents into the chamber.

14. Before discarding the last used tube, proceed to the "Reagent Volume Check" procedure below.

14. After completion of the wash cycle(s) insert a clean, empty tube into the optical chamber.

B. Reagent Volume Check

MATERIALS:

- GEN-PROBE Luminometer Leader 50 instrument
- Clean empty tubes to contain ends of inlet lines
- Clean empty GEN-PROBE polystyrene tubes
- Detection Reagent 1
- Detection Reagent 2
- Volume "Combo" QC tube

1. Frequency: After performing initial washing steps with each use.

2. Procedure: Compare the volume in the last used tube with a "Volume Combo QC" tube that should be kept with the Luminometer.

3. Acceptable Values: The volume in the last used tube will be the same as that marked on the "Volume Combo QC" tube.

4. Corrective Actions:

a. If the volume in the last used tube is less than indicated by the marked line on the "Volume Combo QC" tube then;

- perform a warm water flush (see Weekly Maintenance section) AND
- perform QC of Reagent Dispensing System (see Weekly Maintenance section)

b. If the volume in the last used tube is greater than that indicated by the marked line on the "Volume Combo QC" tube then contact GEN-PROBE.

5. Document volume adequacy on GENPROPE LUMINOMETER MAINTENANCE LOG. If volume inadequate, document corrective action on QC DEVIATION record.

WEEKLY MAINTENANCE:

A. Warm water flush to clean inlet lines

B. Quality Control of Reagent Dispensing System

A. Warm Water Flush

MATERIALS:

- GEN-PROBE Luminometer Leader 50 instrument
- 150mL distilled water in clean glass beaker
- Hot plate set to 50-60°C
- Thermometer calibrated to NIST standard
- 70% Iso-Propyl Alcohol wipe
- Clean empty tubes to contain inlet lines
- Clean empty GEN-PROBE polystyrene tubes

1. Purpose: To flush any built-up precipitation from the Detection Reagents out of the Leader 50 inlet lines. Precipitation will decrease the volume of Detection Reagent delivered to the reaction tube and will affect the result of the assay.
2. Frequency: Weekly or when reagents are changed and as necessary if reagent volume check fails.
3. Procedure:
   a. Place 150 ml of distilled water in a clean beaker, on the hot plate set to 50-60^0 C. **NOTE: It is extremely important that the water is fresh with no contaminants - they may plug the line.** Check the temperature with a thermometer that has been calibrated to the NIST standard and cleaned with a 70% iso-propyl alcohol wipe.
   b. If Detection Reagents are in use, unscrew the caps from both Detection Reagent bottles and remove bottles from bottle holder. Place each inlet line in a separate clean tube. If Detection Reagents are not in use and the inlet lines are stored in water, then jump to step d.

c. Do three wash cycles to clear the lines of the Detection Reagents.

d. Place the inlet lines into the beaker of warm distilled water, and do as many wash cycles as necessary until the foam bubbles turn clear.

Note: Use a clean GEN-PROBE polystyrene tube for each wash cycle (this could take up to 15-20 tubes if the lines contain reagents)

e. Document completion of warm water flush on GENPROPE LUMINOMETER MAINTENANCE LOG.

e. Perform Quality Control of Reagent Dispensing System.

B. Quality Control of Reagent Dispensing System

MATERIALS

- GEN-PROBE Luminometer Leader 50 instrument
- Clean empty GEN-PROBE polystyrene tubes
- Volume "Single" QC tube
- 150mL of warm distilled water in a clean glass beaker
- 2 clean tubes of distilled water at room temperature in which to keep ends of inlet lines when luminometer is not in use

1. Purpose: To ensure the volume of each Detection Reagent delivered to the reaction tube is correct. Inadequate volumes or an incorrect ratio of Detection Reagents will affect the result of the assay.
2. Frequency: Weekly or when reagents are changed and as necessary if reagent volume check fails. Perform directly after Warm Water Flush while inlet lines are still in the beaker of warm distilled water.
3. Procedure:
   a. Run 3 empty GEN-PROBE polystyrene tubes with protocol 10 (Pump Test 1, tests injector 1 only) consecutively.
   b. Compare the volume in each tube with the levels marked on the "Volume Single QC" tube which should be stored near the instrument.
   c. Run 3 empty GEN-PROBE polystyrene tubes with protocol 11 (Pump Test 2, tests injector 2 only) consecutively. (Disregard sample information on Pump Test Luminometer printout, the values are irrelevant.)

d. Compare the volume in each tube with the levels marked on the "Volume Single QC" tube which should be stored near the instrument.

e. Remove the inlet lines from the beaker of warm distilled water; place each line back into a separate clean tube of distilled water.

