Mycobacteriology Laboratory Capacity Building for Tuberculosis Clinical Trials

Webinar, 25th March 2019

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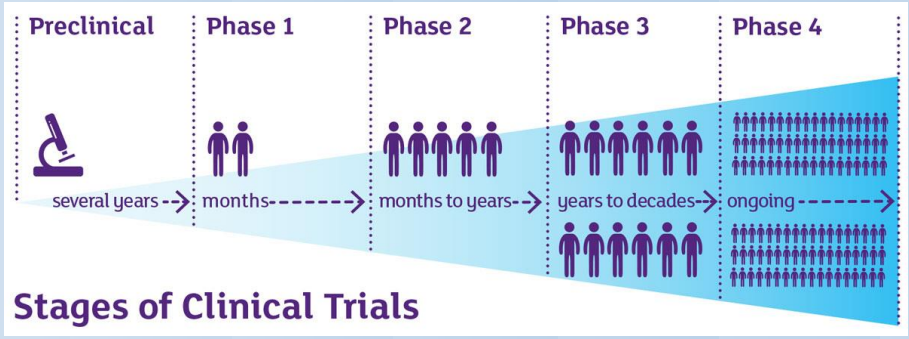
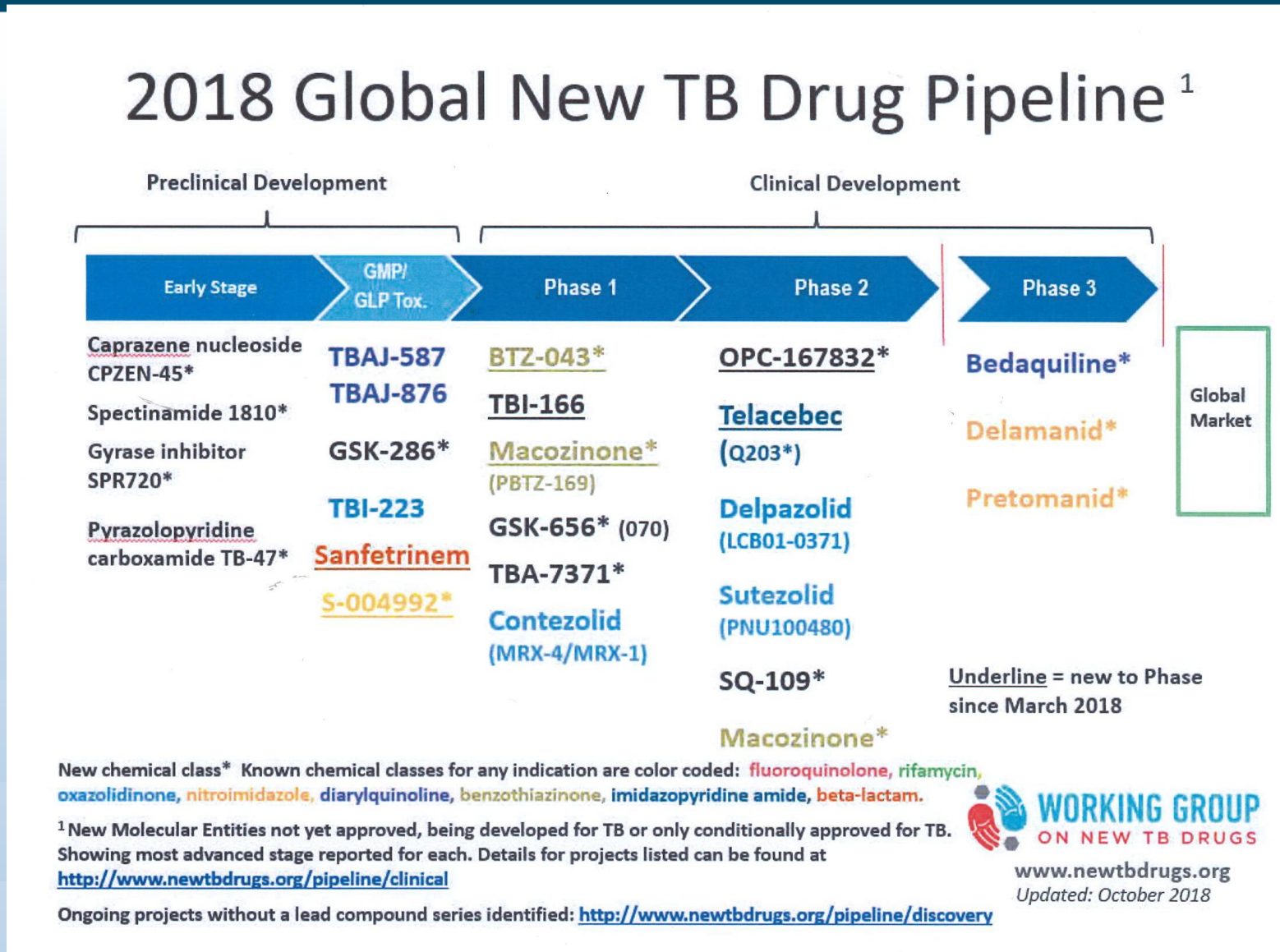
OUTLINE

- Overview of clinical trials
- Laboratory classifications
- Overview of guidelines and initial requirements to conduct clincial trials
- Challenges faced and lessons learned
  - Building capacity for lab procedures required for clinical trials
  - Laboratory infrastructure strengthening
  - Human resources strengthening
  - Quality and data management systems
- Conclusions

