Alternative cost-effective media to facilitate MODS culture for diagnostics of tuberculosis

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Abstract

Background: Tuberculosis (TB) is still one of the leading causes of death from infectious diseases in low-and middle-income countries (LMIC). Efficient and prompt diagnosis is crucial for TB control. Molecular tests show important advantages (i.e. short response time, and high sensitivity/specificity, however, they are relatively expensive and limited to LMIC. Most culture-based methods remain a low-cost option but still requires longer response times (more than 24 days). The MODS culture is a low-cost assay to diagnose TB and determine drug susceptibility, with a mean response time of 10 days and sensitivity/specificity of more than 90%. However, its implementation is limited in LMIC due to the low accessibility to reagents and supplies required for the enriched medium.

Methods: In this study we evaluate alternatives to facilitate the preparation of the MODS culture media. Two easy to produce and transport, dry 7H9-OAD based media were evaluated: A powder-based mixed media (PM), and a lyophilized media (LM). Catalase, PANTA, and gamma irradiation were evaluated as additions to PM and LM to improve growing rate and prevent culture contamination. Culture performance was compared with the MODS standard medium (MM) using *Mycobacterium tuberculosis* isolates and positive acid-fast smear sputum samples.

Results: Bacterial growth was reported as number of micro-colonies and growth-well percentage (GW%). Analysis with isolates showed that catalase promotes bacterial growth regardless of the type of media. Gamma irradiation did not reduce bacterial growth. Overall, no significant difference was observed in the number of micro-colonies between the evaluated media and MM. However, PANTA and gamma irradiation combined reduced bacterial growth significantly in all media variants. A median positivity day of 6 ± 5 days was observed, regardless the type of media. The non-irradiated PM showed high contamination (30% sputum samples), while no significant difference was observed in the GW% between irradiated PM (100%), and LM (100%) with MM.

Conclusion: The preliminary results show that the two variants culture media have a similar performance to the standard MODS medium, aiming their incorporation in a high-accessible *M. tuberculosis* diagnostic kit.

Key words: Tuberculosis, MODS, diagnostics, culture media, irradiation, lyophilization

ABBREVIATIONS

PM_CP: Powder-based mixed medium supplemented with catalase and antibiotic mixture PANTA.

PM_CG: Powder-based mixed medium supplemented with catalase and irradiated with gamma radiation.

PM_CPG: Powder-based mixed medium supplemented with catalase, antibiotic mixture PANTA, and irradiated with gamma radiation.

PM_P: Powder-based mixed medium supplemented with antibiotic mixture PANTA.

PM_G: Powder-based mixed medium irradiated with gamma irradiation.

PM_PG: Powder-based mixed medium supplemented with antibiotic mixture PANTA and irradiated with gamma irradiation.

LM_CGP: Lyophilized medium supplemented with catalase, antibiotic mixture PANTA and irradiated with gamma radiation.

LM_CG: Lyophilized medium supplemented with catalase and irradiated with gamma radiation.

LM_CP: Lyophilized medium supplemented with catalase and antibiotic mixture PANTA.

LM_C: Lyophilized medium supplemented with catalase.

PANTA: Antibiotic mixture of polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin.

1. Introduction

Tuberculosis (TB) is a disease caused by *Mycobacterium tuberculosis*. It is considered one of the top 10 deaths causes by infectious diseases (World Health Organization, 2020). Although the global incidence is decreasing slowly through different strategies for disease control, drug-resistant TB is considered one of the major public health problems in the world (World Health Organization, 2020). Peru is the South American country with the highest number of multidrug-resistant TB cases (MDR-TB), with 3,500 cases reported, being the second country with the highest number of estimated TB cases (37 thousand cases) in the Americas for 2017 (World Health Organization, 2020) (Alarcón et al., 2017) (OPS, 2018).

In low- and middle-income countries, and in particular in Peru, acid-fast smear microscopy remains the main diagnostic method at primary levels of care. However, it is a test with a modest sensitivity (40% - 70% in sputum samples) (Dorronsoro & Torroba, 2014).

