Socios En Salud Sucursal Perú
Laboratorio
ISO9001 Certified — CAP #: 8661499 – Smile Harmonized ID: 0318

FINAL VALIDATION REPORT - *M. tuberculosis* culture in LJ media slants

1. Background

Tuberculosis (TB) is the cause of death for 1.5 million people a year [1] and now possibly, we have an increase of affected people [2]. The National TB Control Programs carry out various actions for diagnostic and therapeutic approaches [3] and the TB treatment is monitored through sputum smear microscopy and periodic cultures [3, 4] with the objective of know the exactly category of treatment outcome [5]. While smear microscopy is the most popular method to detect mycobacteria in clinical specimen, culture of the etiological agent remains the accepted "gold standard" for diagnosing mycobacterial infections [6]. Cultivation on solid media, such as LJ, has a critical role in labs. This method is very frequent because has been designed for the isolation and growth of *M. tuberculosis*. Additionally, it is used for the determination of antibiotic susceptibility and microbiological research. [7]. Given some limitations, commercial products are preferred for improving performance; thus, minimizing variability between assays [8].

Since SES participates in clinical trials and this method is useful for diagnostic and treatment monitoring of patients; this report documents the validation process of the BD LJ media slants in our TB lab by comparison with the previously validated LJ media.

2. Objective

Validate the *M. tuberculosis* culture with BD BBL Lowenstein-Jensen Medium Slants in the SES Peru Lab.

3. References

1. World Health Organization: **Global Tuberculosis Report 2020**. In. Geneva, Switzerland: World Health Organization.; 2021.
2. World Health Organization: **Global tuberculosis report 2023**. In. Geneva: World Health Organization; 2023.
3. Dirección de Control y Prevención de Tuberculosis: **Norma Tecnica de Salud para La Atencion Integral de Personas Afectadas por Tuberculosis**. In. Lima, Perú: Ministerio de Salud del Perú; 2013.
4. Ministério da Saúde do Brasil: **Manual de recomendações para o controle da tuberculose no Brasil / Secretaria de Vigilância em Saúde, Departamento de Vigilância Epidemiológica. – Brasília : Ministério da Saúde**. In. (Série A. Normas e Manuais Técnicos); 2011: 284.
5. World Health Organization: **Definitions and reporting framework for tuberculosis – 2013 revision (updated December 2014 and January 2020)**. In.; 2013.
6. Cheng VC, Yew WW, Yuen KY: **Molecular diagnostics in tuberculosis**. *Eur J Clin Microbiol Infect Dis* 2005, **24**(11):711-720.
7. Organización Panamericana de la Salud: **Manual para el diagnostico bacteriologico de la tuberculosis. Normas y guia tecnica. Parte 2 Cultivo**. In. Washington, D.C: Pan American Health Orgatization; 2008.
8. Parisaca Mamani S, Bautista A, Vasquez Michel A: **Comparación del rendimiento del medio de cultivo Löwenstein-Jensen in house y Löwenstein-Jensen comercial, para el aislamientos de Mycobacterium tuberculosis de pacientes con tuberculosis pulmonar**. *Rev Cs Farm y Bioq* 2015, **3**(1):69-76.

4. Methodology

4.1. Specimens

Spiked simulated samples (sterile water) with standard strains were used for the precision assessments.

- As negative control was used S. aureus (ATCC 25923) The spiked specimen was performed using an individually independent bacillar suspension of this strains at McFarland 0.5 with a dilution to 10^{-4}
- As positive control was used *M. tuberculosis* H37Ra (ATCC 25177). The spiked specimen was performed using an individually independent bacillar suspension of this strains at McFarland 0.5 with a dilution to 10^{-5}

- Additionally, we used a blank or system control using phosphate buffer 0.068 M; pH 6.8

Raw available samples used in this validation were chosen from our cryobank (-80°C) with the following characteristics:
- 15 or more specimens with negative culture result by the standard method.
- 15 or more specimens with positive culture result by the standard method.