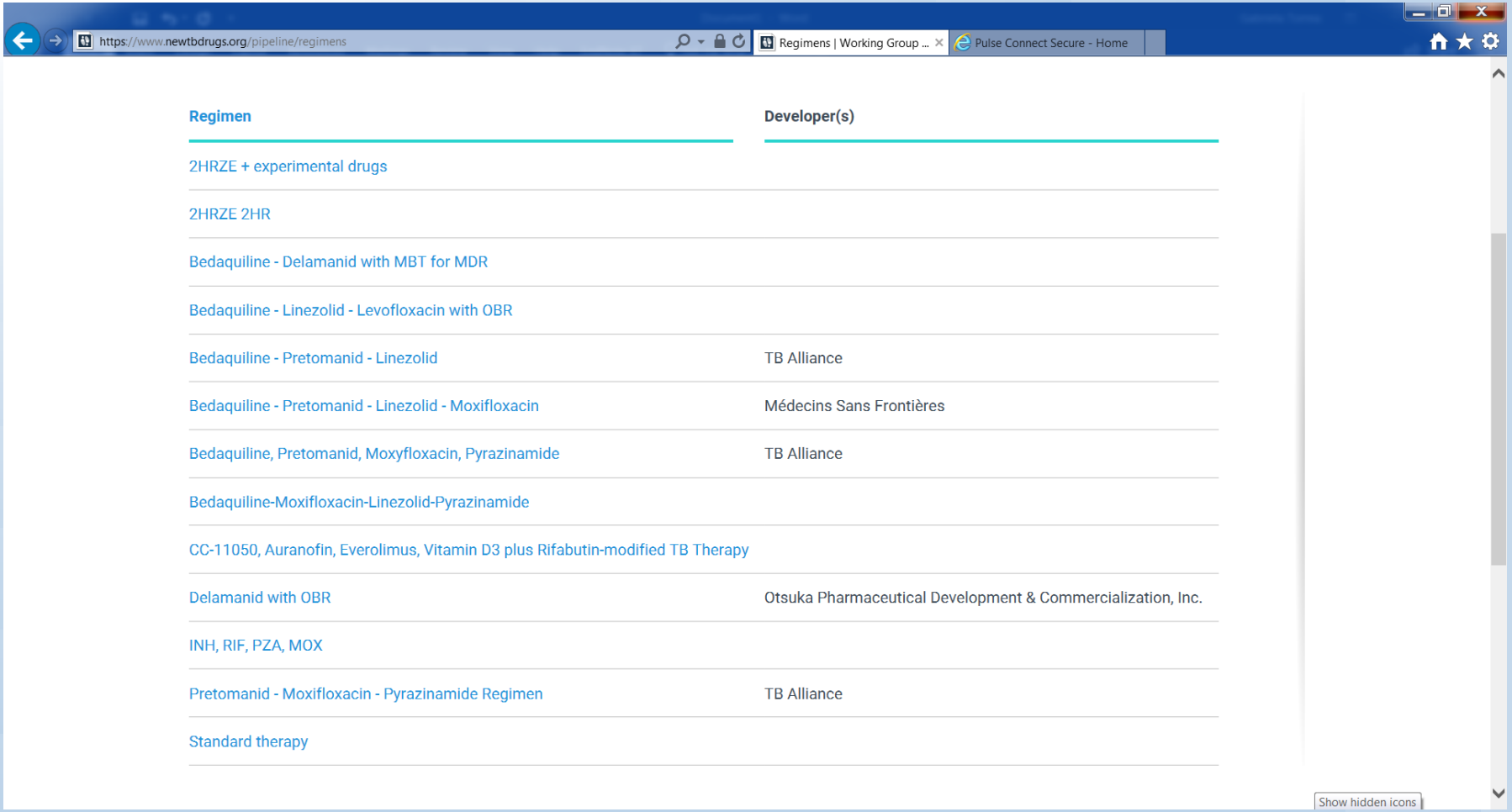
Introduction to Clinical Trials



DRUG DEVELOPMENT

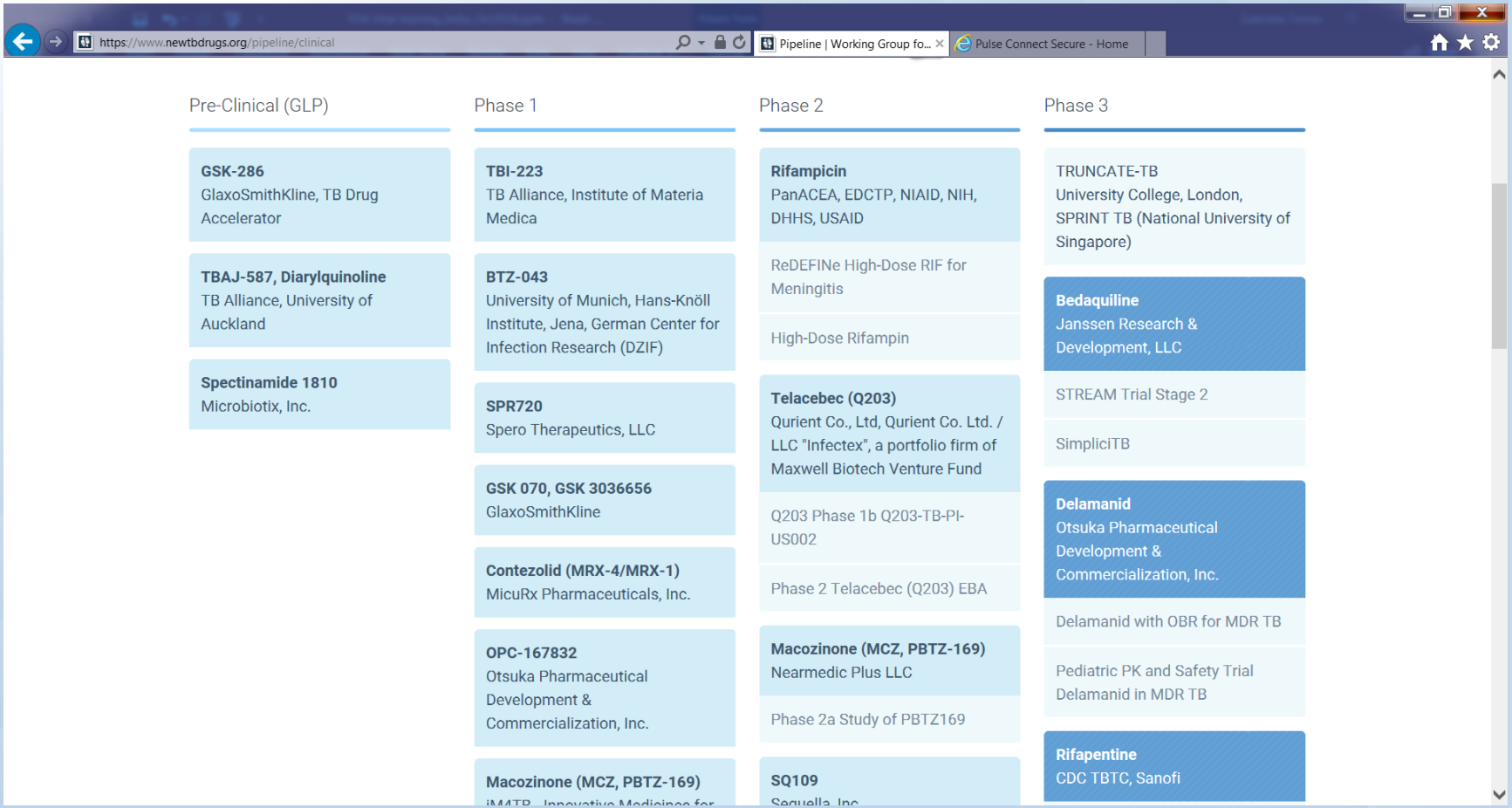


REGIMENS



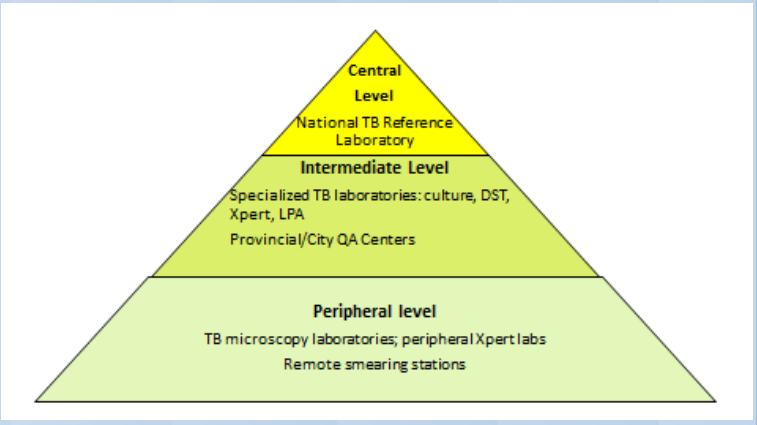
Regimen	Developer(s)
2HRZE + experimental drugs	
2HRZE 2HR	
Bedaquiline - Delamanid with MBT for MDR	
Bedaquiline - Linezolid - Levofloxacin with OBR	
Bedaquiline - Pretomanid - Linezolid	TB Alliance
Bedaquiline - Pretomanid - Linezolid - Moxifloxacin	Médecins Sans Frontières
Bedaquiline, Pretomanid, Moxyfloxacin, Pyrazinamide	TB Alliance
Bedaquiline-Moxifloxacin-Linezolid-Pyrazinamide	
CC-11050, Auranofin, Everolimus, Vitamin D3 plus Rifabutin-modified TB Therapy	
Delamanid with OBR	Otsuka Pharmaceutical Development & Commercialization, Inc.
INH, RIF, PZA, MOX	
Pretomanid - Moxifloxacin - Pyrazinamide Regimen	TB Alliance
Standard therapy	

CLINICAL TRIALS



MYCOBACTERIOLOGY LABORATORY LEVELS

- Site Mycobacteriology (TB) labs performing tests to enroll patients and monitoring of treatment
  - Hospital
  - National Tuberculosis Reference Lab (NRL)
  - Intermediate Reference Lab (IRL)
  - Private