5. Acceptable Values

a. Volumes for each tube should be between the two levels marked on the "Volume Single QC" tube.

b. If volumes are not between the two levels marked on the "Volume Single QC" tube, inform the supervisor and take corrective action.

6. Corrective Action

a. Repeat Warm Water Flush and then repeat Quality Control of Reagent Dispensing System procedure.

b. If volumes still are not between the two levels marked on the "Volume Single QC" tube, contact GEN-PROBE.

b. Document corrective action on QC DEVIATION record.

7. Documentation

a. Document volume adequacy on GENPROPE LUMINOMETER MAINTENANCE LOG.

MONTHLY MAINTENANCE

A. Optics check with Tritium Standard

B. Cleaning the Optical Chamber

C. Software Quality Control

A. Optics check

MATERIALS:

- GEN-PROBE Luminometer Leader 50 instrument
- GEN-PROBE Tritium Standard
- GEN-PROBE Manual Decay Correction Factors worksheet

1. Frequency: weekly; also following any optical chamber cleaning; and if recommended by GEN-PROBE.

2. GEN-PROBE Tritium Standard

a. Storage: upright at 20-25^{0}C, in the dark (no sunlight or UV light)

b. Shelf-life: 6 years from date of calibration

c. When new Standard is received from the company, centrifuge 2-5 minutes at 500-1000 rpm before initial use (to bring all liquid to tube bottom)

d. Sign and date Standard box to indicate Standard has been centrifuged

3. Procedure:

a. Turn GEN-PROBE Leader 50 instrument on - allow 20 minutes warm-up.

b. Press white UTILITIES on keyboard. Then press 1 (for Q/A), and press ENTER. A header prints; the optical chamber cover opens.

c. ENTER TRITIUM STANDARD SERIAL NUMBER, screen displays the alphanumeric choice screen. The serial number is located on the side of the Tritium Standard box. Select each digit on the screen by using the 1 key to move left and the 3 key to move right. Press SEL (the 0 key) when the cursor is under the appropriate digit. If the wrong number is selected, press DELETE (the CLEAR key) Each selected number will show on the screen. When the serial number is complete, press ENTER.

d. ENTER EXPECTED TRITIUM STANDARD ACTIVITY RLU/SEC. Enter the **decay corrected value** for the Tritium Standard using the number keypad directly and press ENTER after the number.

To obtain the **decay corrected value**: Multiply the assigned value of the Standard (located on the side of the Standard tube and on the outside of the Standard box in Net RLU/sec) by the appropriate **decay correction factor** according to the number of months from the Standard's calibration date.

The **decay correction factor** is found on the TRITIUM STANDARD DECAY CORRECTION FACTORS worksheet in the GenProbe QC manual, on page VI-3. Note that this worksheet has an extension received from GenProbe that has been attached, for decay correction factors out to 72 months from the Standard's Calibration Date.

The Standard's calibration date (also found on the outside of the standard box) should be logged on the TRITIUM STANDARD QUALITY CONTROL RECORD.

e. Screen display reads: TRITIUM STANDARD INSERT TUBE, CLOSE COVER. Place the tritium Standard tube in the optical chamber: **the arrow on the Standard MUST face the front of the instrument.** Close the chamber cover. The instrument will measure the Standard's RLU five times and print the average RLU/sec. The cover opens at completion.

f. Screen display reads: BLANK TUBE INSERT TUBE, CLOSE COVER. Place an empty clean GEN-PROBE polystyrene tube in the optical chamber; close cover. The instrument measures the blank's RLU five times and prints the average.

g. The instrument prints out the Net Observed RLU/sec and the Ratio of the Observed Value to the Expected Value.

h. Place a clean empty tube in the optical chamber; close the cover.

4. Acceptable Values:

a. The ratio of Observed/Expected RLU/Sec Values should fall between 0.95 and 1.05

5. Corrective Action for Unacceptable Result

a. First check the standard –liquid on the sides of the tube may explain a low value. Centrifuge the standard for 2-5 min at 500-1000 rpm and retest.

b. If this fails, clean the Optical Chamber (see Monthly maintenance procedures) and repeat Optics Check procedure.

c. If the value is still outside the acceptable range, call GEN-PROBE.

