Africa Regional Training Program (ARTP) on MODS, NRA and CRI

Mycobacteriology Laboratory,
Department of Medical Microbiology, Makerere University College of Health Sciences, Kampala, Uganda

For and in association with the STOP TB Partnership New Diagnostic Working Group

Organized By*:*
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TRAINING CURRICULUM

Preamble

Multidrug-resistant tuberculosis is an increasing public health concern in many parts of the world, especially in low-income countries, where most cases occur. Traditional drug susceptibility testing is either time-consuming, such as the proportion method on solid media, or expensive, such as the BACTEC 460 system. Commercial liquid culture systems and selected molecular assays have been endorsed by World Health organization (WHO) as gold standards for rapid detection of multidrug- resistant tuberculosis (MDR-TB); however, due to technical complexity except Xpert TB MTB/RI, cost and the need for supplicated infrastructure, uptake of these technologies has been limited in many resource constrained settings.

WHO still recommends the use of phenotypic assays to monitor treatment progress and to detect resistance to drugs other than rifampicin.Therefore; several non-commercial culture and DST methods have been developed at the same time targeting laboratories in resource limited settings. Among these,
the following were recently assessed by WHO (WHO Policy statement, July 2010):

- **Microscopic Observation Drug Susceptibility Assay (MODS):** A micro-colony direct method in liquid culture based on inoculation of specimens in media with and without drug followed by microscopic examination of early growth.
- **Nitrate Reductase Assay (NRA):** A direct and/or indirect method based on the ability of M. tuberculosis to reduce nitrate, which is detected by a coloured reaction.
- **Colorimetric Redox Indicator (CRI) Methods:** Indirect testing methods based on the reduction of a coloured indicator added to liquid culture medium in a micro-titer plate after in vitro exposure of M. tuberculosis strains to anti-TB drugs.
- **Thin Layer Agar (TLA):** A micro-colony direct method on solid culture based on inoculation of specimens to media with or without drug followed by microscopic examination of early growth.
- **Phage-based Assays:** Assays which use bacteriophages to infect and detect the presence of viable M. tuberculosis in clinical specimens and culture isolates.

CRI, MODS and NRA methods were subsequently evaluated to have su_cient evidence to consider their use in laboratories that lack access to more sophisticated infrastructure. WHO has recommended the selective use of one or more of the following non commercial culture and DST methods in reference laboratories, and under strict laboratory protocols, and as an interim solution while capacity for genotypic and/or automated liquid culture and DST is being developed:

- **MODS**, as a direct or indirect test for rapid screening of patients suspected of having MDR-TB.

- **NRA**, as a direct or indirect test for screening of patients suspected of having MDR-TB, and acknowledging that time to detection of MDR-TB in indirect application would not be faster than conventional DST methods using solid culture.

- **CRI** methods, as indirect tests on M. tuberculosis isolates from patients suspected of having MDR-TB, and acknowledging that time to detection of MDR-TB would not be faster (but less expensive) than conventional DST methods using commercial liquid culture or molecular line probe assays.

To achieve the above goal the culture subgroup of the STOP TB P a r t n e r s h i p N D W G (N D W G - C S G) recommended our laboratory setup (Mycobacteriology Laboratory, Department of Medical Microbiology, Makerere University, Kampala in Uganda) as a referral training centre for these newly endorsed non-commercial TB diagnostics and Drug Susceptibility Testing in Africa. On behalf of the N D W G - C S G recommendation we organize training to full _ll the above global goal.

Purpose Of the Training

This training has been designed to enlighten participants on the application and implementation of the NRA, MODS and CRI techniques for simple, rapid and inexpensive detection methods of M. tuberculosis in low- resource laboratories/developing countries.

The goal of the programme is training the mycobacteriologists, and technicians working in National TB Programmes (NTP) from African countries who after this training course will be able to carry out high quality practice in the _eld, management of MDR-TB in their representative countries and will be able to train others in these methods based on experience gained during the training course.

What is Included in the Training?

In this advanced training the participants will learn the principles behind tuberculosis diagnostic. Study designs, tools in the pipeline are not covered in this training.

Basically, The first part will be based on theory and it will help participants to understand the next session. Participants will receive Standard Operating Protocols and documentation for laboratory biosafety.

The second main part will involve the basic mycobacterial laboratory exposures and much of practical exposures on NRA, MODS and CRI methods. Participants will have direct, hands-on experience on these methods.

- Principle and hands on workshop on NRA.
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- Principle and hands on workshop on NRA.
- Principle and hands on workshop on MODS.
- Principle and hands on workshop on CRI method.
- Principle and hands on workshop on first and second line DST by MGIT-960.
- Observations and interpretations of test results.

Who should participate in the Training?

The training will be best for Clinical Microbiologists, Mycobacteriologists, and Laboratory technicians who are engaged in tuberculosis diagnosis. This training will help participants to acquire the necessary theoretical knowledge and practical skills on rapid diagnosis of tuberculosis and MDR- TB with the NRA, MODS and CRI methods.