  - Research Institutes

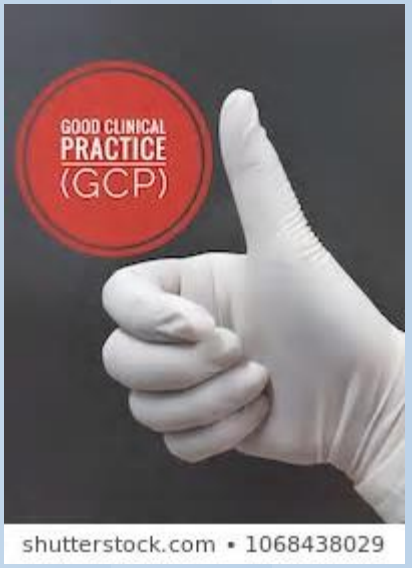


- Central TB laboratory coordinating microbiological aspects
  - Supranational Reference Lab (SRL)
  - NRL
    - Initial assessment and training of site TB labs/manuals/protocols
    - Coordinate and monitor microbiological analyses in site laboratories
    - Perform specific tests for trial analyses

Guidelines

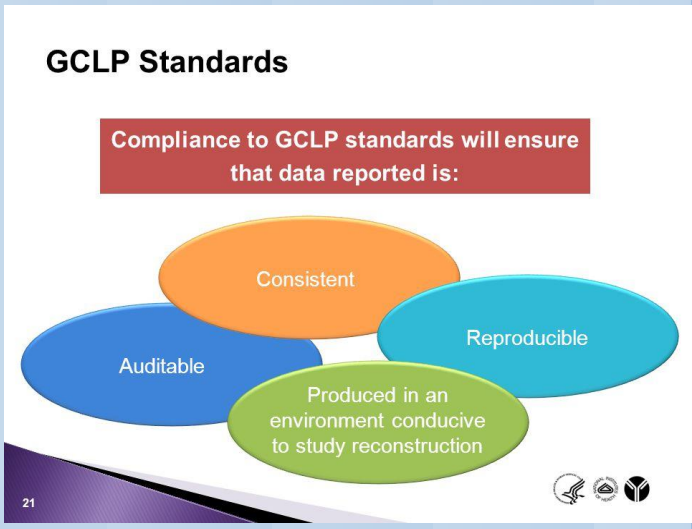
and

Initial lab requirements to conduct clinical trials



OVERVIEW OF GUIDELINES

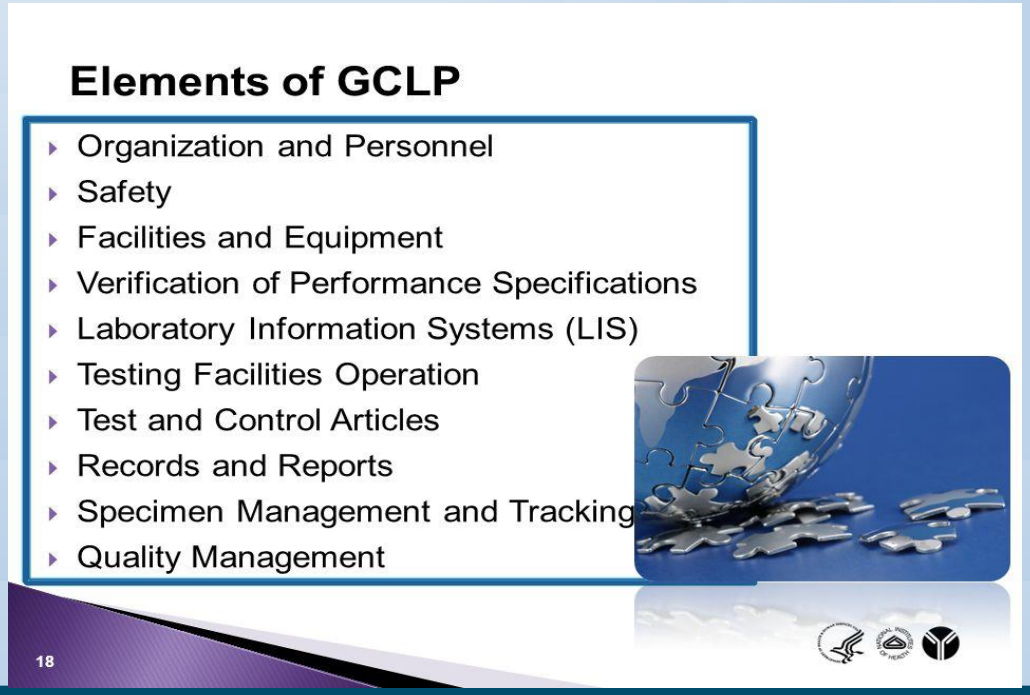
- **ICH-Good Clinical Practice (GCP)**
  - Focus on ethics and scientific quality of trials
  - Designing, conducting, recording and reporting for trials
  - Origin in the Declaration of Helsinki (ICH GCP Guideline)
- **Good Laboratory Practice (GLP)**
  - Focus on quality and validity of test data
  - Planning, conducting, recording and reporting for lab studies
- **Good Clinical Laboratory Practice (GCLP)**
  - GLP principles relevant to the analysis of samples from clinical trials
  - Taking into account the principles of GCP
  - Reliability and integrity of data



