Updated November 06, 2015

Request for Proposals: Establishment of *Mycobacterium tuberculosis* complex (MTBC) Whole Genome Sequencing Reference Centers

Application Due date: December 3, 2015

Submit to: Anne Gaynor, Manager of HHST (Anne.Gaynor@aphl.org)

Summary

The Association of Public Health Laboratories (APHL), in cooperation with the U.S. Centers for Disease Control and Prevention (CDC) Division of TB Elimination (DTBE), is seeking to identify up to six state or local public health laboratories that will serve as reference centers to provide whole genome sequencing (WGS) of 16–64 samples of MTBC per month. The reference centers (WGS Reference Centers) will conduct one of the following approaches to WGS.

Option 1. Sequence samples provided by DTBE to support DTBE selected cluster investigations.

Option 2. Prospectively, a universal WGS surveillance pilot for a defined jurisdiction (e.g., city, county, region, state or multi-state).

Option 3. Provide a combination of both functions.

The WGS Reference Centers will serve as an extension of the CDC DTBE Laboratory Branch and will provide services that are complementary to those at CDC. Funding will be awarded via a contract with APHL.

Background

Since 2004, the Division of Tuberculosis Elimination has conducted genotyping surveillance of MTBC. Genotyping data is integrated with patient demographic and clinical data, and routinely analyzed to identify suspected outbreaks. The discriminatory power of conventional methods (i.e., spoligotyping and MIRU-VNTR) is sometimes insufficient and some suspected outbreaks identified by genotyping include a mix of outbreak and sporadic cases, or do not represent an outbreak at all. Genomic surveillance has the

power to increase the accuracy of outbreak detection systems. In collaboration with the Molecular Epidemiology Activity (MEA) in the DTBE Surveillance, Epidemiology, and Outbreak Investigations Branch, the results and interpretation of the data are shared with state and local TB programs, and experience and feedback from U.S. TB Control Programs demonstrate that WGS data result in more focused targeting of limited public health resources leading to more effective epidemiologic field investigations and an improved ability to identify where public health intervention will have the greatest impact. The demand for WGS to support cluster investigations currently exceeds capacity within the DTBE Laboratory Branch. To increase capacity, APHL in collaboration with CDC seeks to partner with state or local public health laboratories to provide WGS services (Option 1). Isolates from the National TB Genotyping Service Collection located at DTBE will be provided to the WGS Reference Center monthly. WGS Reference Centers will extract and sequence DNA from the samples and electronically deliver fastq sequence files to DTBE within two months using protocols developed in-house. CDC DTBE will only provide an example DNA extraction protocol (Appendix D).

Determining the optimal approach for supporting WGS services for TB Control Programs in the United States is essential. Supporting WGS through state and local public health laboratories, regional reference centers, or a single contract laboratory all have advantages and disadvantages that impact feasibility, turnaround time and cost. Factors determining best practices for conducting real-time, prospective sequencing of MTBC in a public health laboratory environment are not always apparent from retrospective sequencing in a research lab. To evaluate relevant approaches to WGS, APHL in collaboration with CDC seeks to partner with state or local public health laboratories to add prospective, real-time WGS services (Option 2). Applicants may propose any jurisdiction—city, county, region, or state—for which they will sequence one sample from culture positive TB cases as they are identified. The jurisdiction should have 192-768 culture positive cases/year. This work will provide the data necessary to evaluate WGS delivery models based on feasibility, cost and turnaround time. WGS Reference Centers will extract and sequence DNA from the samples and electronically deliver fastq sequence files to DTBE within three months of culture-confirmed MTBC using protocols developed in house. CDC DTBE will only provide an example DNA extraction protocol (Appendix D).

To meet both the increased demand for WGS from TB control partners, the increased demand for experience with WGS from our public health laboratory partners and the need for evaluation of WGS in a public health laboratory, APHL and CDC are seeking, through competitive announcement, to identify up to six state or local public health laboratories to perform WGS on 16–64 MTBC samples each month.

Eligibility

Eligible laboratories include all public health laboratories with the following capabilities and facilities in place. Specific expectations regarding the methodologies to be used by the WGS Reference Centers are outlined in Appendix A. No training will be provided but technical assistance and troubleshooting may be provided by teleconference. All applicants are required to agree to the minimum requirements outlined in Appendix B.

Established capacity for preparing cultures of MTBC

Established capacity for isolating chromosomal DNA from MTBC suitable for WGS

Established and demonstrated capacity for WGS of bacterial pathogens

Sufficient equipment, laboratory space and workforce capacity for the proposed testing volume

Anticipated RFP Schedule

APHL anticipates the following schedule:

October 28, 2015 – RFP issued

November 5, 2015 – Informational teleconference for RFP questions and answers

December 3, 2015 – RFP responses due

December 10, 2015 – Proposal review completed

December 10-17, 2015 – If needed, follow-up interviews and updated proposals due

December 17, 2015 – Final review completed and WGS Reference Center selected

January 15, 2016 – Contracts finalized and work begins

Any modification to this anticipated schedule will be communicated on APHL's procurement website (www.aphl.org/rfp) and via an email blast to the public health laboratories (PHLs).

Award

Funding will be distributed via a contract administered with APHL. Up to six laboratories, depending on strength of applications, funding requested and funds available will be selected. Awards could range from approximately $19, 200 (based on 16 specimens/month X 6 months X ~$200/specimen) to near $76,800 (based on 768 positive cultures in a year X 6 months X ~$200/specimen) based on estimated cost per specimen in proposals.