Current TB control schemes include the use of rapid molecular diagnostic tests (Xpert MTB/RIF and the MTBDR-Plus) with high sensitivity and specificity, that allow early detection of the disease (World Health Organization, 2016). However, the main limitation for its large-scale use in unattended or low-equipped locations is the high cost of implementation (Asencios et al., 2012) (OPS, 2010).

The Microscopy Observation Drug Susceptibility test (MODS) is a fast microbiological culture (7-10 days) with high sensitivity (91% - 98%) and specificity (98% - 99%), low cost ($ 3.50 - $ 4 per sample) (Caviedes et al., 2000; Moore DA et al., 2006). MODS is based on the detection of *M. tuberculosis* growth and phenotypic determination of resistance to isoniazid and rifampicin. This test uses a liquid medium, which reduces the response time to an average of 7-10 days (Caviedes et al., 2000; Moore DA et al., 2006). However, its implementation requires trained personnel to prepare the laborious media. The media is based in a mix of Middlebrook 7H9 sterilized by autoclaving with the OADC (oleic acid, albumin, dextrose and catalase) enrichment reagent (that is combined and sterilized by double filtration), and PANTA, an antibiotic cocktail that is added before use (Agarwal et al., 2019).

In 2014, a diagnostic kit with the MODS test was developed, with presentations of the Middlebrook 7H9 culture medium, OADC and PANTA, ready for use (Hardy Diagnostics). However, the liquid presentation of all the components requires a careful transport and constant cold chain (4°C) resulting in a high-cost kit and limiting the demand and its use in low resource settings.

To optimize the preparation, transportation, and handling, and to improve the sterility of the media, in order to facilitate the implementation of MODS on a larger scale, in this study we present an evaluation of alternate presentations of MODS media. The development of alternate presentations of the culture medium with improved storage capacity, that is easy to use and inexpensive, would facilitate the implementation of the MODS assay as a routine test for the diagnosis of TB and drug susceptibility testing.

2. Material and Methods

2.1. Samples

The media evaluation was done in two stages: First stage, evaluation of media components using *M. tuberculosis* isolates; and a second stage evaluation of selected media using sputum samples from TB patients.

2.2. Standard MODS culture media (MM)

Standard MM was prepared according to the MODS medium composition (Coronel *et al.,* 2008) (Caviedes et al., 2000; Moore DA et al., 2006) with some modifications. OADC was prepared with 0.3 mL of oleic acid (Sigma Aldrich), 25 g of bovine serum albumin (Becton Dickinson), 10 g of glucose (Becton Dickinson), 0.015 g of catalase (Sigma Aldrich) and 4.25 g of sodium chloride (Merck). All components were resuspended in 500 mL of sterile water. The OADC solution was sterilized by filtration with 0.44 µm membrane, followed by a second filtration with 0.22 µm membrane. The OADC solution was aliquoted to 50 mL. Aliquots were incubated at 37°C for 24 hours as a sterility control. Finally, the aliquots were stored at 4°C until use.

Distinct reference was used because *M. tuberculosis* isolates are sterile while sputum samples should be cultured with an antimicrobial otherwise the sample contamination is high. MM was evaluated with and without PANTA (MM_P and MM, respectively). MM was considered as a reference culture medium (*M. tuberculosis* strain growth was considered as 100%) in the first stage of the study (evaluating strains), and MM_P was considered as a reference culture medium in the second stage (evaluating sputum samples), where the decontamination effect of PANTA is required.

2.3. Middlebrook 7H9-OADC culture media variants

The PM and LM were prepared according to the MODS medium composition (Coronel et al., 2008) with some modifications as described below:

2.3.1. Powder-based mixed medium (PM). Mixed medium aliquots were prepared for a final volume of 25 mL with 147.5 mg DIFCO™ Middlebrook

7H9 broth (Becton Dickinson), 31 mg Bacto™ Casitone (Becton Dickinson), 21 mg NaCl (Merck), 125 mg Bovine Serum Albumine (Becton Dickinson), and 50 mg D-glucose (Becton Dickinson). For use, the mix was resuspended in sterilized distilled water, and 387.5 µL of 20% sterilized glycerol (Merck). Six variations of the PM were evaluated based on the addition of catalase (final concentration 750 ng/µL) (Sigma), PANTA antibiotic mixture (Becton Dickinson) and gamma irradiation delivered at 5 KGy (Figure 1).