INITIAL LABORATORY REQUIREMENTS TO CONDUCT CT

- Compliant with requirement ISO standards 15189
  - Mandatory for the central lab
  - BUT not always a reality in site labs

- Good Clinical Laboratory Practice Guidelines WHO/TDR



LABORATORY ORGANIZATION

- Trial facility management responsibilities
- Well organized and trained staff
- Coordinator/Manager
- Trial staff – technician/technologist, quality officer
- Clear Organizational structure
  - To ensure clear communication during trial work

INFRASTRUCTURE AND SUPPLIES

- In line with requirements from the trial (size, construction,location)
  - Proper designed areas
    - BSL2 – BSL3
    - Molecular lab

- Suitable facilities for the preparation of trial supplies

- Appropriate storage areas for samples and supplies

EQUIPMENT, MATERIALS, REAGENTS, AND SOLUTIONS

Equipment should be:

- In good working condition
- Periodically inspected, cleaned, maintained, and calibrated

Instructions for use must be available

Equipment users should be qualified and trained

Materials and consumables should be:

- Stored at appropriate environmental conditions
- Appropriately labelled
  - Identity
  - Concentration
  - Specific storage instructions
  - Stability (e.g. preparation date and expiration date)

TRIAL MATERIALS (TM)

Receipt

- Design procedures to prevent mix-ups and maintain their integrity
- Should be adequately identified
- Maintain records

Chain of Custody

- Maintain TM identification and traceability
- Retrospective evaluation of storage
- TM storage areas should be monitored
- Contingency plans with actions

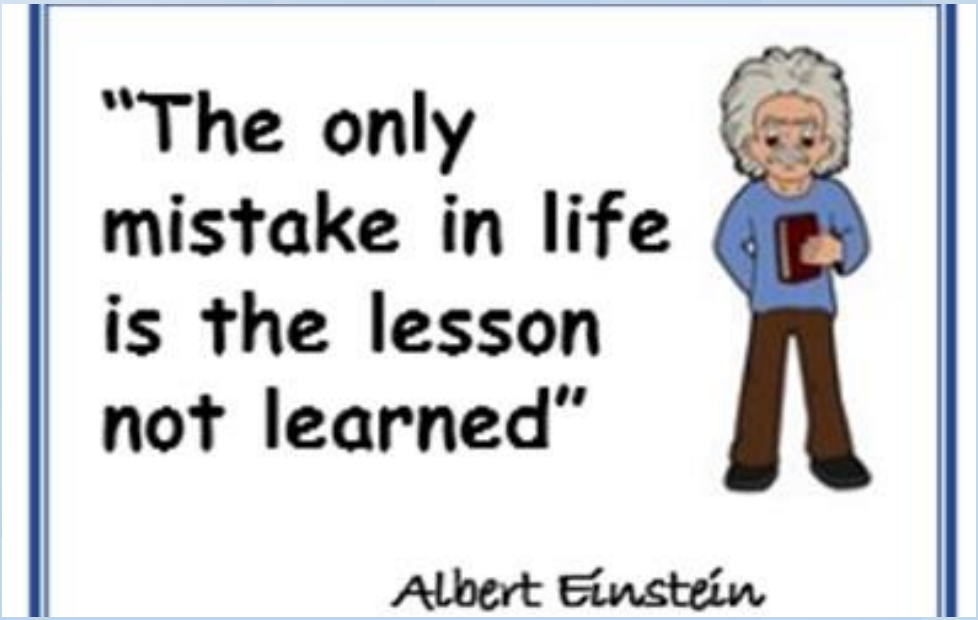
STANDARD OPERATING PROCEDURES (SOPs)

- To ensure uniformity and quality of results
- KISS (Keep it Short and Simple)
- For procedures (pre-analytical, analytical, and post analytical phases)
- For handling test equipment
- For quality control procedures
- For staff training

- Approved by Trial Facility Management
- SOPs should be periodically reviewed
- A list of current SOPs with version numbers
- Published textbooks, articles and manuals may be used as supplements to the SOPs

Challenges and Lessons Learned

Lab Procedures



SITE LABORATORIES

Diagnostics for enrolment

- Smear microscopy
  - Microscope
  - Quality of stains and reagents
- FDA vital staining
  - Fluorescent microscope
  - **Validation/implementation**
- Xpert:
  - Maintenance/calibration
  - Errors not evaluated
- LPA
  - Lab design
  - **Validation/implementation**
  - Technical issues and interpretation

Treatment outcome

- Culture
  - Liquid culture - MGIT960
  - Solid culture – Simple culture method (Kudoh)
    - Media preparation – no inspissator?
      - ✓ Provided by the central lab
      - ✓ Impact of shipment on quality
    - Fresh sputum samples required
    - **Validation/implementation**