6. Documentation

a. Enter the ratio of Observed/Expected RLU/Sec Values on the GENPROPE LUMINOMETER MAINTENANCE LOG.

b. Document all corrective action on a QC DEVIATION FORM.

c. Supervisor will review results monthly.

B. Cleaning the Optical Chamber

MATERIALS

- GEN-PROBE Luminometer Leader 50 instrument
- Clean cotton swab
- Distilled water
- Lint-free lab wipes

1. Frequency: monthly and as needed
2. Procedure:
    a. Press the release button to raise the optical chamber cover.
    b. Turn the instrument power to "**OFF"** and remove the test tube from the optical chamber.
    c. With your finger, pull first the black ring and then the glass well liner out of the chamber. **Do NOT remove the spring assembly in the chamber**.
    d. Using a swab and distilled water, clean as much of the interior chamber suraface as possible. The spring can be pressed down with the swab to clean.
    e. Dry the chamber interior using lint-free lab wipes. Depress the spring to dry as much as possible. However, you do not have to remove every drop of water at the bottom of the liner below the spring.
    f. Wipe the outside of the glass well liner (to remove fingerprints) and replace into the optical chamber.
    g. Replace the black ring in the chamber. The rubber gasket must face upward.
    h. Place a clean empty tube in the chamber; close the cover.
    i. Document cleaning on GENPROPE LUMINOMETER MAINTENANCE LOG.
    j. Turn the instrument power to "**ON"**. Allow 20 minutes warm-up.
    k. Do the Optics Check with Tritium Standard (see weekly maintenance) to verify instrument performance.

C. Software Quality Control

MATERIALS

- GEN-PROBE Luminometer Leader 50 instrument
- List of authorized protocols

1. Frequency: After acquiring the instrument or creating a new protocol, and as a monthly check that protocols have not been changed.
2. Procedure: a. Obtain a printout of all protocols by pressing LIST: key in protocol number, ENTER

   b. Repeat for each protocol programmed in the instrument and compare with original list. Supervisor keeps original print-out list of protocols and any new authorized protocols signed in file cabinet.
3. Acceptable results: Print-out will be the same as the authorized list kept by supervisor. If not, inform supervisor and document on QC Deviation Form.
4. Corrective Action: Supervisor will reprogram instrument according to Operation manual and obtain a new print-out prior to running patient samples.
5. Documentation

   a. Document software program acceptability on GENPROPE LUMINOMETER MAINTENANCE LOG.

"AS NEEDED" MAINTENANCE

Directions for paper replacement and fuse replacement are located in the GEN-PROBE Luminometer Leader 50 manual by the instrument. Document fuse replacement on GENPROPE LUMINOMETER MAINTENANCE LOG.

GEN-PROBE LUMINOMETER LEADER^{TM} 50 OPERATION

MATERIALS

- GEN-PROBE Luminometer Leader 50 instrument
- Clean empty GEN-PROBE polystyrene tubes
- Lint-free lab wipes
- 2 clean empty tubes to contain ends of inlet lines
- Detection Reagent 1
- Detection Reagent 2

- 2 clean tubes of distilled water at room temperature in which to keep ends of inlet lines when luminometer is not in use

A. Instrument Startup/Precautions:

1.The luminometer requires an initial 20 minute warmup before use. ON/OFF switch is on back of machine.

2. NEVER allow tubes to overflow in the measurement chamber. Use a fresh empty tube for each wash cycle.

3. NEVER turn instrument OFF with Chamber Cover open.

4. ALWAYS leave a clean empty tube in the measurement chamber. DON'T CLOSE CHAMBER COVER WITHOUT A TUBE IN THE CHAMBER!

5. Keep measurement chamber cover CLOSED as much as possible. Excess light accumulation while cover is open can cause high background levels.

6. When instrument is not in daily use, keep inlet lines in separate tubes of distilled water instead of in bottles of Detection Reagents to avoid precipitants in the lines. Ensure instrument is flushed with distilled water after use of Detection Reagents, before turning off (see Operation). Ensure instrument is flushed with Detection Reagents again before use (see "Each Use" Maintenance).

B. Operation:

1. Before beginning the procedure record the positive and negative controls and the specimen identification numbers for each sample **in the order they will be tested** on the "Accuprobe Mycobacteria Position Worksheet." Ensure you have circled MTB or MAI as appropriate for the probe you are using.