Term of Project

From date of contract signing (Approximately January 15, 2016) through June 30, 2016. APHL anticipates the potential for annual renewals (with each additional funding year running from July 1 to June 30) for a total of 4 additional years. Each of the potential renewals may involve some adjustment to the scope of work in order to address any change in the funding received by APHL and to accommodate CDC programmatic needs in that funding year. Each WGS Reference Center would be notified in advance of any modification to the anticipated scope of work in a future funding year.

Request for Proposals – Required Submissions

To submit a proposal for consideration as a MTBC WGS Reference Center, please respond to the following questions. Responses should be limited to no more than eight (8) double-spaced pages (font size ≥11 pt and page margins of ≥1 inch) and must comply with the submission requirements set out in Additional Information and Deadlines for Applications Submission below.

1. Please describe the current methodology used in your laboratory for preparing cultures of MTBC.
2. Please describe the current methodology used in your laboratory for isolation of chromosomal DNA from MTBC. Included information on how long the methodology has been in use, how often it is performed, your annual volume, the amount of experience your laboratory staff has in using that methodology, and any training your staff has received. Please describe how the methodology has been validated for providing chromosomal DNA of sufficient quality and quantity for WGS.
3. Please describe the current methodology for WGS of MTBC or other bacterial pathogens in your laboratory. Include information on which assay is used, how long the methodology has been in use, how often it is performed, your annual maximum volume, the amount of experience your laboratory staff has in using that methodology and any training your staff has received.
4. Please describe any existing infrastructure that could be utilized for this project including equipment. Please provide a description of the library and sequencing kits that will be used for this project.
5. Please describe the WGS option selected for this RFP (i.e., prospective, referred from CDC, or combination) and if prospective in nature, the proposed sample set. Include information on the number of samples that would be sequenced each month (minimum of 16 and a maximum of 64) and attach a letter of support from the jurisdiction's TB control program. If providing sequencing of samples from CDC to support outbreak investigations (Option 1), please provide the number of samples that would be sequenced each month (minimum of 16 and maximum of 32). If providing prospective universal WGS for a jurisdiction (Option 2), please define the jurisdiction, provide the number of culture positive cases in that jurisdiction for the year 2014, and describe the planned mechanism for obtaining and identifying those samples for. If isolates will be obtained from other laboratories, please describe this relationship and attach a letter of support from collaborating laboratories.
6. Please describe the plan for DNA extraction and sequencing. Provide a description of the proposed DNA extraction workflow. Provide a description of the proposed library and sequencing workflow.
7. Provide a 6-month budget outlining at least the following line items: a proposed per sample cost that includes reagents, staff time, and any other charges and overhead.
8. Include a signed copy of Appendix B as an attachment.

Evaluation Team

APHL staff, led by the HHST Program Manager, will conduct an initial review of all proposals for completeness. Any incomplete application on the proposal due date specified in Anticipated RFP Schedule section above will not be considered and will not receive a formal evaluation. Complete proposals will be reviewed by a team of three (3) subject matter experts (SMEs) from CDC DTBE and a panel of three (3) APHL members selected from non-applicant public health laboratories. SMEs from CDC will be identified and selected by the Branch Chief of the DTBE Laboratory Branch based on their familiarity with laboratory techniques and project requirements. APHL member experts will be identified from among the non-applicant PHLs by the APHL HHST Program Manager and will have expertise in the laboratory testing methods described in this RFP and familiarity with APHL reference center structure. Once potential reviewers have been identified, APHL's Director of Infectious Disease Programs will have final approval over the review team's composition.

Evaluation Criteria

Proposals will be evaluated based on the responses to the questions above and will receive a numeric score of up to 100 maximum points based on the scorecard template in Appendix C.

Laboratories will be given preference based on more extensive experience with the test methods, ability to handle increased test volume for WGS, existing in-house subject matter expertise, ability to comply with expectations laid out in Appendix A and ability to meet the minimum expectations outlined in Appendix B.

Evaluation Process

The entire review will be conducted via a combination of email communication between APHL's HHST Program Manager and the members of the evaluation team or among the evaluation team members and teleconference and/or webinar evaluation sessions. APHL's HHST Program Manager will coordinate the review process and the evaluation sessions.

The reviewers may request follow-up interviews with all or some of the applicant laboratories and, following these interviews, may request supplemental information on an applicant's proposal. These interviews and any supplemental information would clarify a laboratory's capacity or experience in one or more of the evaluation criteria or to explain other information contained in an applicant's proposal.

There will be no formal evaluation performed by a member of APHL staff. In cases where all other evaluation criteria are substantially similar, APHL will have the ability to advise the evaluation team on selections that would provide geographical spread or otherwise diversify APHL's funding allocations. In addition, the evaluation team may receive documentation from APHL staff on an applicant's past performance in other capacities noted in this RFP as part of the evaluation criteria.

Post-Evaluation Procedures

The selected laboratories will be notified by APHL staff within ten (10) business days of the completion of the evaluation and the names of the recipients will be posted to APHL's procurement website, www.aphl.org/rfp on the same day. Unsuccessful applicants will receive notification of these results by e-mail or by U.S. mail within 30 days of the date the name of the winning vendor is posted.

All applicant laboratories will be entitled to utilize APHL's RFP Appeals Process to formulate a protest regarding alleged irregularities or improprieties during the procurement process. Specific details of this policy are located on the procurement website.