Validations to be done at least 3 months before trial initiation

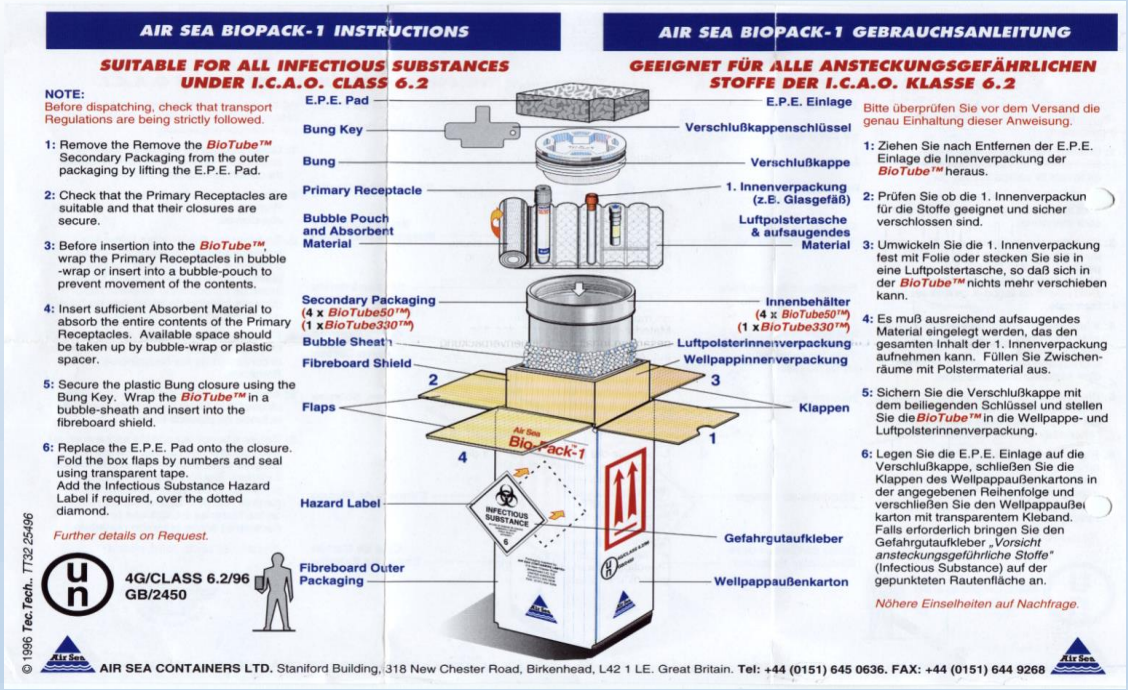
SHIPMENT OF TRIAL MATERIAL

United Nations Model Regulations

- *Mycobacterium tuberculosis* isolates: Category A (UN 2814)
  - Transport medium: dry, 0.5% CPC
  - Room temperature
  - UN 2814 boxes (Class 6.2 and accompanied by a Shipper's declaration)

- Sputum specimen in MGIT not incubated : Category B (UN 3373)
  - MGIT tubes
  - Refrigerated
  - UN 3373 boxes (class 6.2)

PACKAGING AND DECLARATION FORM



AIR SEA BIOPACK-1 INSTRUCTIONS

SUITABLE FOR ALL INFECTIOUS SUBSTANCES UNDER I.C.A.O. CLASS 6.2

NOTE:
Before dispatching, check that transport Regulations are being strictly followed.

1: Remove the Remove the *BioTube™* Secondary Packaging from the outer packaging by lifting the E.P.E. Pad.

2: Check that the Primary Receptacles are suitable and that their closures are secure.

3: Before insertion into the *BioTube™*, wrap the Primary Receptacles in bubble -wrap or insert into a bubble-pouch to prevent movement of the contents.

4: Insert sufficient Absorbent Material to absorb the entire contents of the Primary Receptacles. Available space should be taken up by bubble-wrap or plastic spacer.

5: Secure the plastic Bung closure using the Bung Key. Wrap the *BioTube™* in a bubble-sheath and insert into the fibreboard shield.

6: Replace the E.P.E. Pad onto the closure. Fold the box flaps by numbers and seal using transparent tape. Add the Infectious Substance Hazard Label if required, over the dotted diamond.

Further details on Request.

UN 4G/CLASS 6.2/96 GB/2450

AIR SEA BIOPACK-1 GEBRAUCHSANLEITUNG

GEEIGNET FÜR ALLE ANSTECKUNGSGEFÄHRLICHEN STOFFE DER I.C.A.O. KLASSE 6.2

Bitte überprüfen Sie vor dem Versand die genau Einhaltung dieser Anweisung.

1: Ziehen Sie nach Entfernen der E.P.E. Einlage die Innenverpackung der *BioTube™* heraus.

2: Prüfen Sie ob die 1. Innenverpackun für die Stoffe geeignet und sicher verschlossen sind.

3: Umwickeln Sie die 1. Innenverpackung fest mit Folie oder stecken Sie sie in eine Luftpolstertasche, so daß sich in der *BioTube™* nichts mehr verschieben kann.

4: Es muß ausreichend aufsaugendes Material eingelegt werden, das den gesamten Inhalt der 1. Innenverpackung aufnehmen kann. Füllen Sie Zwischen-räume mit Polstermaterial aus.

5: Sichern Sie die Verschlußkappe mit dem beiliegenden Schlüssel und stellen Sie die *BioTube™* in die Wellpappe- und Luftpolsterinnenverpackung.

6: Legen Sie die E.P.E. Einlage auf die Verschlußkappe, schließen Sie die Klappen des Wellpappaußenkartons in der angegebenen Reihenfolge und verschließen Sie den Wellpappaußen-karton mit transparentem Kleband. Falls erforderlich bringen Sie den Gefahrgutaufkleber *„Vorsicht ansteckungsgefährliche Stoffe"* (Infectious Substance) auf der gepunkteten Rautenfläche an.

Nähere Einselheiten auf Nachfrage.

E.P.E. Pad — E.P.E. Einlage
Bung Key — Verschlußkappenschlüssel
Bung — Verschlußkappe
Primary Receptacle — 1. Innenverpackung (z.E. Glasgefäß)
Bubble Pouch and Absorbent Material — Luftpolstertasche & aufsaugendes Material
Secondary Packaging (4 x *BioTube50™*) (1 x *BioTube330™*) — Innenbehälter (4 x *BioTube50™*) (1 x *BioTube330™*)
Bubble Sheath — Luftpolsterinnenverpackung
Fibreboard Shield — Wellpappinnenverpackung
Flaps — Klappen
Hazard Label — Gefahrgutaufkleber
Fibreboard Outer Packaging — Wellpappaußenkarton

AIR SEA CONTAINERS LTD. Staniford Building, 318 New Chester Road, Birkenhead, L42 1 LE. Great Britain. Tel: +44 (0151) 645 0636. FAX: +44 (0151) 644 9268

SHIPPER:	Air Waybill No Page 1 of 1 Pages Shipper's Reference Number
CONSIGNEE:	

Two completed and signed copies of this Declaration must be handed to the operator

TRANSPORT DETAILS

This shipment is within the limitations prescribed for *(deletion non applicable)*: PASSENGER AND CARGO AIRCRAFT / CARGO AIRCRAFT ONLY

Airport of Departure:

Airport of Destination:

WARNING

Failure to comply in all respects with the applicable Dangerous Goods Regulations may be in breach of the applicable law, subject to legal penalties. This Declaration must not, in any circumstances, be completed and/or signed by a consolidator, a forwarder or an IATA cargo agent.