2. Program functions are accessed thru the eight keys on the righthand pad. Progress through these functions and the assays as prompted and monitored on the display screen.

3. Wipe all sample tubes with a moist lint-free lab wipe before testing.

4. Remove the inlet lines from the distilled water; place each line into a separate clean empty tube.

5. Do three wash cycles to clear the lines of water (see Wash Cycle in "Each Use" Maintenance section)

6. Replace the inlet tubing into and tighten caps on the appropriate Detection Reagent bottles.

AFTER THE DETECTION REAGENTS ARE CONNECTED TO THE INSRUMENT THE WASH CYCLE SHOULD BE RUN THREE TIMES BEFORE ANY SAMPLE ANALYSIS IS PERFORMED. ALSO, IF THE INSTRUMENT HAS BEEN IN OPERATION BUT HAS BEEN IDLE FOR MORE THAN 30 MINUTES, ONE WASH CYCLE SHOULD BE PERFORMED

7. Press START key to begin the MEASURE protocol.

8. Screen display: CHECK REAGENT LEVELS. (Inform supervisor if they are low.) Press START again.

9. Screen display: WASH CYCLE 0=NO 1=YES

10. Before running samples, perform three wash cycles to prime reagent lines.

11. Press 1, and then ENTER key. Chamber lid will open.

12. Place a clean empty tube in the chamber and close lid. Wash cycle will proceed.

13. Discard first tube; repeat wash cycle with a fresh empty tube

14. At completion of second wash cycle, discard used tube.

15. Discard second tube; repeat wash cycle with a fresh empty tube.

16. At completion of third wash cycle, discard used tube.

17. Place a clean empty tube in the chamber. CLOSE the CHAMBER COVER. Press 0, then ENTER key to continue.

18. To bypass wash cycles, select 0 instead of 1.

19. Screen display: Operator

20. Press ENTER if displayed ID is correct.

21. To enter new ID, press 1. (DO NOT HIT ENTER!) Then press CLEAR key to delete display of previous ID.

22. Press <--- or ---> keys to move cursor to desired number or letter on screen display; press SELECT key (NOT ENTER) to accept each selection.

23. Press ENTER key ONLY when selection of the new ID is complete! (If ENTER is inadvertently pressed too soon then press RESUME and go back to step 5, but do not repeat the wash cycles).

24. Kit lot #: Press 1 for "yes", Key in kit lot number, ENTER. (If doing Protocol 10 or 11 for QC of Reagent Dispensing System using water then press 0 for "no")

25. Key in protocol number; press ENTER. Current protocol for mycobacteria is # 4: AP MYCOBAC.

26. Screen display: Select Data Mode: This screen confirms appropriate protocol has been selected. Press 0 to enter new data; press ENTER.

27. Screen calls for first sample. Insert first sample and close chamber cover. Instrument background check, reagent injection, RLU count, and result printing proceeds. RLU result is displayed on screen during assay. Chamber cover opens when test is complete.

28. If Error Message "High Background Count" appears, wipe tubes off again and retest, holding chamber cover closed gently.

29. Insert second sample; close chamber cover. Continue to process samples until all are complete.

30. When all tubes are completed, press STOP. DON'T CLOSE CHAMBER LID YET! See next step.

31. Screen display: Stop Protocol 1? Press 2, and ENTER (2=ABORT). Place a clean empty tube in the chamber and close the cover. Run is terminated and screen display will return to Main Menu.

32. Remove the inlet lines from the bottles of Detection Reagents. Place each line into a separate clean tube of distilled water.

33. Perform the wash cycle at least three times to flush the Detection Reagents from the lines. Use an empty tube for each cycle to avoid spillover in the optical chamber. Stop doing wash cycles when the foam in the tube is minimized.

34. After the last wash cycle insert a clean empty tube into the optical chamber.

35. Double check tube is in optical chamber before closing chamber lid.

36. Do not turn instrument off until tube is in chamber and lid is closed.

C. Reporting Results:

1. Write the RLU and interpretation for each specimen from the luminometer printout tape on the "Accuprobe Mycobacteria Position Worksheet", and give the tape and worksheet to the supervisor.

2. Enter QC organism results in the QC log.

REFERENCE:

Operator's Manual Leader 50i, Rev. D 5/19/99 Catalog #33121. Gen-Probe INC. Calif.

APPENDIX - GENPROPE LUMINOMETER MAINTENANCE LOG.