
بسم الله الرحمن الرحیم

Detection Of *Mycobacterium Tuberculosis* In Blood During Tuberculosis Infection Using Phage Technology

Supervisor: Dr. Poursina

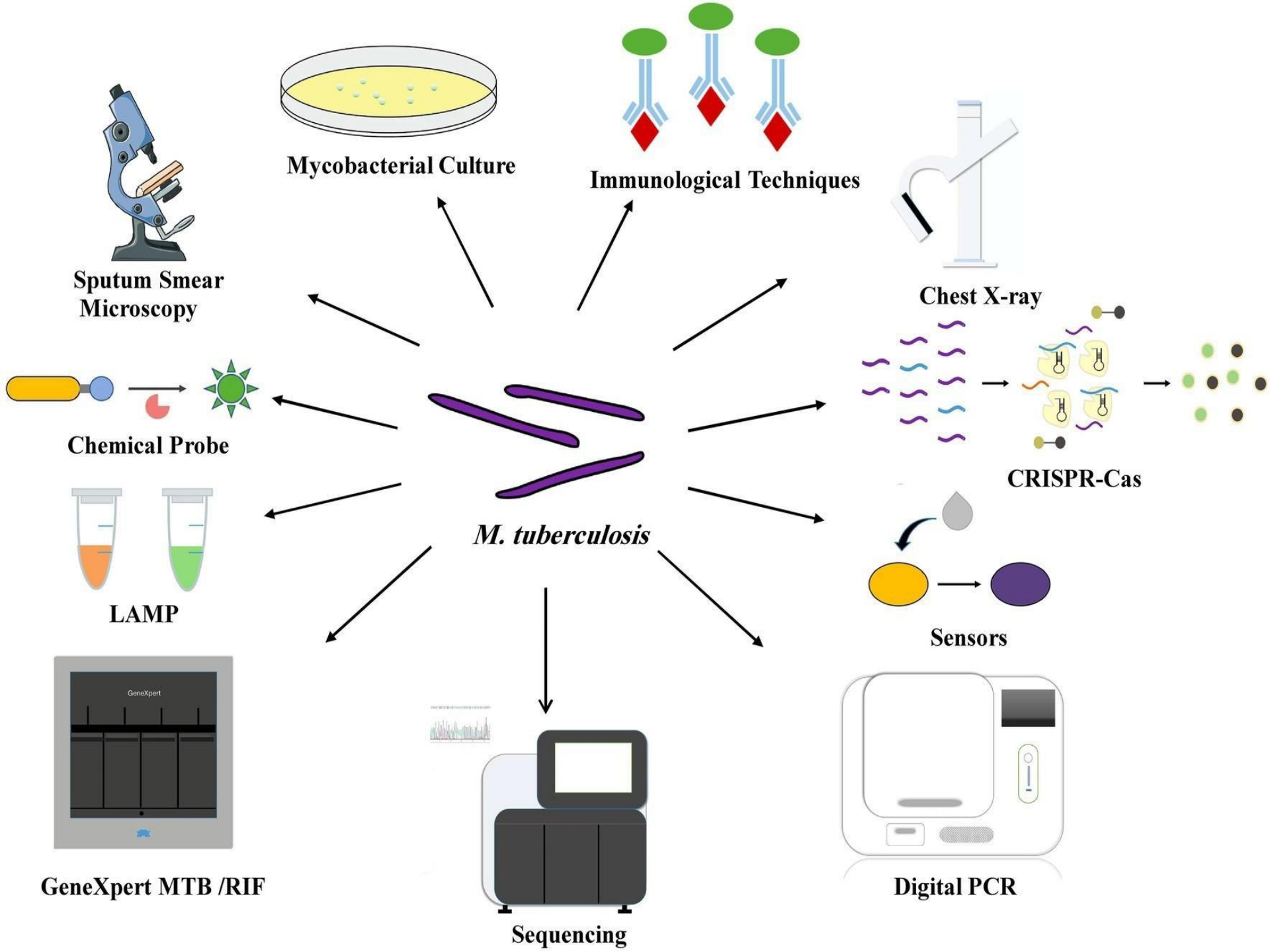
Presentation By: Fatemeh keshavarz

Outline

- Introduction
- Laboratory diagnosis of TB
- Development of TB Diagnostics
- Bacteriophages
- phage amplification method
- Methods
- Advantage and disadvantage
- Results and conculotin
- References

INTRODUCTION

- Aerosol-borne infection
- Cause of death worldwide
- Burden of (MDR) tuberculosis has increased over the past 3 decades.
- Inaccessibility of intracellular Mtb DNA in PBMCs: poor sensitivity of methods