2.3.2. Lyophilized medium (LM). The lyophilized media was prepared as the standard MODS culture medium. After autoclaving and adding the sterile OADC solution as was prepared for the standard MODS media, fresh Middlebrook 7H9 culture media was distributed in 25 mL aliquots and frozen at -70°C for 24 hours. Lyophilization was performed at -46°C with a pressure between 50 x 10^{-3} to 70 x 10^{-3} MBAR in the lyophilizer LYPHLOCK (Labconco). The room was UV irradiated for 15 min before lyophilization. Four culture variations of the LM were evaluated based on the addition of PANTA antibiotic mixture (Becton Dickinson), and gamma irradiation delivered at 5 KGy (Figure 2).

2.4. Culture performance evaluation with a *M. tuberculosis* laboratory strain

A bacterial *M. tuberculosis* isolate (DM097) was obtained from the Mycobacterial Isolates Bank of the Bioinformatics and Molecular Biology Laboratory, Universidad Peruana Cayetano Heredia (Peru). Before performing the assays, the isolate was cultured at 7H10 agar plates supplemented with OADC (Fisher Scientific). The inoculated plates were incubated at 37°C for 30 days. Then, a cellular suspension was prepared with phosphate saline buffer pH 7.4 to a final concentration of 3 x 10^8 CFU/mL (McFarland 1) in 10 mL. The suspension was stored at 4°C for 15 days.

A 1 milliliter-inoculum of 3 x 10^5 CFU/mL and 6 x 10^3 CFU/mL with dilution 1:1000 (used as a control in the standard MODS) and 1:50000, respectively, was prepared from the 3 x 10^8 CFU/mL cellular suspension using each culture medium variant. The culture was done in 24-well plates (Corning). At least eight replicas of each dehydrated culture medium were evaluated. The culture plates were placed in a resealable bag (Ziploc) and incubated at 37°C. After 9 days of incubation, bacterial growth was evaluated in the culture diluted 1:1000 using a 40X magnification in an inverted microscope (TS100-Nikon) and reported as growth percentage, considering the

standard MODS culture medium without PANTA as 100% growth reference (Figure 3). Additionally, the total number of microcolonies was also registered from the culture diluted 1:50000. A microcolony was defined as a small colony that has a characteristic cord-like appearance observed at 100X magnification (Caviedes et al., 2000).

2.5. Culture performance evaluation with clinical sputum samples

Clinical sputum samples were collected between January to February during 2020 in the Regional Tuberculosis Reference Laboratory, Callao, Peru (Ethical approval number 345-15-19). A total of 31 samples with positive acid-fast bacilli smear (AFB) were selected for the study. Sputum samples were decontaminated according to the standardized protocol (Coronel *et al.* 2008). Briefly, two milliliters of sample were mixed with two milliliters of 2% NaLc-NaOH, vortexed roughly, and incubated at room temperature for 15 min. Next, the mix was neutralized by adding 10 mL of phosphate buffer and centrifuged at 3000 x g for 15 min at 17°C. Finally, the pellet was resuspended with 2.5 mL phosphate saline buffer pH 6.8.

From the decontaminated samples, 300 µL were added to two milliliters of each selected culture medium variant and dispensed in two wells of a 24-well plate (Corning). The 24-well plate was placed in a resealable bag (Ziploc) and incubated at 37°C in for up to 30 days. The standard MODS medium (Coronel *et al.* 2008) with PANTA antibiotic mixture (Becton Dickinson) was used as 100% growth reference. Bacterial growth was evaluated since day three using 40X magnification in an inverted microscope (TS100-Nikon) and reported as growth percentage. Culture results were registered the first day that bacterial growth was observed.

