

SATREPS project:

CONTROL OF TUBERCULOSIS AND GLANDERS

Activity report from the Research Institute of Tuberculosis

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Objectives

- To isolate Mycobacterium tuberculosis var. bovis from human with MGIT and Löwenstein-Jensen (L-J glycerol and pyruvate) in the national tuberculosis reference laboratory in National Centre for Communicable Disease (NCCD)
- To identify M. tuberculosis var. tuberculosis and perform drug susceptibility testing (DST) including newly introduced drugs for new WHO regimens
- To introduce next generation sequencing technology into NCCD laboratory
- To perform genotyping of M. tuberculosis in NCCD laboratory
- To develop standard operational procedure (SOP) to identify M. tuberculosis variants
- To perform risk assessment of M. bovis infection among Mongolian people based on the isolation from clinical specimens
- To perform TB screening with LAMP method and IGRA test to workers in slaughterhouses
- To train NCCD trainees for next generation sequencing technologies in RIT

Inputs

The Research Institute of Tuberculosis (RIT) has implemented the followings

1. Regular on-line meeting

1. Regular on-line meetings with mainly three members (Dr. Buyankhishig, Dr. Oyuntuya and Prof. Mitarai) were held since 2020. The progresses and problems of the project was discussed in the meetings.

Inputs

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2. Standard operational procedure development

- Standard operational procedures development is one of the key components of this technical transfer programme. Three different types of SOPs are developed until now.

2.1 SOP for Löwenstein-Jensen (L-J glycerol and pyruvate)

Though *Mycobacterium tuberculosis* var. *bovis* (*M. bovis*) grows on normal Löwenstein-Jensen (L-J) medium with glycerol as carbon source, the use of L-J medium supplemented with pyruvate is recommended.

For the better isolation of *M. bovis*, L-J glycerol and pyruvate have been introduced into NCCD TB laboratory and performed since December 2021.

4. МИКОБАКТЕРИЙН ӨСГӨВӨР

4-1. ӨСГӨВӨРЛӨХ ТЭЖЭЭЛТ ОРЧИН

ЗАРЧИМ

Микобактери нь агаартай, уургаар баялаг орчинд (*M. leprea* аас бусад нь) ургалт өгдөг. *M. fortuitum* ба *M. chelonae* гэх мэт зарим микобактериуд нутриент агар гэх мэт уургаар баяжуулагүй тэжээлт орчинд ургадаг.

M. tuberculosis нь агаартай, уургаар баяжуулсан тэжээлт орчинд 35 – 37°C -д удаан ургадаг, тэжээлт орчинд тарьснаас хойш 2- 3 долоон хоногт нүдэнд харагдах ургалт ажиглагдах ба нөсөө үүсгэхгүй.

M. tuberculosis нь тэжээлт орчны гадаргуугаас төвийсөн, хуурай өрөмтсөн колон өгч ургана. Энэ нь паранитробензойны хүчил (ПНБ)-д мэдрэг байх ба 25°C хэмд ургадаггүй. Сүрьеэгийн микобактери ургуулах өндөг суурьтай болон шингэн зэрэг олон янзын тэжээлт орчингууд байдаг.

4-2. ЛЕВЕНШТЕЙН-ИЕНСЕНИЙ ТЭЖЭЭЛТ ОРЧИН (өндөг суурьтай)

Лабораторид LJ тэжээлт орчинг бэлдэнэ. Тэжээлт орчин бэлдэхдээ шинэ өндөг, хэд хэдэн давс хийж, малахит ногоон, пенициллинийгбохирдолтыг дарангуйлах зорилгоор хийдэг. Тэжээлт орчинг хоёр түбэнд ташуу бэлдэнэ. Нэг ташуу тэжээл нь глицерол агуулсан (LJ G), нөгөө нь пируват (LJ P) агуулна. *M. tuberculosis*-ын хүний төрөл нь L-J G тэжээлд сайн ургах ба үхрийн төрөл (*M. bovis*) нь L-J P тэжээлт орчинд ургадаг.

Тавигдах шаардлага:

Өндөг

Давсны уусмал

(a) Хоёр солигдолт фосфорхүчлийн кали KH_2PO_4

(b) Магний сульфат MgSO_4

(c) Магний цитрат

(d) L-аспирагин

(e) Глицерол

(f) Пируваттай натри

2.2 SOP for MGIT AST for new drugs

Because the World Health Organization recommends new anti-tuberculosis treatment regimens employing new drugs for drug-resistant tuberculosis and has changed the definition of extensively-drug resistant tuberculosis in 2021, we need to introduce a new DST technology to diagnose new drug resistances.