Conditions of Award Acceptance

- The eligible laboratories must be able to contract directly with APHL or have an existing relationship with a third-party organization that can contract directly with APHL on behalf of the laboratory.
- Laboratories must agree to comply with expectations outlined in Appendix A.
- Prior to making the official award, a group of individuals from CDC and APHL reserve the right to tour the facilities to assess compliance with requirements for testing and/or have a teleconference with applicant laboratories. Post award, monitoring site visits may be conducted to include an assessment of continued compliance.

Additional Information and Deadlines for Application Submission

All questions should be directed to Anne Gaynor at anne.gaynor@aphl.org. Questions received from interested PHLs, together with the answers provided by APHL or CDC staff will be posted to APHL's procurement website (www.aphl.org/rfp).

Applications should be submitted to Anne Gaynor at APHL (Anne.Gaynor@aphl.org; 8515 Georgia Ave Suite 700, Silver Spring, MD, 20910; telephone: 240-485-2739; fax: 240-485-2700). For electronic submission, copy Kelsey Vellente (Kelsey.Vellente@aphl.org). Applications must be received at APHL, attention Anne Gaynor by close of business (5:00pm ET) December 3, 2015. Either electronic or physical submission is acceptable. APHL will send an email acknowledging the receipt of your application; if you do not receive an acknowledgement within 48 hours, call 240-485-2739 to confirm receipt.

An optional informational teleconference will be held Thursday November 5, 2015 at 2:00 pm ET. The purpose of this call will be to provide a brief overview of the project and to allow potential applicants to ask CDC and APHL questions. Please come with questions prepared.

For the teleconference there are a limited number of lines, please use only one (1) line per laboratory.

Phone: 866.822.6061 Passcode: 858376#

Appendix A: Expectations for MTBC Sequencing Reference Centers

Methods

1. Prepare a frozen stock of each culture sent to the laboratory from CDC for WGS.
2. If preparing DNA libraries using the Nextera XT kit from Illumina, it is recommended but not required to use the no-drug positive control MGIT tube used for drug susceptibility testing to extract the chromosomal DNA.
3. It is necessary to extract chromosomal DNA from MTBC for WGS. Several methodologies may be found in the literature. The protocol used by the DTBE Laboratory Branch may be found in Appendix
4. The Illumina MiSeq/NextSeq/HiSeq are the selected sequencing platforms for the MTBC sequencing reference centers. Paired end sequencing with read lengths of at least 100 bp is required.

Procurement

The cost of reagents will be incorporated into per specimen reimbursement. There will be no financial support for the procurement of equipment.

Turnaround Time

Fastq sequence files for samples provided by CDC (retrospective) should be provided to CDC within 2 months of receipt at laboratory. Fastq sequence files for samples sequenced for prospective universal WGS should be delivered to CDC within three months of culture-confirmed MTBC.

Data Management

FASTQC or a similar program should be used to calculate and report cluster density, Q30, total sequences per run and theoretical coverage. Minimum acceptable quality scores are those defined in the specifications for the sequencing kit. Minimum acceptable theoretical depth of coverage is 40X for each sample. Data and fastq files will be transferred to CDC electronically.

Performance Management and Evaluation

Performance will be monitored by timeliness of responses to CDC and APHL requests and successful completion of a proficiency panel. The reference center must submit electronic notices of data transfer to APHL and CDC.

<u>Reports</u>

The laboratory will submit to APHL and CDC a monthly line listing of the samples tested and the following variables: sample name or species name, date diagnostic culture was positive, date isolate received in laboratory, type of culture received and type of culture used for DNA extraction (MGIT, LJ, etc.), date of DNA extraction, date sequencing complete, total sequences, theoretical coverage, percent coverage (calculation method available upon request if awarded) and date of fastq transfer to CDC.

The laboratory will submit to APHL and CDC a Sequence run report to include the following information: sequence run identifier, cluster density, clusters passing filter (%), bases >Q30 (%), estimated sequence yield (MTBC)

Site visits and teleconferences

APHL in collaboration with CDC will perform 1-2 site visits as needed. Additional monitoring visits may be needed based on data review and any ongoing challenges mutually identified. Site visits could include data review, review of laboratory workflow, procedural observation, QC information, worksheets for DST, and review of programs (i.e., raw sequencing data).

APHL, CDC and the WGS Reference Centers will participate in monthly teleconferences to review monthly reports, assess successes and challenges and discuss potential resolutions.

Appendix B: MTBC Sequencing Reference Center Minimum Requirements

YES	NO	MINIMUM REQUIREMENT
		Does your laboratory have the infrastructure in place or the ability to adapt it to prepare cultures of *M. tuberculosis*?
		Does your laboratory have the infrastructure currently in place or the ability to adapt it to perform isolation of *M. tuberculosis* chromosomal DNA?
		Does your laboratory have the infrastructure currently in place or the ability to adapt it to perform WGS of bacterial pathogens?
		Does your laboratory have sufficient workforce capacity for the testing volume proposed?
		Does your laboratory have a letter of support from the TB Control Program for the proposed work?

Appendix C: MTBC Sequencing Reference Center RFP Score Card

The following table is a copy of the score card that will be used to evaluate RFP responses.