2.6. Data analysis

Difference in the number of microcolonies and growing percentage between media variations were evaluated by Kruskal Wallis and Dunn test for multiple pairwise comparison. Positivity with CI95% was reported for each media variation compared to the MODS standard medium. All statistical analyses were done using STATA v.16.0 (StataCorp, College Station, TX) considering a significance level of 5%.

3. Results

3.1. Culture performance evaluation with a *M. tuberculosis* laboratory strain

3.1.1. Effect of PANTA addition on culture performance of the standard MODS medium (MM).

PANTA addition to MM resulted in a significant reduction of 20% in *M. tuberculosis* growth compared to its counterpart without the antibiotic mixture ($P < 0.0001$) (Figure 4A, Table 1).

3.1.2. Effect of PANTA addition on culture performance of the PM culture media.

PANTA addition to both PM and LM, resulted in a significant reduction of *M. tuberculosis* growth, when compared to the counterparts non-supplemented with the antibiotic mixture (PM_CG vs PM_CPG, P=0.001; PM_G vs PM_PG, P=0.015; LM_C vs LM_CP, P=0.006; and LM_CG vs LM_CGP, P<0.0001) (Figure 4A, Table 1).

3.1.3. Effect of catalase addition on culture performance of the dry culture media.

PM variants treated with PANTA and gamma irradiation, supplemented with catalase showed percentage of growth values significantly higher than their equivalents without catalase (PM_P vs PM_CP, P=0.005; PM_G vs PM_CG, P=0.003; and PM_PG vs PM_CPG, P=0.046). Similar results were observed in the microcolony numbers, catalase-supplement PM variants reported higher counts of microcolonies ($P < 0.04$) (Figure 4B, table 1).

3.1.4. Effect of gamma irradiation on culture performance of the dry culture media.

Significant reduction of *M. tuberculosis* growth was observed in PM when gamma irradiation and PANTA were used together, regardless the addition of catalase, when compared to PM supplemented with only PANTA (PM_P vs PM_PG, $P < 0.001$; PM_CP vs PM_CPG, $P < 0.001$). Similar results were observed for LM, a lower *M. tuberculosis* growth was observed in the gamma irradiated LM compared to the LM independently if PANTA was added (LM_C vs LM_CG, $P < 0.01$; LM_CP vs LM_CGP, $P < 0.001$). When PANTA and gamma irradiation were used together, the growth percentage decreased more than 60% (Figure 4C, Table 1).

3.1.5. Comparison of culture media performance between the standard MM supplemented with PANTA and dry culture media.

Comparison with standard MM supplemented with PANTA (MM_P) showed that the PM_P ($P = 0.104$), PM_G ($P = 0.850$), and PM_CPG ($P = 0.724$) did not differ regarding the *M. tuberculosis* growth. Instead, the PM_CP and the PM_CG showed significant higher *M. tuberculosis* growth ($P < 0.001$), while the irradiated PM

supplemented with PANTA (PM_PG) showed the lowest *M. tuberculosis* growth for the mixed culture medium variants ($P < 0.001$). Concerning the LM, only the LM_CP did not show significant difference with the MM_P ($P = 0.703$). The LM_C and LM_CG showed higher *M. tuberculosis* growth ($P < 0.01$), while the LM_CGP showed the lowest *M. tuberculosis* growth for all the evaluated dehydrated culture media (Figure 5).

3.1.6. Batch reproducibility

A total of three culture batches were evaluated for each dry culture media with at least three replicas. Significant differences in *M. tuberculosis* growth were observed for the PM_G ($P = 0.038$), and the LM_CP ($P = 0.044$) (Table 2).

3.2. Culture performance evaluation with clinical sputum samples

The dry culture media containing PANTA were selected for a primary evaluation with clinical sputum samples (PM_P, PM_CP, PM_PG, PM_CPG, and LM_CP), except the irradiated LM supplemented with PANTA (LM_CPG) because *M. tuberculosis* growth was significantly lower than those found in the standard MODS medium supplemented with PANTA (MM_P).