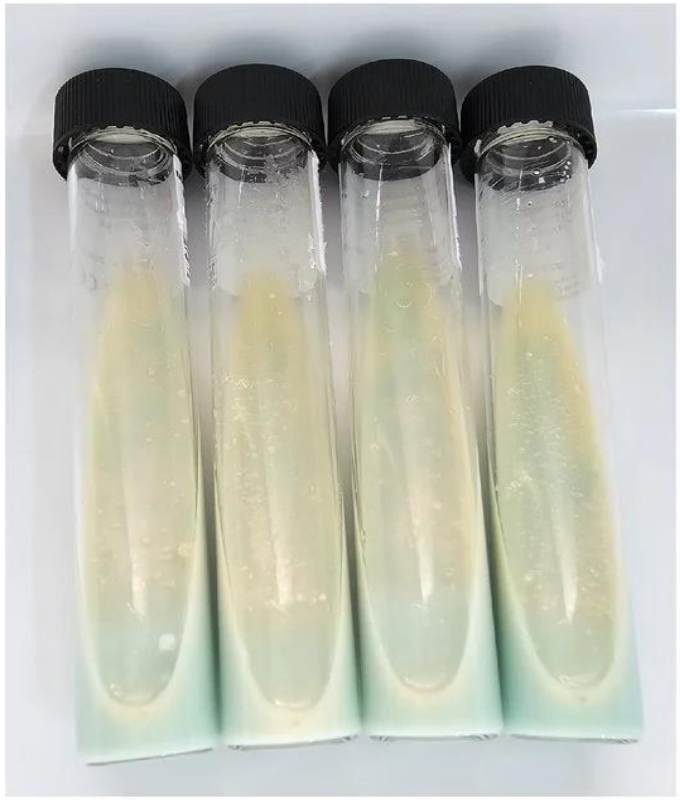

Sputum Smear Microscopy
Mycobacterial Culture
Immunological Techniques
Chest X-ray
Chemical Probe
CRISPR-Cas
M. tuberculosis
LAMP
Sensors
GeneXpert
GeneXpert MTB /RIF
Sequencing
Digital PCR

Barriers to culture-based diagnosis

- Significant delays to a conclusive result
- Relatively poor sensitivity
- Difficulties for obtaining specimens

CULTURE-BASED DIAGNOSIS

- Rates of culture confirmation in 2020
- 75.3% and 44.2% for treated pulmonary and extrapulmonary TB, respectively.

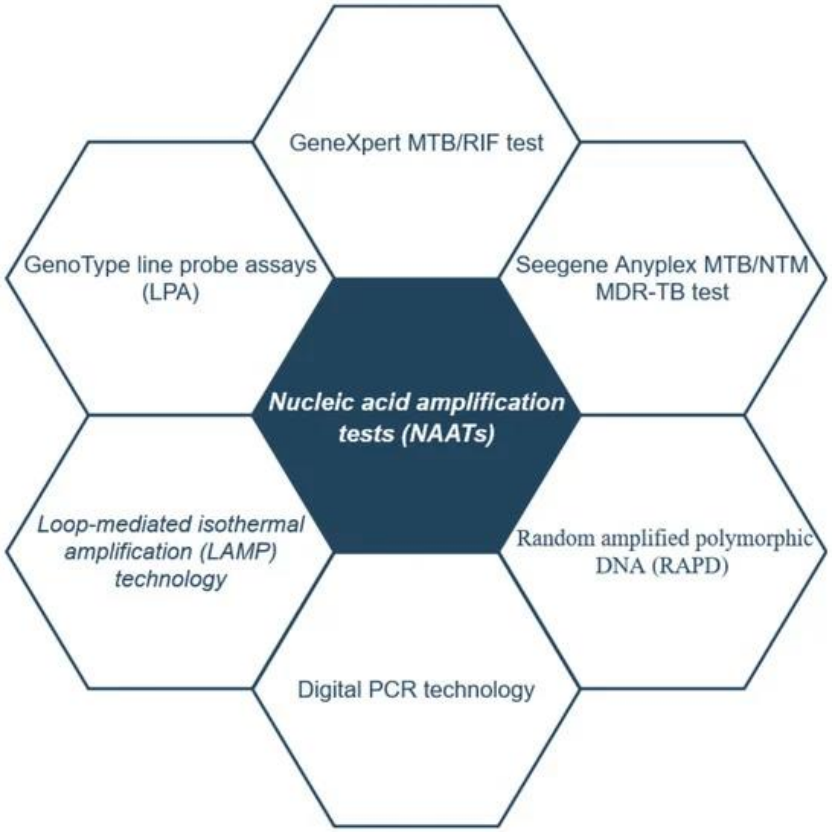


Molecular Methods

- Employing nucleic acid amplification of Mtb gene targets developed as rapid microbiological tools.

- The benefit of nucleic acid based test:

Offering for the first time, reliable, same-day diagnostic capability



Development of TB Diagnostics

The development of TB diagnostics that are ubiquitous—that is, enabling diagnosis from an easily accessible site for all disease phenotypes—is an important unmet need and research priority