Category	Maximum Value	Score	Comments (REQUIRED)
Does the applicant have sufficient capacity and experience to prepare cultures of MTBC? *Evaluate proposed methodology for preparing subcultures of MTBC. Consider the training and experience of existing staff.* Yes (10 points), No (0)	10		Type comments here. (REQUIRED)
Does the applicant have sufficient capacity and experience performing extraction of chromosomal DNA from MTBC to comply with the requirements described in Appendix A of the RFP? *Evaluate proposed methodology for DNA extraction. Consider if the methodology provides DNA of sufficient quantity and quality for WGS, consider training and experience of existing staff.* High: routinely (>10 extractions/month) isolates chromosomal DNA from MTBC and has demonstrated (or others have demonstrated) that the DNA is of sufficient quality and quantity for the proposed sequencing method (21-25 points) Moderate: does not routinely isolate chromosomal DNA from MTBC but has a validated protocol in place and existing staff with experience and training (11-20 points), Limited: does not have a validated protocol for isolating chromosomal DNA from MTBC but demonstrates potential capacity by modifying existing protocols (1-10 points), No experience = 0	25		Type comments here. (REQUIRED)
Does the applicant have sufficient capacity and experience performing WGS of MTBC or other bacterial pathogens to comply with the requirements described in Appendix A of the RFP? *Evaluate their experience with sequencing* MTBC *or other bacterial pathogens. Consider number of bacterial pathogens sequenced each month, experience and training of existing staff.*	25		Type comments here. (REQUIRED)

High: routinely sequences> 50 bacterial pathogens/month (21 -25 points), Moderate: routinely sequences 10-50 bacterial pathogens/month (11 – 20 points) Limited: does not routinely sequence bacterial pathogens but has some experience sequencing bacterial pathogens, and existing staff do have some training and experience (1-10 points), No experience (0 points).			
Does the applicant have a clear plan for meeting the requirements? Has the applicant identified a jurisdiction for prospective universal WGS for TB cases, defined the number of culture positive cases in the jurisdiction, and defined a mechanism for obtaining the cultures, described how isolates shipped from CDC will be received? Has the applicant described a plan for the extraction of chromosomal DNA? Will samples be batched? How often will DNA be extracted and how will DNA concentration be determined? Has the applicant described a plan for sequencing? Does the plan describe the library and sequencing kit that will be used? Is the library kit compatible with the DNA purification protocol? Have the number of samples per sequencing run been defined and will this allow for the required 40X coverage of the MTBC samples? Will MTBC samples be sequenced with other bacterial pathogens? Will the proposed workflow meet the turnaround time expectations outlined in Appendix A? High likelihood of success (31-40 points), Medium likelihood of success (11 – 30 points), Low likelihood of success (1-10 points), No (0 points)	40		Type comments here. (REQUIRED)
TOTAL SCORE	**100**	**_____**	

Appendix D: Example DNA Extraction Protocol

	Division of Tuberculosis Elimination Applied Research Laboratory		
Mycobacterium tuberculosis **complex (MTBC) DNA Isolation Using the ZR Fungal/Bacterial DNA MicroPrep Kit**			
Doc. No. ART.DR.C.0005	**Rev. No.** 02	**Effective Date:** 10/14/2014	**Page 1 of 12**

1.0 **Purpose/Principle**

The ZR Fungal/Bacterial DNA MicroPrep Kit is used for the simple, rapid isolation of DNA from M. *tuberculosis.* The purified DNA can be used for downstream molecular-based applications.

2.0 **Scope**

This procedure is used by the ART personnel for making MTBC DNA preparations by this method.

3.0 **Related Documents**

Title	Document Control Number
DNA Preparation Worksheet using the ZR Fungal/Bacterial DNA MicroPrep Kit	ART.DR.C.0005.F01
Guide for DNA Isolation Using the ZR Fungal/Bacterial DNA MicroPrep Kit	ART.DR.C.0005.J01
Training Checklist for the ZR Fungal/Bacterial DNA MicroPrep Kit	ART.HR.F.0007
ISAW Camera Operation	MLB.EQ.C.0002

4.0 **Responsibility**

Position	Responsibility
ART Staff	Only staff trained and cleared to work in the BSL-3 area and trained to perform this procedure may do so.
ART Team Lead or Designee	Oversees all ART activities.

5.0 **Definitions**

Term	Definition
BSL	Biological Safety Level - MTBC isolate manipulations must be done in the BSL-3 laboratory using good laboratory practices and wearing appropriate PPE for the job.
DSR	Division of Scientific Resources
FSE	Four Seasons Environmental Services is responsible for maintaining, monitoring and repairing both the building systems and laboratory (freezers and refrigerators) equipment.
MTBC	*Mycobacterium tuberculosis* complex includes *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, *M. microti*, *M. caprae*, *M. pinnipedii*, *M. mungi*, *M. orygis* and *M. canetti*.
PAPR	A powered air purifying respirator consists of a facepiece, breathing tube, battery-operated blower, and particulate filters. The PAPR uses a blower to pass contaminated air through a HEPA filter which removes the contaminant and supplies purified air to a facepiece.