3.2.1. Sputum samples growth and culture media contamination.

From 31 sputum samples, three samples did not show bacterial growth and two samples showed contamination during culture incubation in all culture media. The analysis was done over 26 sputum samples. No contamination was observed in the dry culture medium except in PM_CP where eight culture samples showed contamination (8/26).

3.2.2. Culture median response time, dry culture media positivity and growth percentage of M. tuberculosis.

No significant difference was observed for the response time ($P = 0.991$) between the control and the evaluated culture media. A median response time of 6 ± 3 days was observed for the all the evaluated culture media, ranged 4 to 14 days. The dry culture media positivity did not show significant difference from the standard MODS medium ($P > 0.05$) (Table 3). Significant differences in growth percentage were observed for PM_P ($P < 0.001$), and PM_PG ($P < 0.001$) (Figure 6).

4. Discussion

In this study we evaluated two alternative dry presentations of the culture medium for MODS, which are simpler to produce, store, and transport. One is a powder-based

non-sterilized mixed media, and the other a sterile lyophilized liquid medium. Both demonstrated to be as effective as the standard media for MODS.

The effect of PANTA is difficult to evaluate in sputum culture because the sputum contains contaminating flora that cannot be completely inactivated during decontamination. In this study, we used a pure *M. tuberculosis* strain as well as sputum samples to evaluate the relative effect of PANTA. We evaluated the effect of PANTA based on its interaction with the type of medium. Thus, the effect of PANTA on *M. tuberculosis* strain growth was evaluated when using the standard MODS medium, PM and LM dry culture mediia. Growth rates comparisons were made according to presence/absence of PANTA (Figure 4A). In all cases, the addition of PANTA was associated to a reduction of approximately 20%, except while evaluating LM with gamma radiation and PANTA (LM_CGP), where a 40% reduction in the number of microcolonies and more than 60% in the percentage of growth was observed. Therefore, although PANTA is important to decontaminate the sputum samples (i.e. inactivate non tuberculous bacteria), it also has an effect against *M. tuberculosis*, partially impairing its growing.

It has been shown, that the nalidixic acid, which is one component of PANTA, exerts some degree of inhibition in the growth of *M. kansasii* isolates (Conville et al.). That study recommended not to increase PANTA concentration to control the overgrowth of contaminants from clinical specimens, as it also compromises the recovery of *M. kansasii* (Conville et al.). Another study showed that the culture positivity of *M. tuberculosis* from sputum samples, conducted in growth indicator tube (MGIT) using PANTA (35%) was higher than positivity in Ogawa (25.9%). It was also demonstrated that increasing PANTA 2x folds its concentration did not affect the culture positivity of MGIT (Peres et al, 2011).

PANTA has demonstrated to reduce the contamination percentages in MODS-sputum culture (2.2%) in comparison to Lowenstein Jensen culture (6%) (Martin et al. 2014) as well as in MGIT (Peres et al, 2011). Further studies are required to better understand the direct effect of PANTA on the sputum MODS positivity, as for example comparing with different individual antibiotics and cocktails.

Catalase, an oxidoreductase enzyme, acts as a catalyst for some reactive oxygen species (Céspedes et al., 1996) that are continuously produced by living organisms, such as hydrogen peroxide (H_2O_2) produced by aerobic organisms under natural stress, causing damage and inactivation of proteins as well as mutations in the DNA (Imlay, 2013). Although *M. tuberculosis* has detoxification mechanisms against H_2O_2

production as a defense mechanism (Bhat et al., 2012), the use of catalase in the culture medium helps the transformation of H_2O_2 into water and oxygen (Céspedes et al., 1996) helping *M. tuberculosis* to have a better development in the medium. A concentration of 7 mM and not 2 mM H_2O_2 resulted in the death of *M. smegmatis* (Li et al., 2015). Catalase (a powder mix) needs to be stored at -20°C, representing a limitation in the PM preparation. For this reason, catalase was specifically evaluated only in the PM media.