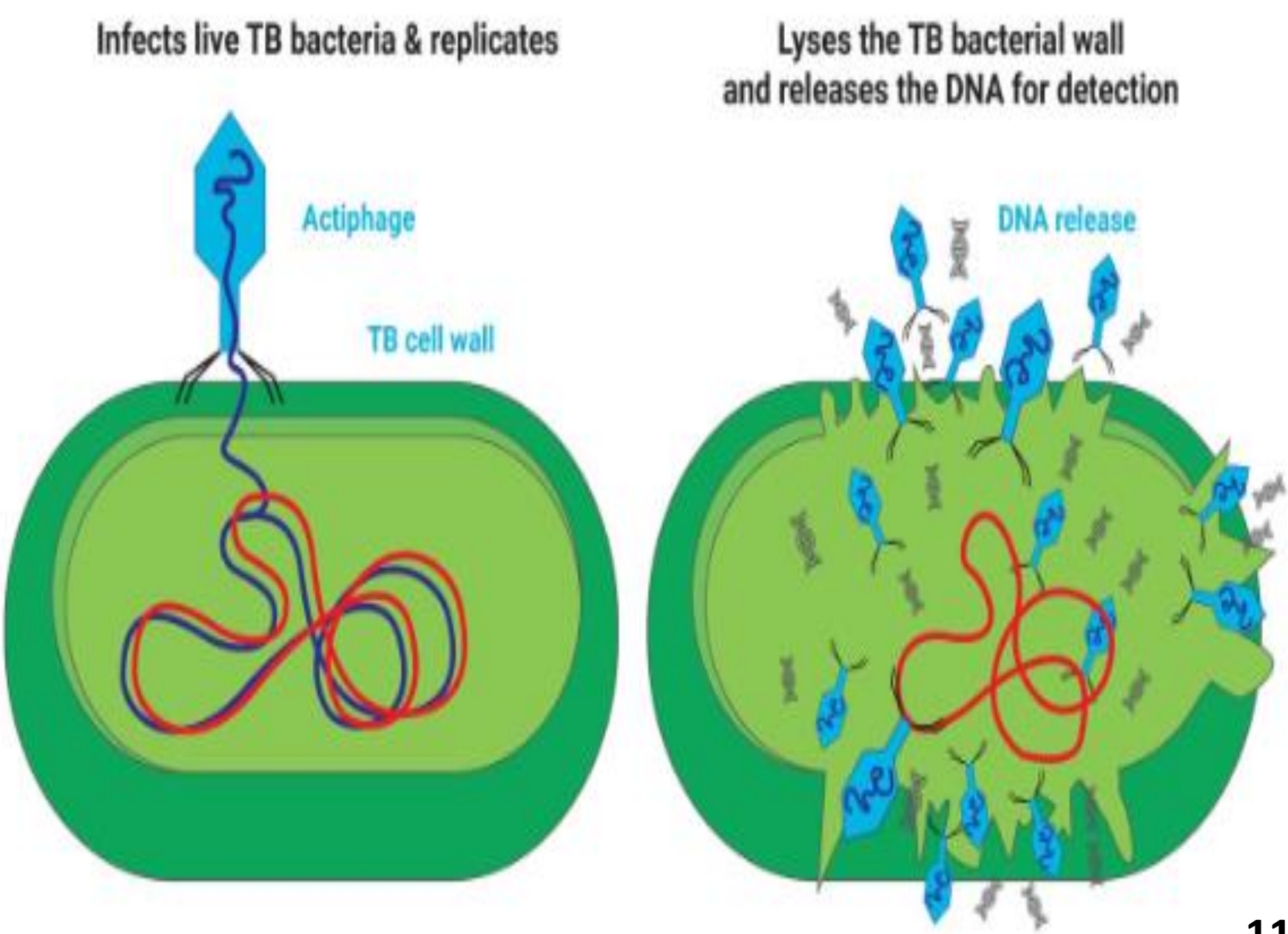
Bacteriophages

Bacteriophages (phages)

The specificity of phage → Rapid diagnostic tests

A mycobacteriophage diagnostic test was first developed to detect *Mtb* in sputum

(FASTPlaqueTB assay, Biotec Laboratories Ltd, UK)


Infects live TB bacteria & replicates
Actiphage
TB cell wall
Lyses the TB bacterial wall
and releases the DNA for detection
DNA release