6.0 Equipment

Item	Any required maintenance - See ART General Equipment Calibration and Maintenance (ART.EQ.C.0002) for additional information.
Aerosolve Canisters or Equivalent	Used for centrifuging viable biological agents in the Allegra centrifuge. A base that holds a tube rack with a cap and O-ring seal.
Allegra 6R Bench Top Centrifuge with Rotor GH-3.8 Horiz with 4 Arm Bkt or Equivalent	See separate procedure for maintenance requirements**.**
Autoclave	FSE is responsible for autoclave maintenance. Any run that has a process failure must be re-autoclaved after the problem is resolved, unless failure occurred while opening the door on a process complete run. A biological indicator is tested monthly as part of the monthly autoclave monitoring program (LB and DSR staff). The BSL-3/3+ laboratory area has a pass-through autoclave.
Biological Safety Cabinet (BSC)	Magnehelic gauge reading and direction of tape on glass shield is recorded before using BSC. Decontaminate the BSC work surfaces before and after each use. BSCs are re-certified each year by a qualified technician. Motor or filter problems must be corrected and re-certified before the BSC can be used. Contact ESHCO for BSC issues.
Camera (ISAW)	See the *ISAW Camera Operation* (MLB.EQ.C.0002*)* for additional information.
Eppendorf 5418 Microfuge 120V or Equivalent	See separate procedure for maintenance requirements**.** In *BSL-3+ laboratory, the microfuge is equipped with an aerosol rotor.*
MPBio Fast Prep 24	FastPrep 24 should be checked for damage before using.
Freezer	Set to -19 to -25°C. Freezer should be defrosted if frost/ice buildup interferes with storage useable space. Four Seasons Engineering services (FSE) is responsible for changing filters on each unit. The freezer temperature is monitored by FSE.
Freezer (Ultralow)	Freezer set at -65^{o} to -75 ^{o}C. Freezer should be defrosted if frost/ice buildup interferes with storage useable space. The unit must be wiped down with a tuberculocidal disinfectant whenever contaminated, defrosted (at least annually), moved, or if repairs are needed. Freezer temperatures are monitored by FSE.
Incubator	Incubator set at 35 to 38^{o}C. Incubator should be wiped down with a tuberculocidal disinfectant annually or whenever contaminated, moved, or if repairs are needed. The incubator temperatures are monitored by FSE.
Micropipettes	Calibration and checks for any needed repairs are done

	annually.
Refrigerator	Set to 2° to 8°C. Refrigerator should be wiped down with a tuberculocidal disinfectant whenever contaminated, moved, or if repairs are needed. Refrigerator temperatures are monitored by FSE.
Spectrophotometer (Nanodrop 2000 or Qubit 2.0)	
Vortex mixer	Clean if spill occurs.

7.0 Reagents and Media

Item	Storage requirements
Middlebrook 7H9 Broth (6 mL)	Store at 2 to 8°C. May be used until expiration date. Either made by DSR or by ART staff.
Nuclease free water	Store at RT.
Tuberculocidal Disinfectant (Vesphene or Lysol III)	Store at RT. May be used until expiration date.
ZRFungal/Bacterial DNA MicroPrep Kit	Store at RT. Integrity of kit components is guaranteed for up to one year from date of purchase.

ZR Fungal/Bacterial DNA MicroPrep Kit Components (50 preps.)	Quantity
Collection Tubes	150
DNA Elution Buffer	10 ml
DNA Pre-Wash Buffer*	15 ml
Fungal/Bacterial DNA Binding Buffer**	100 ml
Fungal/Bacterial DNA Wash Buffer	50 ml
Lysis Solution	40 ml
ZR BashingBead Lysis Tubes	50
Zymo-Spin IC Columns	50
Zymo-Spin IV Spin Filters (orange tops)	50

* A precipitate may have formed in the DNA Pre-Wash Buffer during shipping. To completely re-suspend the buffer, incubate the bottle 30 – 37 ˚C for 30 minutes and mix by inversion. DO NOT MICROWAVE.
**For optimal performance, add beta-mercaptoethanol to 0.5% (v/v) i.e., 500 µL per 100 mL

8.0 Supplies, Other Materials

Item
1.5 mL microtube
10 mL serological pipette
15 mL polypropylene centrifuge tubes
Appropriate PPE for the BSL-3+ and BSL-2 laboratories
Biological safety container or carrier (to move preparations from BSL-3 to BSL-2)

Electronic pipetting device
Screw cap glass test tube
Sterile filtered pipette tips
Test tube rack

9.0 **Safety Precautions**

9.1. *BSL-3+ Laboratories*:

9.1.1. Viable *Mycobacterium tuberculosis* is an infectious agent and must be handled with care by trained personnel working under appropriate conditions.

9.1.2. All procedures involving manipulation of live organisms (i.e., inoculum preparation, making dilutions, and inoculations of media) must be performed in a BSC (or equipment with aerosol containing devices) in a BSL-3+ laboratory using BSL-3 practices. Use appropriate PPE (scrubs, gown, PAPR, gloves, dedicated shoes, shoe covers and hair cap).

9.1.3. All containers and equipment must be sprayed with tuberculocidal disinfectant (Lysol III) before removing from the BSC.

9.1.4. Tubes should be contained within aerosolve containers or similar biosafety devices for all centrifugation steps. **Following centrifugation, these containers should be transferred into the BSC (microfuge is used in the BSC) before they are opened.**

9.1.5. *Recommended set up for BSC*: Items are arranged in the BSC to avoid contaminated items being passed over clean items. Items or equipment in direct contact with the etiologic agent remain in the BSC until surface decontaminated.

9.1.6. All biological waste is placed in a discard pan lined with a biohazard bag for autoclaving.

9.2. *BSL-2 Laboratories*:

9.2.1. A lab coat and gloves is worn when working in a BSL-2 laboratory with DNA preparations.

9.3. See the LB Site Specific BioSafety Document for PPE exceptions, biological spills, waste disposal and other biosafety information.

9.4. *PAPR requirements*

9.4.1. Inspect the breathing tube, body of the HEPA filter, hood for damage before using.

9.4.2. Use a battery that has sufficient charge for the activities to be performed.

9.4.3. Check for adequate airflow prior to use. Attach the breathing tube to the PAPR outlet then turn on the power. Insert the tapered end of the airflow meter.