Oleic acid was not included because it is not essential for *M. tuberculosis* growth (Butler & Kilburn, 1982) (Dubos, 1946) (Becton Dickinson and Company, 2015). We confirmed that in PM, the absence of catalase in the media variants decreases the number of microcolonies and the percentage growth of *M. tuberculosis* strain in media with PANTA, gamma irradiation and with both.

Gamma irradiation exposure could be considered an ideal alternative as a physical means of sterilization (Singh et al., 2016)(Snadle & Raju, n.d.), since this method is suitable for materials that cannot be exposed to high levels of temperature or chemical agents. In addition, gamma irradiation does not compromise important factors such as pressure and humidity that could alter the components of the medium (Vargas et al., 2014). The sterilization of rehydrated Mac Conkey agar with 1.5 Mrad gamma radiation containing sodium thioglycolate as a radio-protectant, reduced media contamination and did not interfered with microorganism growth, therefore it was recommended to use in kits for detection of bacteriuria (Bogokowsky 1983). Despite its advantages, we found that gamma irradiation reduces significantly the growth of *M. tuberculosis* strain in the PM and LM. Its effect could be attributed to an interaction between the products generated by lyophilization, irradiation and mixture of PANTA with the powder media (PM and LM).

It is worth mentioning that the multiple comparisons shown in Figures 4 and 5, correspond to data obtained from a single experiment, where the same samples were processed in simultaneous. For this reason, it is valid to perform the stratified comparisons (by PANTA, catalase, and gamma irradiation (Figure 4)), as well as the overall comparison against the standard MODS with PANTA (MMP) culture (Figure 5).

Important to highlight that comparisons between the equivalent dry culture media, showed that PANTA and gamma irradiation reduced *M. tuberculosis* growth, but in contrast catalase increased *M. tuberculosis* growth (Figure 4). The overall comparisons between the dry culture media variants with the control media with PANTA (MMP), showed that the variants PM_CPG, PM_P, PM_G and LM_CP, had a similar *M.*

tuberculosis growth compared to MMP. In contrast, the variants PM_CP, PM_CG, LM_C and LM_CG, showed an increased growth compared to MMP (Figure 5). Two variants, the irradiated PM combined with PANTA without catalase (PM_PG) and the irradiated LM combined with PANTA and catalase (LM_CGP), showed a reduced *M. tuberculosis* growth (Figure 5). Interestingly, the growth rate of the PM_PG was improved when catalase was added (PM_CPG). Further studies are required to understand the molecular causes of these effects.

In sputum samples, the PM and LM culture medium variants containing PANTA (except LM_CGP) were compared with the standard MODS media with PANTA. In MM_CP, 25.2% of the sputum samples were contaminated, probably due to the protection of the bacterial contaminants to the toxic metabolites or PANTA components themselves, or eventually due to the presence of non-sterile catalase. However, the contamination was controlled when the media was sterilized by autoclaving (LM_CP) o by gamma radiation (PM_CPG).

In all the media tested, the days of response time and positivity to detect *M. tuberculosis* in sputum samples was similar to the standard MODS media (96-100%). However, those media without catalase (PM_P and PM_PG) had a slightly lower positivity compared to the standard MODS media. These results are agreement with the significantly lower growth percentage found in PM_P and PM_PG compared to the standard MODS media.

This study shows that catalase has a moderate effect in the sputum culture positivity but a more important effect in the growth percentage. MODS is a qualitative culture method where a dichotomous (positive or negative) result is reported. Therefore, it would be necessary to perform further studies with a larger population, to confirm the importance of catalase in the growth of *M. tuberculosis* in dry media-culture.

5. CONCLUSIONS

In conclusion, MM_CPG and LM_P showed the highest percentage of growth to detect *M. tuberculosis* in sputum samples. The PM_P medium is the simplest to prepare since it does not require any sterilization process and shows a time-to-positivity and sensitivity comparable to the standard MODS medium with PANTA, although it is not associated to a high percentage of growth. We believe that the implementation of these culture variants media may facilitate MODS in low resource settings in developing countries.