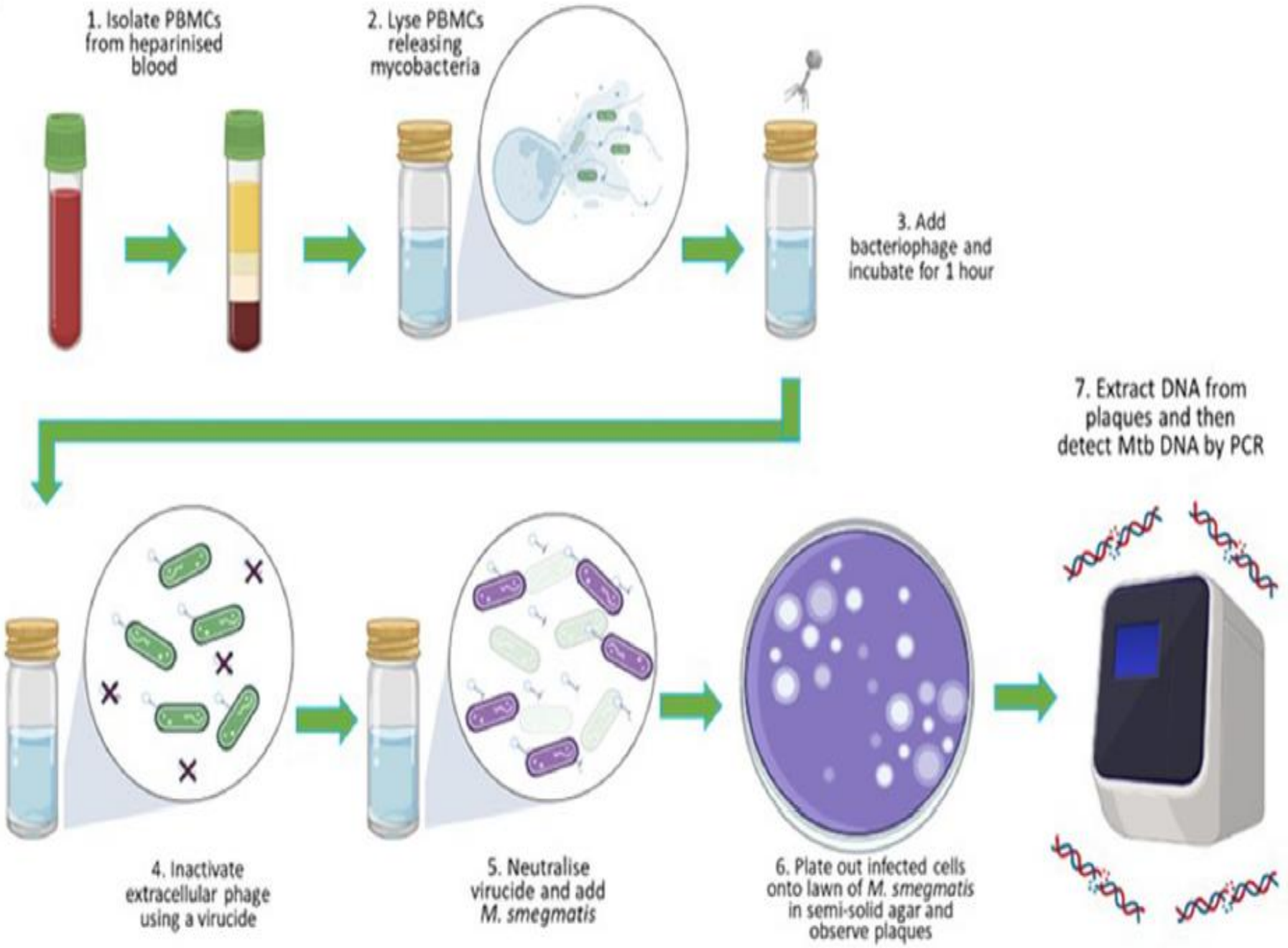
Mycobacteriophage Diagnostic Test

- Mycobacteriophage **D29** : range of different pathogenic mycobacterial species.
- Detects the growth of the phage on host cells present in the sample
- Emergence of Plaques

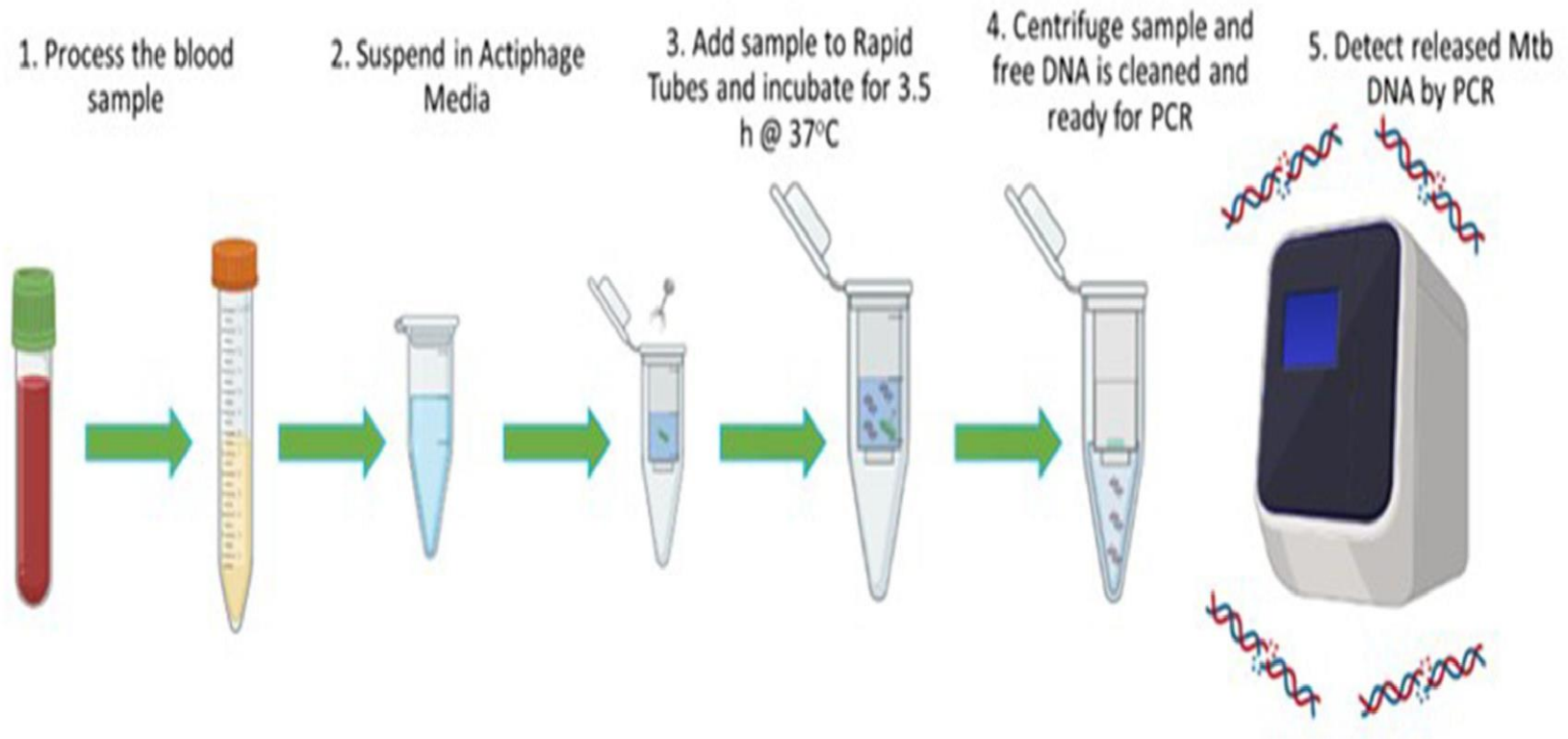
High specificity but similar sensitivity to sputum microscopy, and **therefore provided no real advantage over conventional tests**.