4.2. Materials and methods

The *M. tuberculosis* culture procedure has performed using:
- BD BBL Lowenstein-Jensen Medium Slants REF: 220909;

with the following SOPs:
- SES-GLB-PR-02 Preparación de Buffer Fosfato v.10
- SES-GLB-PR-03 Descontaminación de Muestras Respiratorias por el Método NALC-NaOH v. 2.0
- SES-GLB-PR-19 Procesamiento de Muestras Para Cultivo en Medio Löwenstein Jensen por el método NALC v. 1.6

4.3. Precision

Precision is the agreement of the measurements of replicate runs of the same sample. It is the process of determining the range of random error.

4.3.1. For short-term, *M. tuberculosis* H37Ra, *S. aureus* as non-mycobacteria species and a negative control containing only phosphate buffer were used in triplicate run (individually independent bacterial suspensions), by 2 operators in the Lab.

4.3.2. For long-term, *M. tuberculosis* H37Ra, *S. aureus* as non-mycobacteria species and a negative control containing only phosphate buffer were used in triplicate run (individually independent bacterial suspensions), by 2 operators and for three days in the Lab.

Acceptability criteria: Results are approved if the determined reproducibility is 100%.

4.4. Accuracy

Accuracy is the true value of a substance being measured and this verification is the process of determining that the test system is producing true, valid results.

4.4.1. At least 30 raw samples were used. These samples were chosen by the results on ZN smear and Xpert MTB/RIF Ultra.

Acceptability criteria: Results are approved if the determined grown is obtained between the 90 - 100% of the specimens.

4.5. Time to Detection (TTD)

For precision and accuracy, TTD was registered for each specimen. This assessment is not applicable, because the specimens used were frozen and the characteristics will be compromised.

4.6. Analytical Sensitivity

This assessment is not applicable, because the specimens used were frozen and the characteristics will be compromised.

4.7. Method Approval

The final decision on methodology validation and acceptance is made by the Laboratory Director after review of all the studies performed as part of the complete method validation process.

4.8. Operators

OPE 01: MUF - Mariella Uchima (Biologist)
OPE 02: DAO – Diana Alonso (Biologist)

4.9. Supervisor

Nadia Barreda – Biologist, MSc.

5. Results

5.1. Short term (with-in run) precision

5.1.1. Results

Table N° 1. Short term precision results of culture performed on BD LJ Medium Slants using spiked specimens.

Specimen	Inoculation date	LJ media Lot #	LJ media - expiration date	Operator	Read 72 h	Read 1st w	Read 2nd w	Read 3rd w	Read 4th w	Read 5th w	Read 6th w	Read 7th w	Read 8th w	Final Result Date LJ slant	Final Result LJ slant	Expected result LJ prepared
S. aureus (1)*	9/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	No grow
S. aureus (2)*	9/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	
S. aureus (3)*	9/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	
S. aureus (4)*	9/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	
S. aureus (5)*	9/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	
S. aureus (6)*	9/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	
Blank (1)*	9/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	No grow
Blank (2)*	9/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	
Blank (3)*	9/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	
Blank (4)*	9/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	
Blank (5)*	9/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	
Blank (6)*	9/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	
H37Ra (1)*	9/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	col	+	NA	NA	NA	13/11/2023	Positive	Positive
H37Ra (2)*	9/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	col	+	NA	NA	NA	13/11/2023	Positive	
H37Ra (3)*	9/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	col	+	NA	NA	NA	13/11/2023	Positive	
H37Ra (4)*	9/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	col	+	NA	NA	NA	13/11/2023	Positive	
H37Ra (5)*	9/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	col	+	NA	NA	NA	13/11/2023	Positive	
H37Ra (6)*	9/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	col	+	NA	NA	NA	13/11/2023	Positive	

* Bacterial suspension individually prepared for this test. Lot #: Lot number of the LJ slant used for this validation. NG: No grow. COL: Colonies observed, not conclusive. NA: Not applicable.

5.1.2. Assessment of short term (with-in run) precision

Operator MUF 100 %
Operator DAO 100 %

5.1.3. Acceptability of short term (with-in run) precision

Operator MUF APPROVED
Operator DAO APPROVED

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5.2. Long term (between-day) precision

5.2.1. Results

Table N° 1. Long term precision results of culture performed on BD LJ Medium Slants using spiked specimens.