6. ACKNOWLEDGEMENTS

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TABLES

Table 1. Bacterial growth reported in the evaluated dry culture media. Growth percentage and number of microcolonies were estimated by comparison of each culture medium to the standard MODS media without PANTA antibiotic mixture.

Type of dry culture medium		Growth percentage[#]	Number of microcolonies[†]
Standard MODS medium	MM[*]	-	78 ± 15
	MM_P	80 (60 – 90)	64.5 ± 11
Powder based mixed medium	PM_CP	100 (90 – 100)	84 ± 12
	PM_CG	100 (70 – 100)	94 ± 20
	PM_CPG	80 (60 – 80)	68 ± 16
	PM_P	80 (70 -100)	65 ± 19
	PM_G	80 (60 – 90)	69 ± 8
	PM_PG	60 (40 – 70)	49 ± 21
Lyophilized medium	LM_CGP	10 (10 – 30)	14 ± 4
	LM_CG	90 (80 – 100)	71 ± 11
	LM_CP	80 (60 – 90)	61.5 ± 9
	LM_C	100 (90 – 110)	84 ± 14

*Standard MODS medium (MM) considered as reference.

Median (p50) and the minimum and maximum range are reported.

†Median (p50) and interquartile range are reported.

Table 2. Culture reproducibility for three dry culture media batches. Growth percentages from a *M. tuberculosis* culture using an inoculum of 6 x 10^3 CFU/mL

Type of medium	Growth percentage			
	Batch 1	Batch 2	Batch 3	P-value*
PM_CP	100 ± (90 – 100)	100 ± (90 – 100)	100 ± (90 – 110)	0.783
PM_P	80 ± (80 – 100)	80 ± (80 – 80)	90 ± (70 – 100)	0.530
PM_CG	100 ± (70 – 100)	90 ± (90 – 100)	100 ± (100 – 100)	0.238
PM_G	80 ± (70 – 90)	70 ± (60 – 70)	80 ± (80 – 90)	0.038
PM_CPG	80 ± (60 – 80)	80 ± (80 – 80)	70 ± (70 – 70)	0.055
PM_CG	60 ± (40 – 60)	60 ± (60 – 70)	50 ± (50 – 60)	0.176
LM_C	100 ± (100 – 100)	100 ± (90 – 110)	100 ± (100 – 100)	0.801
LM_CP	90 ± (80 – 90)	80 ± (70 – 90)	60 ± (60 – 70)	0.044
LM_CG	100 ± (80 – 100)	80 ± (80 – 90)	90 ± (80 – 90)	0.396
LM_CPG	10 ± (10 – 10)	20 ± (20 – 30)	10 ± (10 – 20)	0.056

*P-value from Kruskall – Wallis non-parametric comparison test

Table 3. Statistical *M. tuberculosis* positivity comparison.

Dry culture medium	n/N	Positivity (%)	p-value*
LM_CP	26/26	100	1.000
PM_P	25/26	96	0.3173
PM_CP	18/18	100	1.000
PM_PG	25/26	96	0.3173
PM_CPG	26/26	100	1.000

*P-values correspond to rank-sum Wilcoxon test compared to the standard MODS medium supplemented with PANTA.