original phage amplification method combined with PCR

A)


1. Isolate PBMCs from heparinised blood
2. Lyse PBMCs releasing mycobacteria
3. Add bacteriophage and incubate for 1 hour
4. Inactivate extracellular phage using a virucide
5. Neutralise virucide and add *M. smegmatis*
6. Plate out infected cells onto lawn of *M. smegmatis* in semi-solid agar and observe plaques
7. Extract DNA from plaques and then detect Mtb DNA by PCR

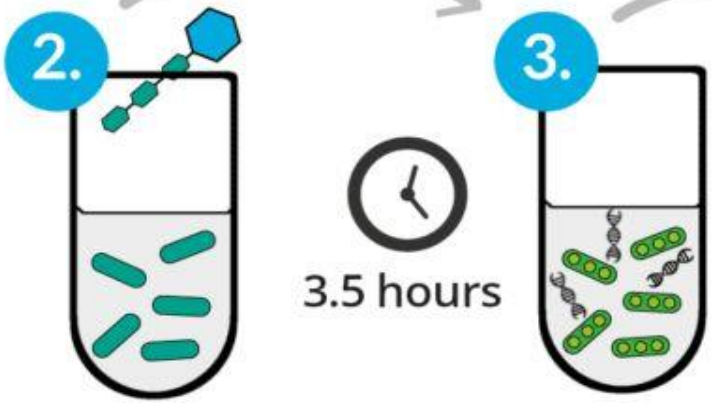
B)


1. Process the blood sample
2. Suspend in Actiphage Media
3. Add sample to Rapid Tubes and incubate for 3.5 h @ 37°C
4. Centrifuge sample and free DNA is cleaned and ready for PCR
5. Detect released Mtb DNA by PCR

The Actiphage® process

1. Isolation of white blood cells

Cells lysed to release Mycobacteria

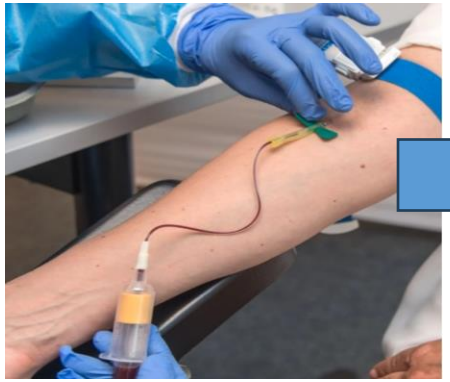


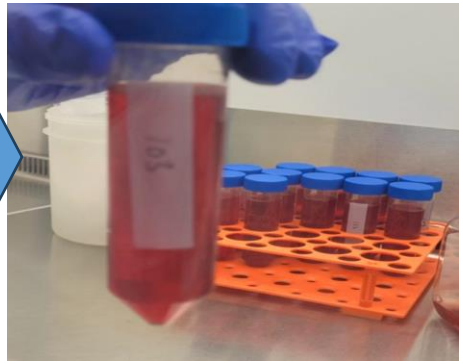
2. Actiphage reagent added to release DNA from bacteria

3.5 hours

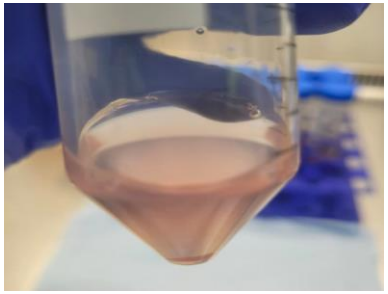
3. DNA recovery

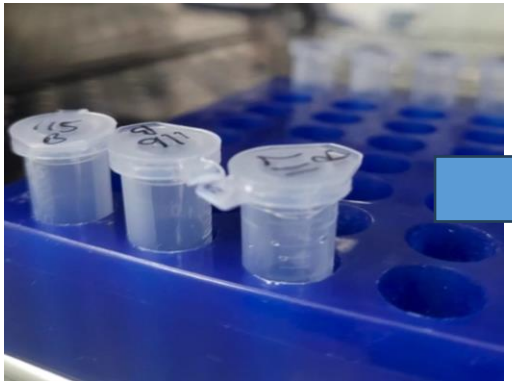
4. DNA from viable Mycobacteria detected using qPCR