Number of Participants

Maximum of six participants will be selected/called to attend the training programme per round. Training duration will be two weeks, implying that 12 participants can be trained per quarter. Any more than this number can hinder the opportunity for each member to have adequate time to share and hands-on exposure.

When is the training conducted?

Quarterly	Month
Quarter 1	March
Quarter 2	June
Quarter 3	September
Quarter 4	November

How to apply

For application and inquiries please contact Francis Mumbowa francmumbowa@yahoo.co.uk or Geraldine Nalwada, mbl@chs.mak.ac.ug. You can also download the form from www.mbl.mak.ac.ug/PDFform.pdf

Training Agenda

LABORATORY TRAINING ON

Identification and Drug Susceptibility Testing of Mycobacterium tuberculosis Reference Training Centre for African region in association with culture subgroup of the STOP TB Partnership NDWG (NDWG-CSG). at Mycobacteriology Laboratory, Department of Medical Microbiology, Makerere University College of Health Sciences, Kampala, Uganda

Training summary

DAYS	SESSION	TITLE
Day-1 (Monday)	Forenoon	Introduction and opening the training
	Afternoon	Basic concepts and Biosafety practices
Day-2 (Tuesday)	Forenoon	Principles and preparation of MODS and NRA
	Afternoon	Demonstration of specimens processing of MODS and NRA
Day-3 (Wednesday)	Forenoon	Practice of MODS processing
	Afternoon	Practice of NRA processing
Day-4 (Thursday)	Forenoon	Practice of CRI assay-1st line drugs
	Afternoon	Practice of CRI assay-2nd line drugs
Day-5 (Friday)	Forenoon	Demonstration of 1st line DST by MGIT-SIRE kit
	Afternoon	Practice of MODS processing.
Day-6 (Saturday)	Forenoon	Demonstration of 2st line DST by MGIT
	Afternoon	**Free**
Day-7 (Sunday)		**Free day**
Day-8 (Monday)	Forenoon	MODS reading and interpretation*
	Afternoon	NRA reading and interpretation *
Day-9 (Tuesday)	Forenoon	CRI reading and interpretation *
	Afternoon	Open questions and general review
Day-10 Wednesday)	Forenoon	MODS reading and interpretation
	Afternoon	NRA reading and interpretation
Day-11 (Thursday)	Forenoon	CRI reading and interpretation
	Afternoon	Teoric test of key concepts
Day-12 (Friday)	Forenoon	MGIT, MODS, and NRA reading
	Afternoon	Final review and training closing

*Readings MODS, NRA and CRI of test prepared previously (a week early) for the training.

Day 1: (MONDAY)

Opening and Basic Concepts

Session	Title	Estimated Time
1	Welcome and opening of the course	10.00 -10.30am
	Introduction of participants	10.30 - 11.00am
	Introduction to TB diagnosis and training programme	11.00 - 1.00pm
Lunch		
2	Biosafety practice in the laboratory (General and BSL2 & 3)	2.00 - 2.30pm
	Introduction with essential equipments: uses and routine maintenance	2.30 - 3.00pm
	Introduction to Liquid culture handling: Skill Assessment, pipetting, taped work areas, pouring etc)	3.00 - 4.00pm
	Overview of Participant Experiences and Basic Concepts	4.00 - 5.00pm

Day 2: (TUESDAY)

Principles and preparation of NRA and MODS implements, and demonstration of the process

Session	Title	Estimated Time
1	**Introduction and principle of NRA and MODS**	09.00 -10.00am
	Provide copies of specimen processing Standard Operating Procedure manual for NRA and MODS	-
	Required equipments, supplies, reagents and sterilization of materials to be used. Biosafety and working area	10.00 -10.30am
	Preparation of materials and reagents	10:30 -11.00am
	Preparation of drugs • Drug potencies • Preparation of antibiotics stocks solutions • Storage of drug stock solutions	11.00 -12.00am
	Preparation of Middle-brook 7H9 media	12.00-12.30pm
	Preparation of the media (describe) • Preparation of egg-based media Löwenstein-Jensen (LJ) media without drugs • Preparation of egg-based media Löwenstein-Jensen (LJ) media containing drugs • 7H11 media	12.30-1.00pm

Lunch		
2	Demonstration of specimen processing: MODS, NRA	2.00 -4.30pm
	Storage of decontamination samples of NRA and MODS as a back-up	4.30 -5.00pm

Day 3: (WEDNESDAY)

Practice of MODS and NRA procedures

Session	Title	Estimated Time
1	**Practice MODS** Preparation of media aliquot for: • Specimens, negative and positive controls and antibiotic working solutions.	9.00-9.30am
	Decontamination of specimens	9.30-10.30am
	Preparation of final sample suspension and back-up. Final MODS plate preparation	10.30-11.00am
	Plating out the positive internal quality control strains	11.30-12.00am
	* Plate reading for MODS	12.00-1.00pm
Lunch		
2	**Practice NRA** Preparation of McFarland 1.0 inoculums: • Inoculum from growth on solid media • Inoculum from a liquid media • Dilution of the inoculums	2.00 -3:30pm
	Inoculation and incubation of NRA tubes	3:30 – 4:00pm
	*Reading NRA tubes	4:00 – 5:00pm

*Readings MODS, NRA and CRI of test prepared previously (a week early) for the training.