9.4.4. The ball in the airflow meter should reach the level for the type of PAPR used (4 cfm for facepiece and 6 cfm for hood).

9.5. Verification testing has been done to show that MTBC organisms, at the volumes used in this procedure, are inactivated or depleted at the completion of the steps in this method. **If greater volumes or modifications are made to this method, verification testing must be performed. See the *Verification of An Inactivation or Depletion Method* procedure (ART.DR.C.0009) used by ART for MTBC Organisms.**

10.0 **Sample Information / Processing**
Frozen aliquot of MTBC organism of interest.

11.0 **Quality Control** – Not Applicable

12.0 **"Two Key" Approach to Critical Control Point**

12.1. The critical control point (step) for inactivation of the sample is the Fast Prep step.

12.2. To document this critical step, the ART staff member performing the procedure activates the camera to video Fast Prep steps. See the *ISAW Camera Operation* (MLB.EQ.C.0002) procedure for camera operation.

12.3. At the completion of the critical step, turn the camera off. Press the silver button on the top of the black box to transfer video.

12.4. Once BSL-3 work is complete, DNA samples are stored in the refrigerator in room 4079CC until the video is reviewed and the *DNA Preparation Worksheet using the ZR Fungal/Bacterial DNA MicroPrep Kit* (ART.DR.C.0005.F01) is signed off by the team lead or designee.

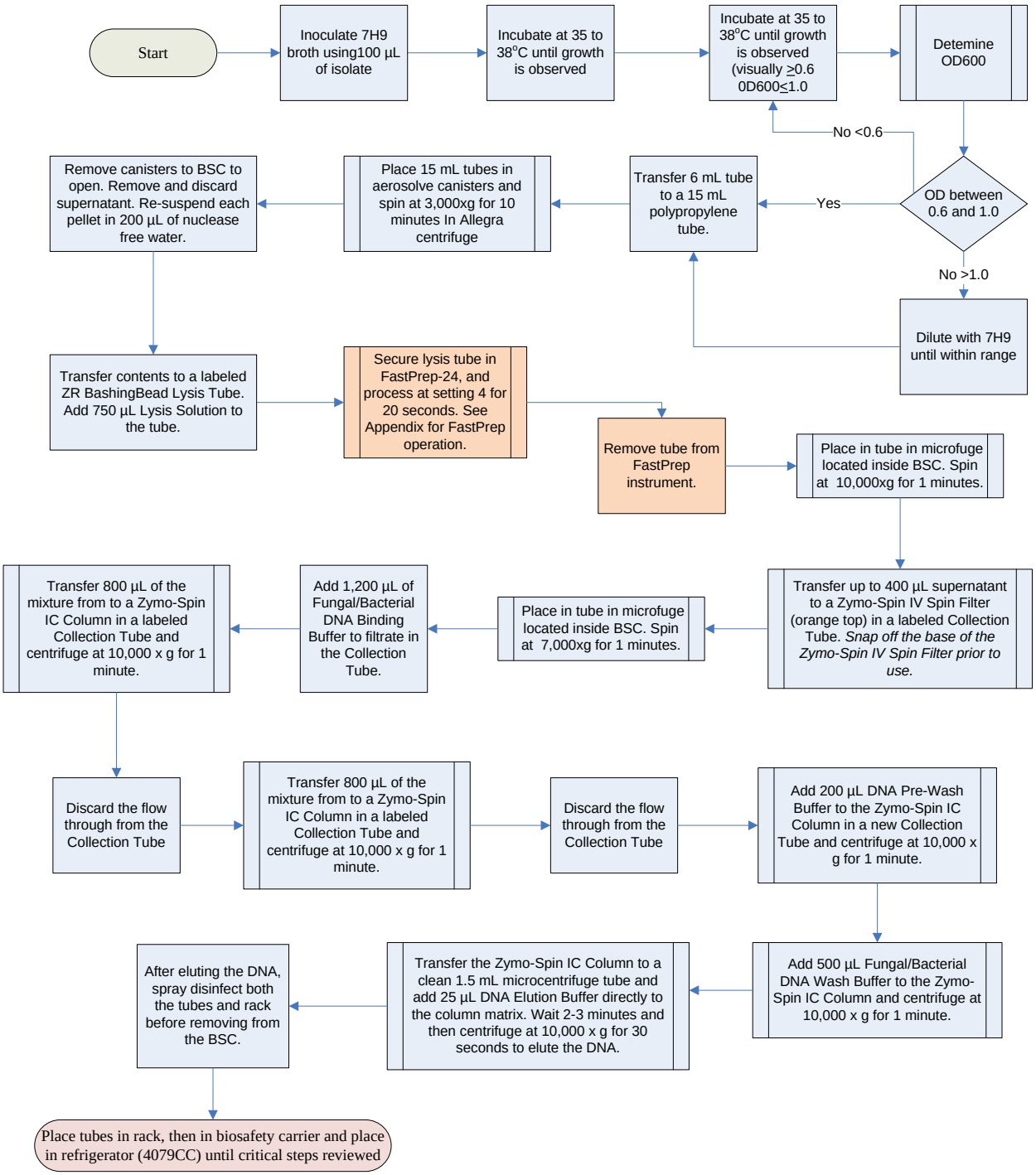
12.5. *Documentation of the critical step*:

12.5.1. The staff member attests that the materials were prepared (in the BSL-3+) according to the stated protocol on the worksheet.