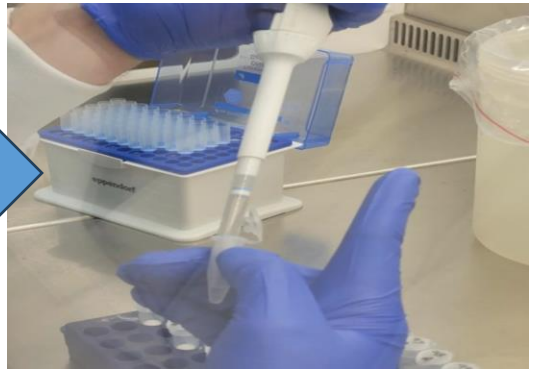


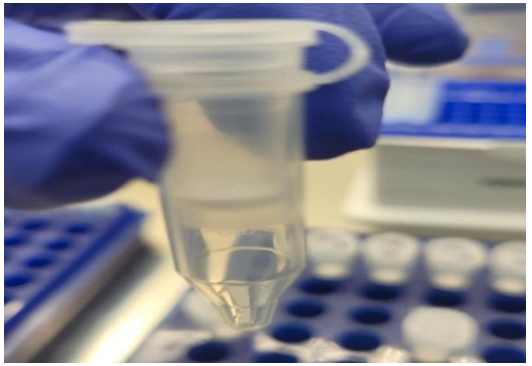


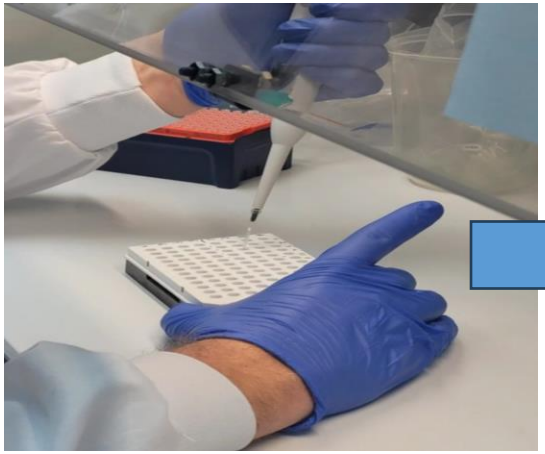

eppendorf
Centrifuge 5920 R

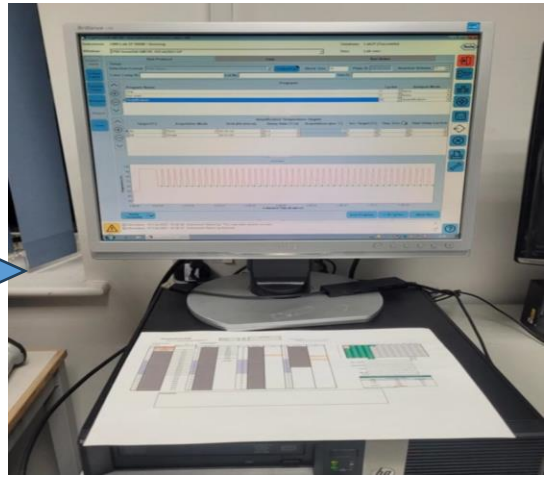












Mycobacteriophage diagnostic test

- Phage-PCR blood assay : remove the need for agar plates
- 6-12 hours
- Phage cannot replicate in dead hosts :advantage ;only detects viable cells but retains the sensitivity and specificity of PCR.
- Limit of detection :less than 10 viable mycobacteria in a 2 ml blood sample.

Clinical studies using phage-based detection of *Mycobacterium tuberculosis* in the blood

Methods

Participants

The study recruited participants to four groups:

1. Pulmonary TB
2. Non-TB Acute respiratory illness
3. IGRA-positive house-hold contacts of the PTB)LTBI)
4. Negative control group