Specimen	Inoculation date	LJ media Lot #	LJ media - expiration date	Operator	Read 72 h	Read 1st w	Read 2nd w	Read 3rd w	Read 4th w	Read 5th w	Read 6th w	Read 7th w	Read 8th w	Final Result Date LJ slant	Final Result LJ slant	Expected result LJ prepared
S. aureus (1)*	9/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	No grow
S. aureus (2)*	9/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	
S. aureus (3)*	9/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	
S. aureus (4)*	9/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	
S. aureus (5)*	9/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	
S. aureus (6)*	9/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	
Blank (1)*	9/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	No grow
Blank (2)*	9/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	
Blank (3)*	9/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	
Blank (4)*	9/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	
Blank (5)*	9/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	
Blank (6)*	9/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	4/12/2023	Negative	
H37Ra (1)*	9/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	col	+	NA	NA	NA	13/11/2023	Positive	Positive
H37Ra (2)*	9/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	col	+	NA	NA	NA	13/11/2023	Positive	
H37Ra (3)*	9/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	col	+	NA	NA	NA	13/11/2023	Positive	
H37Ra (4)*	9/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	col	+	NA	NA	NA	13/11/2023	Positive	
H37Ra (5)*	9/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	col	+	NA	NA	NA	13/11/2023	Positive	
H37Ra (6)*	9/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	col	+	NA	NA	NA	13/11/2023	Positive	
S. aureus (1)*	11/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	6/12/2023	Negative	No grow
S. aureus (2)*	11/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	6/12/2023	Negative	
S. aureus (3)*	11/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	6/12/2023	Negative	
S. aureus (4)*	11/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	6/12/2023	Negative	
S. aureus (5)*	11/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	6/12/2023	Negative	
S. aureus (6)*	11/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	6/12/2023	Negative	
Blank (1)*	11/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	6/12/2023	Negative	No grow
Blank (2)*	11/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	6/12/2023	Negative	
Blank (3)*	11/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	6/12/2023	Negative	
Blank (4)*	11/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	6/12/2023	Negative	
Blank (5)*	11/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	6/12/2023	Negative	
Blank (6)*	11/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	6/12/2023	Negative	
H37Ra (1)*	11/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	col	+	NA	NA	NA	15/11/2023	Positive	Positive
H37Ra (2)*	11/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	col	+	NA	NA	NA	15/11/2023	Positive	
H37Ra (3)*	11/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	col	+	NA	NA	NA	15/11/2023	Positive	
H37Ra (4)*	11/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	col	+	NA	NA	NA	15/11/2023	Positive	
H37Ra (5)*	11/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	col	+	NA	NA	NA	15/11/2023	Positive	
H37Ra (6)*	11/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	col	+	NA	NA	NA	15/11/2023	Positive	
S. aureus (1)*	13/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	8/12/2023	Negative	No grow
S. aureus (2)*	13/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	8/12/2023	Negative	
S. aureus (3)*	13/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	8/12/2023	Negative	
S. aureus (4)*	13/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	8/12/2023	Negative	
S. aureus (5)*	13/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	8/12/2023	Negative	
S. aureus (6)*	13/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	8/12/2023	Negative	
Blank (1)*	13/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	8/12/2023	Negative	No grow
Blank (2)*	13/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	8/12/2023	Negative	
Blank (3)*	13/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	NG	NG	NG	NG	NG	8/12/2023	Negative	
Blank (4)*	13/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	8/12/2023	Negative	
Blank (5)*	13/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	8/12/2023	Negative	
Blank (6)*	13/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	NG	NG	NG	NG	NG	8/12/2023	Negative	
H37Ra (1)*	13/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	col	+	NA	NA	NA	17/11/2023	Positive	Positive
H37Ra (2)*	13/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	col	+	NA	NA	NA	17/11/2023	Positive	
H37Ra (3)*	13/10/2023	2181557	30/12/2023	MUF	NG	NG	NG	NG	col	+	NA	NA	NA	17/11/2023	Positive	
H37Ra (4)*	13/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	col	+	NA	NA	NA	17/11/2023	Positive	
H37Ra (5)*	13/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	col	+	NA	NA	NA	17/11/2023	Positive	
H37Ra (6)*	13/10/2023	2181557	30/12/2023	DAO	NG	NG	NG	NG	col	+	NA	NA	NA	17/11/2023	Positive	

* Bacterial suspension individually prepared for this test. Lot #: Lot number of the LJ slant used for this validation. NG: No grow. COL: Colonies observed, not conclusive. NA: Not applicable.