Shipment type: *(deletion non applicable)* — NON-RADIOACTIVE / RADIOACTIVE

NATURE AND QUANTITY OF DANGEROUS GOODS

Dangerous Goods Identification						
UN or ID No.	Proper Shipping Name	Class or Division (Subsidiary Risk)	Pack-ing Group	Quantity and Type of packing	Packing Inst.	Authorization

Additional Handling Information

EMERGENCY CONTACT : +32 (0)3 247 65 03 L. CASIER

I hereby declare that the contents of this consignment are fully and accurately described above by the proper shipping name, and are classified, packaged, marked and labelled/placarded, and are in all respects in proper condition for transport according to applicable international and national governmental regulations. I declare that all of the applicable air transport requirements have been met.

Name/Title of Signatory
L. CASIER / EXPORT MANAGER
Place and Date
ANTWERPEN, 12.11.2008
Signature
(see warning above)

SHIPMENT LIST

STREAM Study Specimen Packing List

Country		Total Number of cryotubes :	
Laboratory		Responsible for the shipment :	
Date of shipment		E-mail	
Name of the person who prepared the list		Date:	
Name of the person who prepared the package for the shipment		Date:	
Name of the reviewer		Date:	

List all specimens to be shipped:

	Specimen type (Sputum, sediment, colonies, DNA extract)	Study number	Week visit	Local lab accession number	Transport medium (Ethanol, CPC, MGIT, None, Other : specify)	Sputum collection date	Smear results *	Culture results *	LPA resutls **							ITM ID
									rpoB	katG	inhA	gyrA	gyrB	rrs	eis	
1																
2																
3																
4																
5																
6																
7																

SHIPMENTS

Challenge	Resolution and follow-up
Local issues with preparation of documents	To be solved by the site with the support of the ITM shipment department
Issue of biodiversity for sending isolates (isolated after Octobre 2014)/ Nagoya Protocol	To be solved by the site together with the Sponsor/ Please check: https://absch.cbd.int/countries/ET

CENTRAL TB LABORATORY

DST Methods:

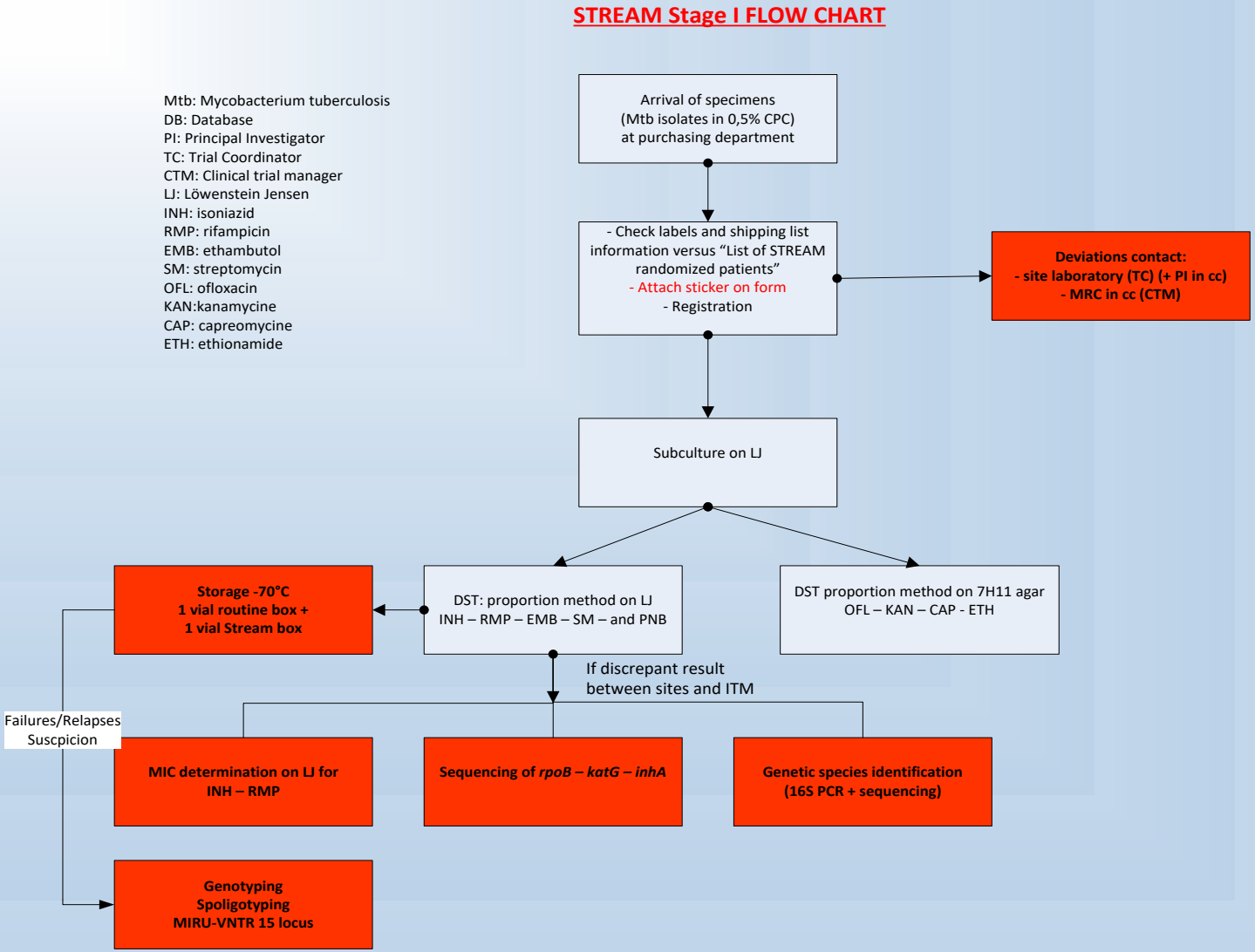
- First line drugs: H, R, E, S, Z
  - Proportion Method (PM) on LJ or agar
  - Z: MGIT/Pzase assay/pncA seq.
- Second line drugs: FQL, SLI
  - PM on LJ/agar/MGIT
  - MIC on agar/MGIT
  - MIC by REMA
- New drugs: BDQ, DLM, CFZ
  - PM on MGIT/agar
  - MIC on MGIT/agar
  - MIC REMA