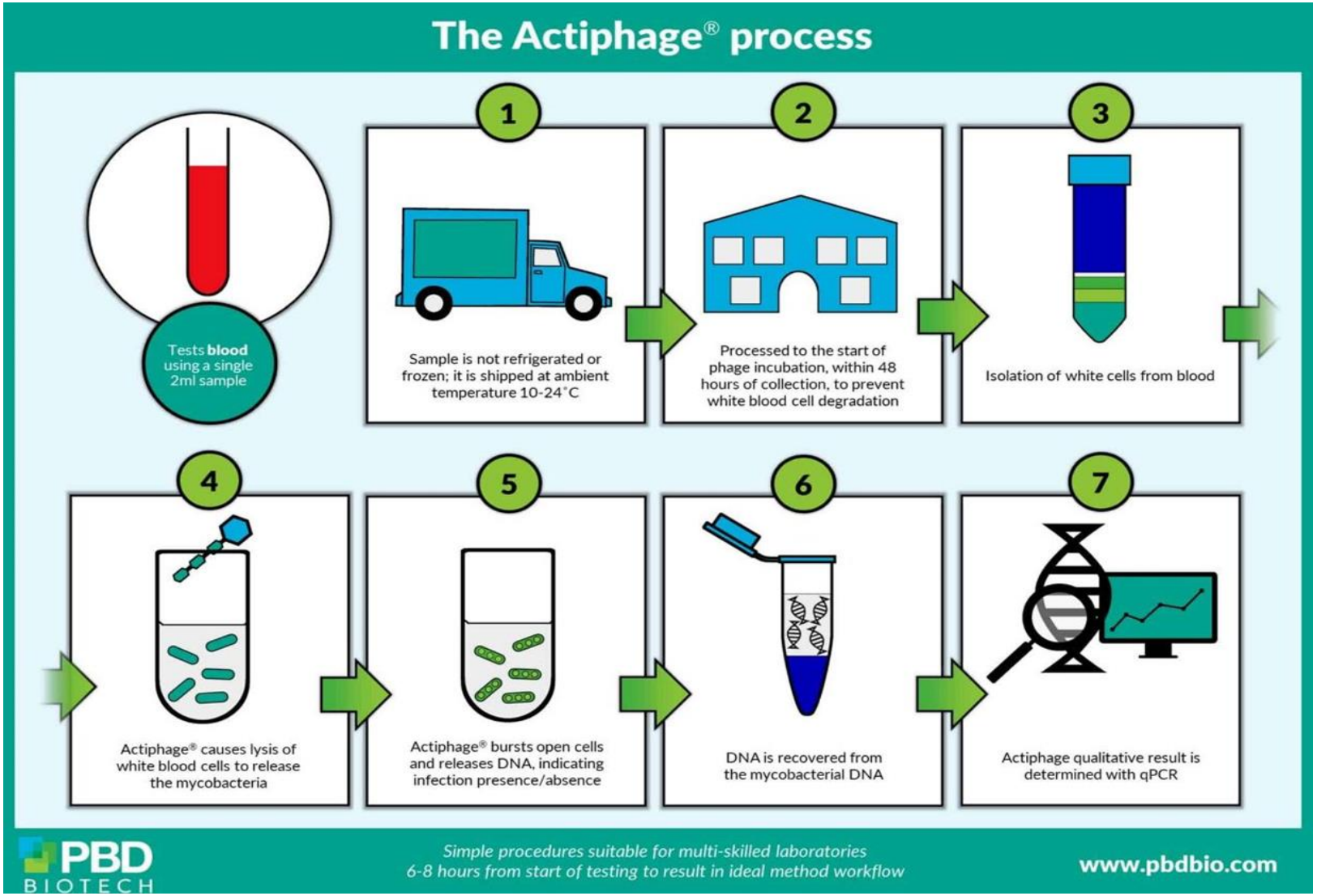
All recruited participants were immunocompetent, HIV-uninfected, adults with no previous history of TB disease.

Methods

Blinded testing with the Actiphage assay was performed in all groups, before any treatment at the baseline visit

All participants provided blood samples for **Actiphage testing** on recruitment and received a 12-month prospective, clinical follow-up.

Methods


The Actiphage® process
Tests blood using a single 2ml sample
1
Sample is not refrigerated or frozen; it is shipped at ambient temperature 10-24˚C
2
Processed to the start of phage incubation, within 48 hours of collection, to prevent white blood cell degradation
3
Isolation of white cells from blood
4
Actiphage® causes lysis of white blood cells to release the mycobacteria
5
Actiphage® bursts open cells and releases DNA, indicating infection presence/absence
6
DNA is recovered from the mycobacterial DNA
7
Actiphage qualitative result is determined with qPCR
PBD BIOTECH
Simple procedures suitable for multi-skilled laboratories
6-8 hours from start of testing to result in ideal method workflow
www.pbdbio.com

Results

Actiphage Result

	Active Pulmonary TB (n = 15)		Non-TB Acute Respiratory Illness (n = 5)
	Positive (n = 11)	Negative (n = 4)	All Negative
	5 (45.5)	2 (50)	2 (40)
	31.5 (±13.9)	38.8 (±13.5)	50 (±21.7)
)	3 (27.2)	1 (25)	2 (40)
Yes, n (%)[a]	4 (36.4)	2 (50)	2 (40)
Unknown, n (%)	0	0	0
	19.9 (±3.6)	20.9 (±3.0)	25.7 (±5.3)
Smear positive	7	0	0
Smear negative	4	4	0
Xpert-Ultra grade	Medium–high	Very low–low	All negative
CRP, median (IQR)	63 (36–65)	41 (27–45.5)	84 (45–110)
Days to positive culture, median (IQR)	15 (10.5–22)	21 (21–21)	1 blood culture (*S. aureus*); 1 sputum culture (*M. avium*, 6 days)

Results

Actiphage Result

	Pulmonary TB Contacts With LTBI (n = 18)		Healthy Controls: No LTBI (n = 28)
	Positive (n = 3)	Negative (n = 15)	All Negative
	1 (33.3)	10 (55.6)	11 (39.3)
	25.3 (±6.4)	54.7 (±12.3)	38.9 (±14.6)
	1 (33.3)	5 (33.3)	10 (35.7)
	2 (66.7)	7 (63.6)	12 (50)
	0	4 (26.7)	4 (14.3)
	21.9 (±2.0)	26.2 (±6.9)	27.1 (±8.2)
Positive Smear	0	N/A	N/A
Negative Smear	2	N/A	N/A
Xpert-ultra grade	Medium[b]	N/A	N/A
	5 (5–5)[c]	10 (5–13.75)	5 (5–10)
	26 (23.5–28.5)[b]	N/A	N/A