12.5.2. The worksheet is scanned into the computer in room 4085 and emailed to the ART team lead or designee.

12.5.3. The ART team lead or designee documents on a copy of the scanned worksheet that they have reviewed the video and the samples can be moved to BSL-2.

13.0 Workflow Chart



Critical Steps in orange must be videoed.

14.0 Procedure

Step	Action
The following Steps are Performed in a BSL-3 Lab Wearing Appropriate BSL-3 PPE	
1	Working in a BSC, inoculate 100 µL of a frozen stock (thawed) to a labeled 7H9 broth (6 mL). *Return the aliquot stock to the freezer when BSL-3+ work is complete.*
2	Spray the tubes and the rack with tuberculocidal disinfectant before removing from the BSC. Place the rack inside the plastic container on the orbital shaker inside the 35 to 38˚C incubator. Proceed to step 3 when slightly turbid growth is observed.
3	When it appears to have achieved OD600 remove broth tube from incubator and proceed to step 4.
4	Determine OD600 using spectrophotometer using the 7H9 broth tube.
4.1	If OD600 is 0.6 to 1.0, proceed with purification (step 5).
4.2	If OD600 is <0.6, return broth tube to incubator until sufficient OD is achieved.
4.3	If OD600 is >1.0, dilute with 7H9 broth until OD is between 0.6 to 1.0. Proceed with purification (step 5).
4	Transfer 6 mL of culture to a labeled 15 mL polypropylene centrifuge tube. Spray disinfect the centrifuge tubes before removing from the BSC.
5	Place the centrifuge tubes in aerosolve canisters in the centrifuge making sure to balance the tubes. Replace the aerosolve caps. Ensure that swinging buckets are properly seated and spin at approximately 3,000 x g for 10 minutes to pellet the growth.
6	After centrifugation, the aerosolve canisters must be opened in the BSC. Remove the supernatant with a 10 mL pipette and discard into biohazard discard pan.
7	Re-suspend each pellet in 200 µL of nuclease free water and transfer contents to a labeled ZR BashingBead Lysis Tube. Add 750 µL Lysis Solution to the tube. Spray disinfect the lysis tube before removing from the BSC.
Start of '2 key' Critical Step	
8	Turn on the camera to record the critical step. (See appendix 23.2 for video recording steps.) See the *ISAW Camera Operation* (MLB.EQ.C.0002) procedure for detailed instructions on the camera/router/laptop operations.
9	Secure lysis tube in FastPrep-24. Distribute evenly amongst the available holes allowing at least one empty hole between tubes. Process at setting 4 for 20 seconds. See Appendix 23.1 for FastPrep operation.
10	Remove tube from FastPrep.

11	Turn off the camera. Press the silver button on the top of the black box to transfer video. **Seven minutes after the silver button is hit, the camera can start a new video if necessary.**
End of '2 key' Critical Step	
12	Place in microfuge located in BSC and centrifuge at 10,000 x g for 1 minute.
13	Transfer up to 400 µL supernatant to a Zymo-Spin IV Spin Filter (orange top) in a labeled Collection Tube. *Snap off the base of the Zymo-Spin IV Spin Filter prior to use.*
14	Centrifuge at 7,000 rpm for 1 minute.
15	Add 1,200 µL of Fungal/Bacterial DNA Binding Buffer to filtrate in the Collection Tube from Step 13.
16	Transfer 800 µL of the mixture from Step 15 to a Zymo-Spin IC Column in a labeled Collection Tube and centrifuge at 10,000 x g for 1 minute.
17	Discard the flow through from the Collection Tube and repeat Step 16.
18	Add 200 µL DNA Pre-Wash Buffer to the Zymo-Spin IC Column in a new Collection Tube and centrifuge at 10,000 x g for 1 minute.
19	Add 500 µL Fungal/Bacterial DNA Wash Buffer to the Zymo-Spin IC Column and centrifuge at 10,000 x g for 1 minute.
20	Transfer the Zymo-Spin IC Column to a clean 1.5 mL microcentrifuge tube and add 25 µL DNA Elution Buffer directly to the column matrix. Wait 2-3 minutes and then centrifuge at 10,000 x g for 30 seconds to elute the DNA.
21	After eluting the DNA, spray disinfect both the tubes and rack before removing from the BSC.
22	When ready to leave the BSL-3+ lab, change outer gloves and place rack in biological safety carrier (on cart outside BSL-3+ lab). *At this point, the worksheet is signed for the BSL-3+ steps and scanned into the computer in room 4085.*
23	Email the ART team lead or designee requesting review of the video.
24	Place biological safety carrier in the refrigerator located in room 4079CC on the shelf labeled "ART DNA or Whole Cell Lysate Sample Storage - Pending Review of Critical Steps." **The video must be reviewed and the critical steps confirmed before the samples can be removed from the BSL-3 area.**
Documenting Review of the Critical Steps	
25	The team lead or his designee reviews the critical steps on the video.
25.1	*If the critical steps were performed correctly*, the team lead or designee signs and dates the emailed worksheet.
25.2	*If the critical steps were not performed correctly,* the samples must be

	discarded as biohazardous waste. Follow up actions are documented on the worksheet. **A review of the procedure and/or additional training is done before the individual can perform this procedure unsupervised.**
26	The file name of the video is changed to indicate the assay performed/tech.
27	The reviewed worksheet is kept in the office of the team lead for a minimum of 2 years.
If Critical Steps were Performed Correctly	
28	The team lead or designee notifies the person performing the assay that they can remove the samples to the BSL-2.
29	Place tube in either a 2 to 8^{o}C refrigerator (short term storage) or a $\leq$-20 ^{o}C freezer until ready to use.
30	Return the biological safety container to the BSL-3+ area.