The new method is based on MGIT automated liquid culture technology and requires to prepare drug containing medium in-house. The SOP includes procedures for levofloxacin, moxifloxacin, bedaquiline, linezolid, clofazimine and delamanid.

MGIT Culture and DST_TB 05-02_V1.0.doc	
Place logo here	
Баримт бичгийн төрөл: САЗ	ШИНГЭНИЙ ӨСГӨВӨР БОЛОН ЭМЭНД МЭДРЭГ ЧАНАРЫН СОРИЛ
Баримт бичгийн код: ТВ 05-02	
Нууцлал: Үгүй	
Агуулга	
1. Танилцуулага	
2. Хамрах хүрээ	
3. Тодорхойлолт ба товчлолууд	
4. Үүрэг	
5. Зөвлөмж	
6. Журам тогтоох	
5.1. Ерөнхий аюулаас урьдчилан сэргийлэх	
5.2. Сорьц хүлээн авах, боловсруулах, түрхэц бэлтгэх	
5.3. Үндсэн өсгөвөр	
5.3.1. PANTA дахин ашиглах	
5.3.2. MGIT тэжээлт орчинд өсгөвөр тарих	
5.3.3. Инкубаци	
5.3.4. MGIT-ийн эерэг өсгөвөрийн тойм	
5.3.5. MGIT-ийн сөрөг өсгөвөрийн тойм	
5.3.6. Бохирдолтой тэмцэх	
5.3.7. Төрөл зүйлийн таних	

2.3 SOP for Genome DNA extraction

This SOP describes a quick and easy extraction method of high molecular weight (HMW) DNA from MTB. While short-read sequencing, which sequences a massive number of short fragments (< several hundred bp), can yield draft whole genome sequences, it has restrictions including uncertain mapping in repetitive regions. Long-read sequencing is expected to overcome the restrictions by sequencing longer fragments (> several thousand bp), especially for high-GC content genomes such as TB.

Although extracting HMW DNA is required for successful long-read sequencing, the method is not well established in MTB. Here, the quick and easy way to extract HMW DNA from TB that is suitable for long-read sequencing is described.

Institution Laboratory name Location Head/Responsible person	Standard Operating Procedure (SOP) Drug Susceptibility Testing, Minimum Inhibitory Concentration measurement	Code: Version: No Date: of releasing Page : 1 of 10
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 - 4.2 Samples
 - 4.3 Equipment and materials
 - 4.4 Reagents and solutions
 - 4.5 Detailed stepwise instructions for the process/procedure
 - 4.6 Reading, interpretation, recording and reporting
 - 4.7 Quality control
 - 4.8 Waste management and other safety precautions
5. Related documents
6. Rationale for change for SOP version

	Compiled by	Examined by	Approved by	Replaced	New version
Name				Code:	Code:
Date					
Signature					

Laboratory Area:	No of copies:	Reason for change:
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1. Objectives and scope
This SOP describes a quick and easy extraction method of HMW DNA from MTB. While short-read sequencing, which sequences a massive number of short fragments (< several hundred bp), can yield draft whole genome sequences, it has restrictions including uncertain mapping in repetitive regions. Long-read sequencing is expected to overcome the restrictions by sequencing longer fragments (> several thousand bp), especially for high-GC content genomes such as TB. Although extracting HMW DNA is required for successful long-read

2.4 SOP for MinION sequencing

This procedure is involved in the method to perform long-read sequencing with MinION Mk1B. Most of procedure is conducted by kits and equipment sold by Oxford Nanopore Technologies.

First, HMW DNA is fragmented and barcodes are attached to the end of fragments, simultaneously.

Then, the barcoded samples are pooled, and sequencing adapters are attached to the pooled samples.

Finally, the DNA library is loaded into the flow cell and start a run. The sequence data obtained will be analysed separately.

Байгууллага Лабораторийн нэр Байршил Удирдагч/Хариуцагч	Стандарт ажиллагааны заавар (CA3) Геномын дараалал тогтоох шинжилгээ, MinION	Код: Хувилбар: Үгүй Огноо: гарсан өдөр Хуудас: 1-ээс 7
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Агуулга

1. Зорилго, хамрах хүрээ
2. Тодорхойлолт болон товчилсон үг
3. Боловсон хүчний чадавх
 - 3.1 Эрүүл мэндийн байдал
 - 3.2 Боловсрол, сургалт
4. Аргачлал
 - 4.1 Шинжилгээний аргын үндсэн зарчим
 - 4.2 Сорьц
 - 4.3 Шаардлагатай тоног төхөөрөмж, материал
 - 4.4 Шаардлагатай урвалж бодис
 - 4.5 Шинжилгээний аргын нарийвчилсан заавар
 - 4.6 Үр дүнг унших, тайлагнах, бүртгэх, мэдээллэх
 - 4.7 Чанарын хяналт
 - 4.8 Орчны болон аюулгүй байдлын хяналт
5. Холбогдох баримт бичиг
6. Стандарт ажиллагааны журамд өөрлөлт оруулах үндэслэл