5.2.2. Assessment of long term (between-day) precision

Operator MUF 100 %
Operator DAO 100 %

5.2.3. Acceptability of long term (between-day) precision

Operator MUF APPROVED
Operator DAO APPROVED

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5.3. Accuracy

Table N° 2. Accuracy results of culture performed on BD LJ Medium Slants using frozen available specimens.

Specimen ID	ZN Smear result	Xpert MTB/RIF Ultra Result	Inoculation date	Read 72 h	Read 1 w	Read 2 w	Read 3 w	Read 4 w	Read 5w	Read 6w	Read 7w	Read 8w	Result date	Final result in LJ slant	Final result LJ prepared
001096-0590-M	1+	Medium	11/12/2023	NG	NG	NG	col	1+	NA	NA	NA	NA	8/01/2024	Positive 1+	Positive 1+
001096-0672-M	2+	Medium	11/12/2023	NG	NG	NG	col	1+	NA	NA	NA	NA	8/01/2024	Positive 1+	Positive 1+
001096-0824-M	3+	Medium	11/12/2023	NG	NG	NG	col	1+	NA	NA	NA	NA	8/01/2024	Positive 1+	Positive 1+
019096-0338-M	3+	Medium	11/12/2023	NG	NG	NG	col	1+	NA	NA	NA	NA	8/01/2024	Positive 1+	Positive 1+
019096-0459-M	2+	Medium	11/12/2023	NG	NG	NG	col	1+	NA	NA	NA	NA	8/01/2024	Positive 1+	Positive 1+
019096-0517-M	1+	Medium	11/12/2023	NG	NG	NG	col	1+	NA	NA	NA	NA	8/01/2024	Positive 1+	Positive 1+
019096-0654-M	1+	Medium	11/12/2023	NG	NG	NG	col	1+	NA	NA	NA	NA	8/01/2024	Positive 1+	Positive 1+
019096-2730-M	1+	Medium	11/12/2023	NG	NG	NG	col	1+	NA	NA	NA	NA	8/01/2024	Positive 1+	Positive 1+
019096-0778-M	1+	Low	11/12/2023	NG	NG	NG	NG	NG	col	1+	NA	NA	22/01/2024	Positive 1+	Positive 1+
028148-0082-M	7 AFB	Low	11/12/2023	NG	NG	NG	NG	NG	col	1+	NA	NA	22/01/2024	Positive 1+	Positive 1+
028148-0440-M	5 AFB	Low	11/12/2023	NG	NG	NG	NG	NG	col	1+	NA	NA	22/01/2024	Positive 1+	Positive 1+
019096-0532-M	4 AFB	Very Low	11/12/2023	NG	NG	NG	NG	NG	NG	NG	13 col	13 col	29/01/2024	13 colonies	13 colonies
019096-1948-M	Negative	Low	11/12/2023	NG	NG	NG	NG	NG	col	1+	NA	NA	22/01/2024	Positive 1+	Positive 1+
019096-2006-M	Negative	Low	11/12/2023	NG	NG	NG	NG	NG	col	1+	NA	NA	22/01/2024	Positive 1+	Positive 1+
019096-2113-M	Negative	Low	11/12/2023	NG	NG	NG	NG	NG	col	1+	NA	NA	22/01/2024	Positive 1+	Positive 1+
019096-1844-M	Negative	Very Low	11/12/2023	NG	NG	NG	NG	NG	NG	NG	10 col	10 col	29/01/2024	10 colonies	11 colonies
019096-2100-M	Negative	Very Low	11/12/2023	NG	NG	NG	NG	NG	NG	NG	15 col	15 col	29/01/2024	15 colonies	15 colonies
019096-3482-M	Negative	Very Low	11/12/2023	NG	NG	NG	NG	NG	NG	NG	NG	NG	5/02/2024	Negative	Negative
001416-0001-M	Negative	Negative	11/12/2023	NG	NG	NG	NG	NG	NG	NG	NG	NG	5/02/2024	Negative	Negative
001416-0002-M	Negative	Negative	11/12/2023	NG	NG	NG	NG	NG	NG	NG	NG	NG	5/02/2024	Negative	Negative
001416-0003-M	Negative	Negative	11/12/2023	NG	NG	NG	NG	NG	NG	NG	NG	NG	5/02/2024	Negative	Negative
001416-0005-M	Negative	Negative	11/12/2023	NG	NG	NG	NG	NG	NG	NG	NG	NG	5/02/2024	Negative	Negative
001416-0006-M	Negative	Negative	11/12/2023	NG	NG	NG	NG	NG	NG	NG	NG	NG	5/02/2024	Negative	Negative
001416-0007-M	Negative	Negative	11/12/2023	NG	NG	NG	NG	NG	NG	NG	NG	NG	5/02/2024	Negative	Negative
001416-0008-M	Negative	Negative	11/12/2023	NG	NG	NG	NG	NG	NG	NG	NG	NG	5/02/2024	Negative	Negative
001416-0010-M	Negative	Negative	11/12/2023	NG	NG	NG	NG	NG	NG	NG	NG	NG	5/02/2024	Negative	Negative
001416-0011-M	Negative	Negative	11/12/2023	NG	NG	NG	NG	NG	NG	NG	NG	NG	5/02/2024	Negative	Negative
001416-0012-M	Negative	Negative	11/12/2023	NG	NG	NG	NG	NG	NG	NG	NG	NG	5/02/2024	Negative	Negative
001416-0015-M	Negative	Negative	11/12/2023	NG	NG	NG	NG	NG	NG	NG	NG	NG	5/02/2024	Negative	Negative
001416-0017-M	Negative	Negative	11/12/2023	NG	NG	NG	NG	NG	NG	NG	NG	NG	5/02/2024	Negative	Negative
001416-0019-M	Negative	Negative	11/12/2023	NG	NG	NG	NG	NG	NG	NG	NG	NG	5/02/2024	Negative	Negative
001416-0020-M	Negative	Negative	11/12/2023	NG	NG	NG	NG	NG	NG	NG	NG	NG	5/02/2024	Negative	Negative
001416-0021-M	Negative	Negative	11/12/2023	NG	NG	NG	NG	NG	NG	NG	NG	NG	5/02/2024	Negative	Negative
001416-0022-M	Negative	Negative	11/12/2023	NG	NG	NG	NG	NG	NG	NG	NG	NG	5/02/2024	Negative	Negative