15.0 **Method Performance Specifications -** Not Applicable

16.0 **Calculations** - Not Applicable

17.0 **Reference Values, Alert Values -** Not Applicable

18.0 **Interpretation of Results -** Not Applicable

19.0 **Results Review and Approval -** Not Applicable

20.0 **Reporting Results; Guidelines for Notification -** Not Applicable

21.0 **Sample Storage**
21.1. Short term storage is at 2 to 8°C
21.2. Long term storage is at $\leq$ -19°C

22.0 **References**
22.1. MPBio website Fast Prep-24 Instrument Product Information section. 2011.
22.2. MPBio Fast Prep Users Guide **(www.MPBio.com/product_info)**
22.3. ZR Fungal/Bacterial DNA MicroPrep Kit Instruction Manual. Ver. 1.0.4.

23.0 **Appendices**
23.1. **Fast Prep Operation (MPBio Fast Prep Users Guide)**
23.1.1. *Fast Prep Information*:
23.1.1.1. The FastPrep®-24 Instrument is a high-speed bench top homogenizer for the lysis of biological samples. The FastPrep®-24 Instrument uses a unique, optimized motion to

disrupt cells through the multidirectional, simultaneous beating of specialized Lysing Matrix beads on the sample material.

23.1.1.2. Specifications Controls: Microcontroller control panel, with LCD screen and membrane-printed keyboard

23.1.1.3. Microcontroller clock 16 MHz Time: 1- 60 seconds, programmable in 1 sec increments Speed: 4 - 6.5 m/s, programmable in 0.5 m/s increments

23.1.1.4. Acceleration: <2 seconds to maximum speed

23.1.1.5. Deceleration: <2 seconds to stop

23.1.1.6. Duty cycle: 6.5 m/s for 60 seconds with 60 seconds rest period between runs

23.1.1.7. Noise level: <70dB

23.1.1.8. Standard environmental temperature operating range: 4 – 40 °C

23.1.1.9. Safety features: automatic stop on lid opening; emergency stop button; sealing of the sample handling compartment

23.1.2. *Controls*

Set Key	Press this key to select: Speed - 4.0 m/n to 6.2 m/s (Default 4.0 m/s) Time – 1 sec to 60 sec (Default 20 sec)
Run Key	To start or stop the instrument.
▲ Key	To increase the selected value of speed, tube holder & time.
▼ Key	To decrease the selected value of speed, tube holder & time.

23.1.3. *Loading and securing the samples*

23.1.3.1. Remove the securing knob that holds down the sample holder by rotating counter-clockwise.

23.1.3.2. Remove the sample holder from the instrument.

23.1.3.3. Slightly lift the spoke plate (top portion of the sample holder) and rotate it clockwise until the retention spokes move away from the hole openings.

23.1.3.4. Load the sample tubes into the sample holder wells, so that they fit snugly. Make sure that the tubes are balanced.

23.1.3.5. Replace the sample holder in the instrument making sure to align the aluminum locking pin, on the aluminum assembly below the tube holder, to the hole in the tube holder.

23.1.3.6. Rotate the spoke plate counter-clockwise until the retention spokes are above each tube.

23.1.3.7. Replace the securing knob and tighten by rotating until secure.

23.1.3.8. Close the dome and secure the dome clip.

23.2. **Video Recording**

See (*MLB.EQ.C.0002) ISAW Camera Operation* for Detailed Setup Instructions for the Camera/Router/Laptop	
1	Check that the router is plugged in and turned on. Router lights should be lit if on.

2	Check the camera status and turn on if asleep. • *Awake* – the display on the back of the camera will show a picture. • *Asleep* – press the top button on camera briefly and firmly to change camera to awake status.
3	View the picture display to confirm that the camera location will capture the activity(s) that need to be recorded. If not, adjust the camera angle until correct. *Make sure that the location will not interfere with the activities to be performed.*
4	When ready to record make sure camera is awake. (See (MLB.EQ.C.0002) ISAW Camera Operation step 4 if no picture is displayed on back of camera).
5	When the camera is awake, press the top button on the camera and recording will start. The camera is recording when the timer begins recording and a red dot is seen in the lower right area of the display. **Do not bump or disturb the camera while recording.** *If a timer, thermometer or another item needs to be recorded, position the item until it is in view. If a critical step to be recorded involves a reagent, the reagent container should have the name of the reagent displayed.*
6	When finished recording, hold down the top button briefly (2 -3 seconds) to turn the camera off.
7	Press the silver button on the top of the black box. The video will transfer to the laptop to the predetermined directory for viewing. One hour of video transfers in approximately 1.5 minutes, 2 hours in 2.5 minutes, etc. ***Seven minutes after the silver button is hit, the camera can start a new video.***

24.0 Revision History

Rev #	DCR #	Changes Made to Document	Author	Date
01	2014-06	New document	Paige Chopra	7/29/2014
02	2014-31	Modified workflow charts and procedural steps to document storage of DNA samples in BSL-3 until after review of the critical step video. Added procedural steps of what occurs if critical steps not performed correctly. Added worksheet storage requirements. Added camera operation to appendices.	Lois Diem (Revised)	10/10/2014

25.0 Approval Signatures

Approved By: _____ Date: _____
Author or Revised By

_____
Print Name

Approved By: _____ Date: _____
Team Lead or Designee

_____
Print Name

Approved By: _____ Date: _____
QMS Officer

_____
Print Name