	Боловсруулсан	Шалгасан	Баталсан	Өөрчлөлт оруулсан	Шинэ хувилбар
Нэр				Код:	Код:
Огноо					
Гарын үсэг					
Лабораторийн хэсэг : Хуулбарын тоо: Өөрчилсөн шалтгаан:					

1. **Зорилго, хамрах хүрээ**
Энэхүү стандарт ажиллагааны заавар нь сүрьеэгийн үүсгэгчээс ялган авсан өндөр молекул жинтэй ДНХ-ээс MinION Mk1B уртаар унших(long read) геном дарааллыг эхлүүлэх аргыг тайлбарласан.
2. **Тодорхойлолт болон товчилсон үг**
өндөр молекул жинтэй ДНХ
Энэ өндөр молекул жинтэй ДНХ-ийн хэлтэрхийн урт нь хэдэн арван кб гэсэн утгатай бөгөөд энэ нь Long-read дарааллаар цаашид дүн шинжилгээ хийхэд хангалттай юм. Өндөр молекул жинтэй ДНХ -ийн тодорхойлолт нь хэрэглээ, организм болон бусад хүчин зүйлээс хамааран хэд хэдэн кб-аас Мб хооронд хэлбэлздэг.

Inputs

The Research Institute of Tuberculosis (RIT) has implemented the followings

3 On-line training

We conducted on-line new DST training connecting NCCD TB laboratory and RIT on July 5th and 6th. This SOP describes the use of the BACTEC MGIT 960 TB System for liquid culture and drug susceptibility testing of *Mycobacterium tuberculosis*. The BACTEC MGIT 460 TB System has been found to boost culture positivity by 15–20% relative to conventional solid media and to substantially reduce the time to positivity. Liquid culture, however, is more prone to contamination. Liquid culture is now approved by WHO for use in low to middle income countries.

The training was conducted using 20 standardized *M. tuberculosis* strains which drug resistances are already known (standard results from Institute of Tropical Medicine, Antwerp, Belgium). The results are not validated as of October 19, 2022.

Inputs

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4 Introduction of interferon gamma release assay (IGRA)

For the screening of *M. tuberculosis* infection, IGRA is introduced into NCCD TB laboratory. QuantiFERON-TB Gold in Tube (QFT-4G, QIAGEN) is the 4th generation IGRA detecting interferon gamma release from CD4/8 cells. This is a standard method for IGRA with ELISA system, however, due to the delay of transportation of equipment necessary of ELISA to NCCD, the standard QFT system is not installed yet. After consultation with QIAGEN, the company proposed a simple semi-quantitative version of QFT (QIAReach system) which employs lateral flow immunoassay for the quantification of released IFN-gamma.

The QIAReach system has been installed into NCCD in September 2022, and validation test was performed using blood samples collected from bacteriologically confirmed TB cases, TB contacts and healthy volunteers (12 samples in total). The test was successful.

QIAreach QuantiFERON-TB



- Simple QFT-4G test with 1ml blood
- With lateral flow immunoassay
- 5-20 min to results
- Positive or negative results
- Ability to reach people in remote areas for efficient preventive TB programs with digital QIAreach technology

Inputs

The Research Institute of Tuberculosis (RIT) has implemented the followings

5 Overseas training

Dr Oyuntuya Tumenbayar, head of national tuberculosis reference laboratory (NTRL) in NCCD, visited RIT from August 29 to September 30, 2022. RIT staff conducted a training course for the whole genome sequencing of *M. tuberculosis* using Illumina MiSeq and MinION/GridION systems.

The programme included growth of *M. tuberculosis* on solid medium, DNA extraction from *M. tuberculosis* using glass-beads dispersion method followed by organic solvent purification, DNA quality and quantity measures, library preparations, and genome sequencing. The base calling is translated to sequence and analysed basically using TBProfiler solution.

Inputs

The Research Institute of Tuberculosis (RIT) has implemented the followings

6 Ethical process

The protocols for IGRA study, isolation and identification of *M. bovis* in human sample, genetic analysis (typing) of *M. tuberculosis* isolates including *M. bovis*, and genotypic DST are prepared and submitted to corresponding IRB (NCCD or Ministry of Health or both) in collaboration with NTRL/NCCD. The protocols have been approved.