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terium avium; N/A, not applicable; S. aureus, Staphylococcus aureus; SD, standard deviation;

Results

After 12 months of follow-up

no Actiphage-negative participants had developed TB

In the PTB cohort, Actiphage-positive results were associated with sputum smear positivity, higher baseline C-reactive protein levels, and shorter times to mycobacterial culture

Sensitivity and specificity

- As a clinical diagnostic tool in symptomatic patients with suspected PTB:

Actiphage testing had a **sensitivity of 73.3%** and a **specificity of 100%**

Sensitivity and specificity

- When applied to the whole cohort at baseline

Its specificity for detecting PTB was **94.2%** with **no change in sensitivity**.

Previous studies

- using culture and NAAT in blood samples from patients with active TB have been disappointing.

- With 1 study reporting that Mtb was detected in the blood of only **21%** of HIV-infected individuals with severe miliary disease using Xpert MTB/RIF is detectable.

Advantages

- Simplify sample preparation
- High sensitivity)detect fewer than 10 live mycobacteria in a 2ml blood sample)
- Rapid time
- Low-cost assay
- High specificity (high-level specificity controls)
- High accuracy) live and dead mycobacteria)

Disadvantages

- Only for Blood Sample
- Need Conventional PCR

Animal Study

Table 1. Determining the Limited of detection of the Actiphage assay.

	MAP[b]		*M. bovis* BCG[b]		*M. bovis*[b]	
Number of cells[a]	Phage assay	Actiphage® method	Phage assay	Actiphage® method	Phage assay	Actiphage® method
10^4	+	+	+	+	+	+
10^3	+	+	+	+	+	+
10^2	+	+	+	+	+	+
10^1	+	+	+	+	+	+
10^0	–	+	+	+	–	+
0	–	–	–	–	–	–

Results represent the detection of each mycobacteria in three independent samples. For both the phage amplification assay and the Actiphage® method, '+' denotes positive molecular detection in all three replicates;'–' denotes no detection of mycobacteria in all three replicates.

[a]Number of cells added to each sample was determined using the phage amplification enumeration method described by Rees and Botsaris (2012).

[b]For details of the PCR assay used for the different types of bacteria see the method section.

Actiphage TB awarded US Patent grant for diagnostic kit

25-Jul-2023 10:50 PM EDT, by 2023 AACC Annual Scientific Meeting Press Program

PBD Biotech at AACC

Product › Details

Actiphage® blood test (TB test)

	Next higher product group	diagnostic test, indication
	Status	2022-10-17 development pc existent
	Organisation	PBD Biotech Ltd.
	Group	PBD Biotech (Group)

Conclusion

For the first time:

- This study used phage-based DNA extraction (Actiphage), combined with polymerase chain reaction, to detect low levels of viable Mtb in blood.

- High sensitivity & High specificity

References

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Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Perspective

State-of-the-art detection of *Mycobacterium tuberculosis* in blood during tuberculosis infection using phage technology

Catherine ED Rees[1], Benjamin MC Swift[2], Pranabashis Haldar[3,*]

[1] School of Biosciences, University of Nottingham, Nottingham, UK
[2] Royal Veterinary College, Department of Pathobiology and Population Sciences, Herts, UK
[3] NIHR Leicester Biomedical Research Centre, Department of Respiratory Sciences, University of Leicester, Leicester, UK

microbial biotechnology

Open Access

The development and use of Actiphage® to detect viable mycobacteria from bovine tuberculosis and Johne's disease-infected animals

Benjamin M. C. Swift[1,*] Nathan Meade,[2] Elsa Sandoval Barron,[3] Malcolm Bennett,[3] Tania Perehenic,[2] Valerie Hughes,[4] Karen Stevenson[4] and Catherine E. D. Rees[2]

[1] *Pathobiology and Population Sciences, Royal Veterinary College, Hawkshead, Herts AL9 7TA, UK.*

infected cattle. This method provides a revolutionary new tool for the study of infections caused by these difficult to grow pathogens.

Introduction

Clinical Infectious Diseases

BRIEF REPORT

A Novel, High-sensitivity, Bacteriophage-based Assay Identifies Low-level *Mycobacterium tuberculosis* Bacteremia in Immunocompetent Patients With Active and Incipient Tuberculosis

Raman Verma,[1] Benjamin M. C. Swift,[2] Wade Handley-Hartill,[3] Joanne K. Lee,[1] Gerrit Woltmann,[4] Catherine E. D. Rees,[3] and Pranabashis Haldar[1]

[1]Department of Respiratory Sciences, National Institute for Health Research Respiratory Biomedical Research Centre, University of Leicester, [2]Royal Veterinary College, Hawkshead Campus, Herts, [3]School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leicestershire, and [4]Department of Respiratory Medicine, University Hospitals of Leicester National Health Service Trust, Glenfield Hospital, United Kingdom


Thank
you !