Day 4: (THURSDAY)

Demonstration and inoculation of CRI assay for first and second line anti-tubercular drugs

Session	Title	Estimated Time
1	**Introduction and principle of CRI assay**	9.00-9.30am
	Provide copies of specimen processing Standard Operating Procedure manual for CRI	
	Sterilization of materials to be used, biosafety and working area	9:30 - 10:00am
	Preparation of working drug solutions	10.00 - 11.00am
	Preparation of inoculums: • Inoculum from solid medium • Inoculum from liquid medium	11.00 - 12.00pm
	Demonstration and inoculation of the CRI (REMA/MTT/Alamar Blue) plate • For first-line drugs: SM, INH, RMP, EMB	12.00 - 1.00pm
Lunch		
2	Demonstration and inoculation of the CRI (REMA/MTT/Almar Blue) plate • For second-line drugs: PAS, ETH, KAN, OFLO, CAP	2.00-4.00pm

Day 5: (FRIDAY)

Demonstration of first line DST by MGIT SIRE kit and practice of MODS processing

Session	Title	Estimated Time
1	**Introduction and principle of the DST by SIRE kit**	10.00 -10.30am
	Provide copies of specimen processing Standard Operating Procedure manual for SIRE	-
	Preparation of reagents and materials	10.30 -11.00am
	Sterilization of materials to be used, biosafety and working area	11.00 -11.30pm
	Preparation of drugs • Drugs reconstructions	11.30 -12.00pm
	Performance of DST (SIRE)	12.00 -1.00pm
Lunch		
2	Practice of MODS processing	2.00 -5.00pm

Day 6: (SATURDAY)

Demonstration of second line DST by MGIT

Session	Title	Estimated Time
1	**Introduction and principle of the second line DST by MGIT**	10.00-10.30am
	Provide copies of specimen processing Standard Operating Procedure manual	
	Preparation of reagents and materials	10.30-11.00am
	Sterilization of materials to be used, biosafety and working area	11.00 -11.30am
	Preparation of drugs • Drugs reconstructions	11.30-12.00am
	Performance of DST (ETH, OFL, KAN,AMK,CPM)	12.00-1.00pm

Day 7: (SUNDAY) – Free day

Day 8: (MONDAY)

Reading and interpretation of NRA and MODS results

Session	Title	Estimated Time
1	**Reading and Interpretation of CRI results ***	10.00am-12.00am
	Questions	12.00am-1.00pm
Lunch		
2	Open questions and general review	2.00-3.00pm.

*Readings MODS, NRA and CRI of test prepared previously (a week early) for the training.

Day 9: (TUESDAY)

Reading and interpretation of CRI / Open questions and review

Session	Title	Estimated Time
1	**Reading of MODS plates *** After 7 days of incubations, will be repeated at day 9 or 11 days if required.	9:00 – 12:00am
	Interpretation of results	12:00- 1:00pm
Lunch		
2	**Reading of NRA tubes** * After 7 days of incubation If not color change occurs, the procedure will be repeat at day 10 or 14 days if required.	2.00- 3:00pm
	Interpretation of results	3:00 – 4:00pm

*Readings MODS, NRA and CRI of test prepared previously (a week early) for the training.

Day10: (WEDNESDAY)

Reading and interpretation of NRA and MODS results

Session	Title	Estimated Time
1	**Reading and interpretation of MODS**	10.00am-12.00am
	Questions	12.00am-1.00pm
Lunch		
2	**Reading and interpretation of NRA**	2.00-3.00pm.
	Questions	3.00-4.00pm.

*Readings MODS, NRA and CRI of test prepared previously (a week early) for the training.

Day 11: (THURSDAY)

Reading and interpretation of CRI and Teoric Test

Session	Title	Estimated Time
1	**Reading of CRI plates**	10.00am-12.00am
	Results and interpretation - Questions	12.00am-1.00pm
Lunch		
2	Teoric test of key concepts (NRA, CRI, MODS**)**	2.00-2.45pm.
	Questions	3.00-4.00pm.

Day12: (FRIDAY)

Reading, interpretations of MGIT, MODS, NRA results and closing the training

Session	Title	Estimated Time
1	**MGIT reading**	9.00am-9.30am
	Interpretation of results	9.30am-10.00am
2	**Observation of MODS plate and NRA tubes**	10.00am-12.00pm
	Interpretation of results	12:30 – 1:00pm
Lunch		
3	**Final review and closing** • reviewed the key concepts of the training • Post-training Quarries • **Knowledge exchange:** principles of implementation research, collecting evidence for scale-up, cost-effectiveness analyses and modeling studies in TB diagnostics and practice of diagnostic research focused on accuracy of tests. • Implementation of current pipeline of TB diagnostics methods and WHO policies on new diagnostic	2.00pm-5.00pm