Validations:

- Pzase assay vs. MGIT/pncA seq.
- BDQ MIC in 7H11 agar
- MFX and LFX MIC in 7H10 agar
- CFZ MIC in 7H10 agar
- REMA for KAN, AM, S and LZD

Validations to be done at least 6–12 months before trial initiation

Central Laboratory Sample Flow-chart



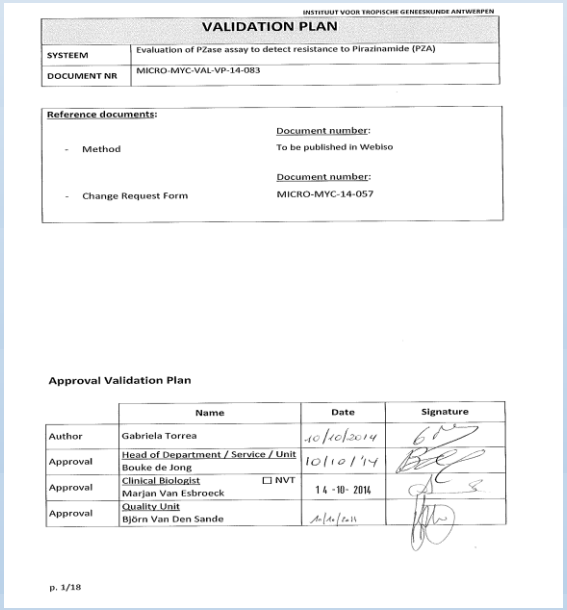
VALIDATION / IMPLEMENTATION

- Clinical Methods
  - Commercial methods
  - Approved methods or published methods (peer reviewed)
  - In-house developed methods
  - Approved methods or published methods (peer reviewed) with modifications

- Depending on nature of clinical method:
  - **Validation** – Are we doing the correct test?
  - **Verification** – Are we doing the test correctly?

VALIDATION / IMPLEMENTATION

- Validation dossier
  - **Validation/verification plan**: approved and **signed off before starting validation** and implementation
  - **Validation/verification Report**: ideally **signed off before start of the trial activities**



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VALIDATION PLAN

SYSTEEM	Evaluation of PZase assay to detect resistance to Pirazinamide (PZA)
DOCUMENT NR	MICRO-MYC-VAL-VP-14-083

Reference documents:

- Method	Document number: To be published in Webiso
- Change Request Form	Document number: MICRO-MYC-14-057

Approval Validation Plan

	Name	Date	Signature
Author	Gabriela Torrea	10/10/2014	
Approval	Head of Department / Service / Unit Bouke de Jong	10/10/'14	
Approval	Clinical Biologist ☐ NVT Marjan Van Esbroeck	14-10-2014	
Approval	Quality Unit Björn Van Den Sande	14/10/2014	

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VALIDATION REPORT

DIR_F_0137 v1.4 Template Validation Report – Methods (Eng).doc

METHOD	Evaluation of PZase assay to detect resistance to Pyrazinamide (PZA)
DOCUMENT NR	MICRO-MYC-VAL-VR-14-083

Reference documents:

- Method	Document number: To be published in Webiso
- Change Request Form	Document number: MICRO-MYC-14-057
- Validation Plan	MICRO-MYC-VAL-VP-14-083

Overall Conclusion Validation / Verification

☐	The Validation / Verification of the method is **successfully completed**. All requirements specified in the Validation Plan were met. Remarks (if any) are mentioned in this report. The method **is suitable** for implementation in the lab.
☐	**Not all test results are in line with the specifications** as defined in the Validation Plan. **Some remarks** were made in this Validation Report. Taking into account these remarks and/or limitations, the method **is suitable** for implementation in the lab.
☒	Test results **are not in line** with the specifications as defined in the Validation Plan. The method **is not suitable** for implementation in the lab.

Method Implementation Date:

Approval Validation Report

	Name	Date	Signature
Author	Gabriela Torrea	16/12/2015	
Approval	Head of Department / Service / Unit Bouke De Jong	16/12/2015	
Approval	Clinical Biologist ☐ NVT		
Approval	Quality Unit Tijs Van Poucke	23/01/2016	

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TIMING OF VALIDATIONS

- **Prospective validation/verification:** before implementation of the method in the lab
  - Ideal but difficult to achieve
  - At least 3 to 12 months before

- **Concurrent validation/verification:** exceptionally, validation/verification can be performed during the first month(s) after implementation of the method in the lab

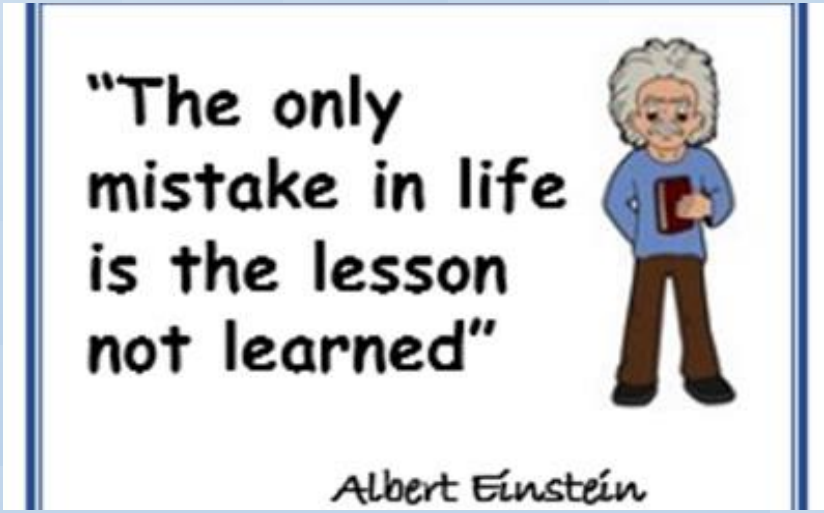
- **Retrospective validation:** method that is already implemented in the lab for a long time and for which historical data can be used to support the performance characteristics of the test

ACTION PLAN

Action plan STREAM stage 2 validations	Responsible	2014												2015											
		Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dic	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dic			
Modified Ogawa medium Stability at 2-8°C																									
Validation plan	GT																								
Experimental work	CD																								
Final Report	GT																								
REMA DST for Amikacin and Streptomycin																									
Addendum	GT																								
Experimental work	CD																								
Intermediate Report	GT																								
Final Report	GT																								
MIC determination of MFX on 7H10																									
Addendum	GT																								
Experimental work	CD																								
Intermediate report	GT																								
Final Report	GT																								
PZase assay																									
Validation plan	GT																								
Experimental work	MD																								
Final Report	GT																								