* Bacterial suspension individually prepared for this test. Lot #: Lot number of the LJ slant used for this validation. NG: No grow. COL: Colonies observed, not conclusive. NA: Not applicable.

5.3.1. Assessment of accuracy precision

Operator MUF 100 %
Operator DAO 100 %

5.3.2. Acceptability of accuracy precision

Operator MUF	APPROVED
Operator DAO	APPROVED

6. Final considerations

Expectations for culture outcomes, although not perfect, may be reasonable. It is important to note that the samples were chosen for their initial results of ZN smear and Xpert MTB/RIF Ultra, which were performed relatively close to the collection and with fresh samples. Our culture results were obtained for processing of frozen specimens and the bacilli distribution possibly are not the same as fresh samples.

Likewise, it must be considered that although the samples analyzed were raw sputum, they were frozen and it is possible that the thawing process has hydrolyzed the sample and especially the bacilli, with the possibility of viability loss during this process.

However, the results obtained have been quite consistent. We have not found significant differences in the capacity to recover bacilli in the samples analyzed and we hope that the change will not generate any change in the performance of the method in the diagnosis of TB.

With all this, a great ability to practically detect the available positives and an almost perfect similarity between the repetitions carried out by each analyst has been demonstrated, so we consider that the findings are satisfactory to establish this procedure as acceptable.

7. Method Approval

For all the above, the auramine staining method developed by both operators in the SES Lab shows values of reproducibility, accuracy and acceptable detection limits.

FINAL RESULT: ***M. tuberculosis*** **culture method using BD BBL Lowenstein-Jensen Medium Slants REF: 220909 APPROVED.**

8. Signatures

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