Challenges
and
Lessons Learned

- Infrastructures and Equipment
- Human Resources
- QMS & DMS



INFRASTRUCTURE AND EQUIPMENT

Mycobacteriology lab

- Space and ventilation: BSL2, BSL3?
- Safety cabinets
  - Class 1 or 2
  - Certification
- Centrifuges
  - Speed (rcf) Not well set up
  - Temperature
- Incubators
  - Space
  - Temperature monitoring
- Autoclave
  - Replacement
- Inspisssator
  - Solid media provided by central lab
- Microscope
  - Replacement
- Freezers
  - Not sufficient number

INFRASTRUCTURE AND EQUIPMENT

Molecular lab:

- 4 separate work areas
  - Not properly designed
- Equipment and consumables allocated to each area not interchangeable
  - Pipettes shared in all areas
- Different set of PPE with different colours
  - Not implemented
- Thermal cycler calibration
- Twincubator
  - better than GTBlot 48?
  - Calibration
- Mini spin
  - Not used
- Water bath
  - Not existing

INFRASTRUCTURE AND EQUIPMENT

- List of supplies with specifications before the start of the trial
- Maintenance and Service of equipment

HUMAN RESOURCES

Staff development

- Training plan — Not always
- Refresher training — Rarely done
- GCLP training — Not followed by all staff
- IATA certification — Non valid certificates

Staff experience

- Number of years
- Full time/part time
- Motivation

- **Workload**

Smear microscopy	Number staff per lab	
	No. of smears / year / staff	
Culture	Number staff per lab	
	No. of cultures / staff/day	
Phenotypic DST	Number staff per lab	
	No. of DST / year / staff	
Genotypic DST	Number staff per lab	
	No. of DST / year / staff	

- The current lab staff
  - Technician overwhelmed
  - Quality officer not existing
  - Coordinator (communication)
- Recruitment of new staff
  - Ideally with TB experience
  - Trained and proficiency assessed

QUALITY MANAGEMENT SYSTEM

- NTP guidelines for QA
  - Microscopy:
    - IQC Not properly done
    - EQA
      - ✓ PT Widely used
      - ✓ Rechecking Not always in place
  - Culture
    - IQC Not properly done – Indicators
    - EQA Not always in place
  - DST
    - IQC Not fully done
    - EQA
      - Phenotypic Few labs
      - Genotypic Not always implemented
        - ✓ Extract DNA Sent by central lab

DATA MANAGEMENT SYSTEM

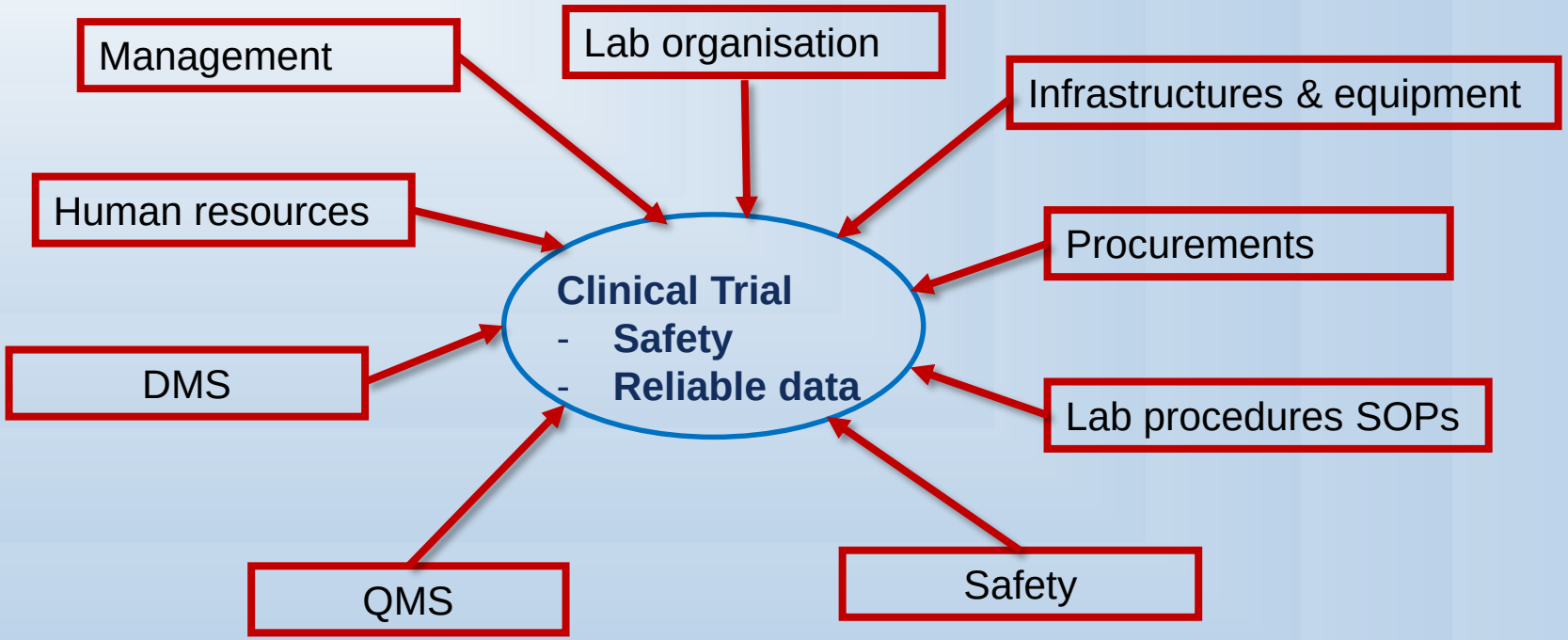
- Request form : Transfer data lab - clinician
  - Hard copy/electronic Clerical errors
  - Trial specific Not always

- Registers (microscopy, culture, DST)
  - Trial specific Difficult to harmonise
  - Hard copy Not complete
  - Electronic Transfer to central lab

- TAT Meet standards
  - Smear microscopy
  - Culture
  - DST

- Review of data Not done

CONCLUSION


Management
Lab organisation
Infrastructures & equipment
Human resources
Procurements
Clinical Trial
- Safety
- Reliable data
DMS
Lab procedures SOPs
QMS
Safety

QUESTIONS

Acknowledgments

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