FIGURES

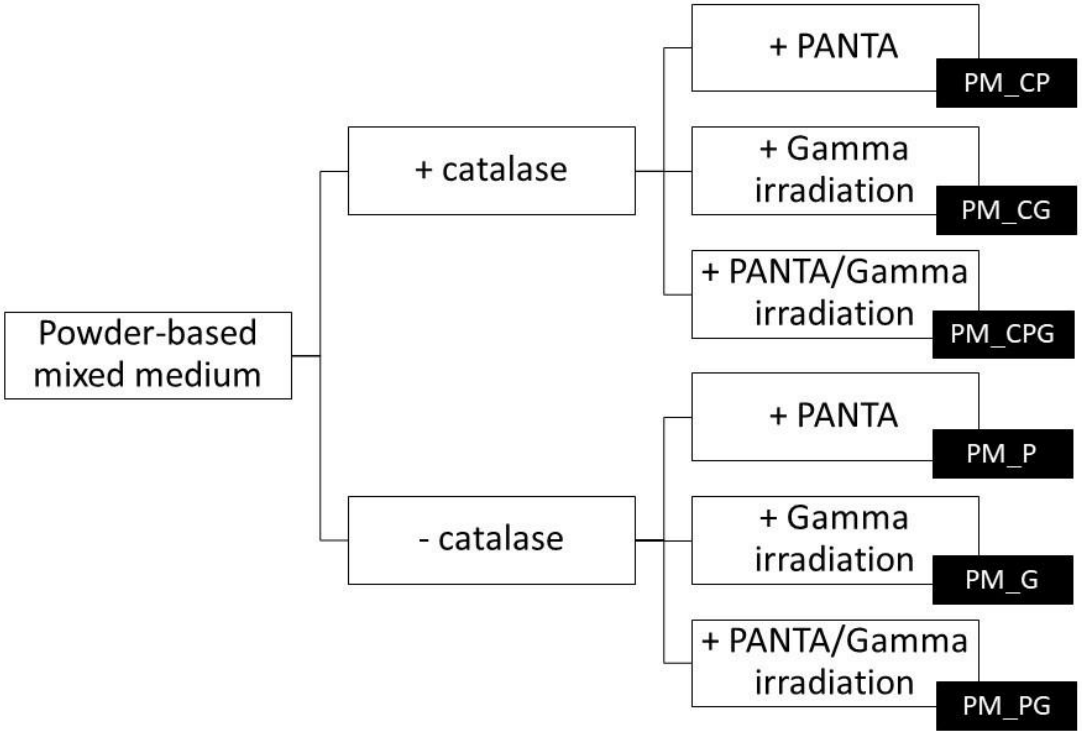

Figure 1. Schematic representation of the power-based mixed culture media (PM). Addition of catalase, antibiotic mixture PANTA, and exposure to gamma irradiation were evaluated.

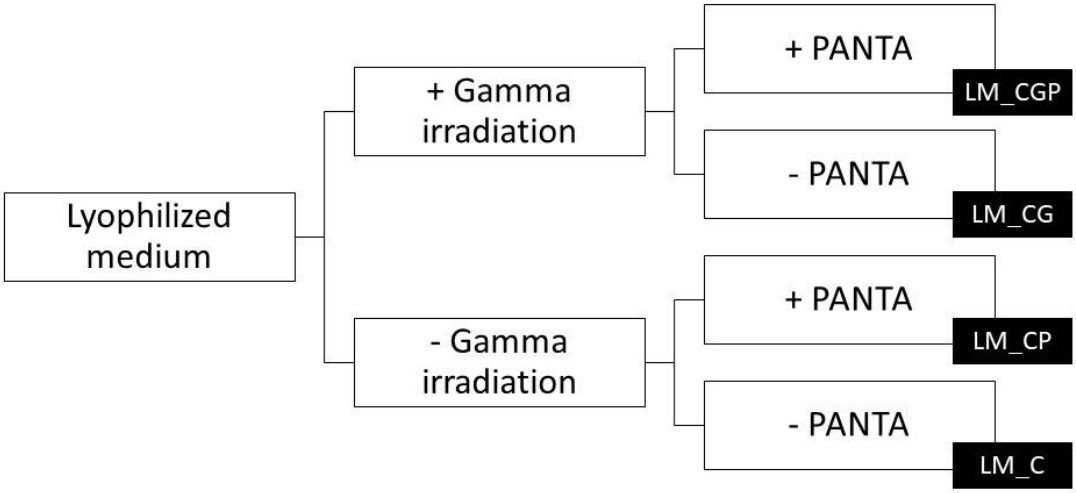

Figure 2. Schematic representation of the lyophilized culture media (LM). Addition of antibiotic mixture PANTA and exposure to gamma irradiation were evaluated. All LM culture media contained catalase in their composition.

Standard MODS culture media	Dry culture media			
100%	90%	50%	25%	10%

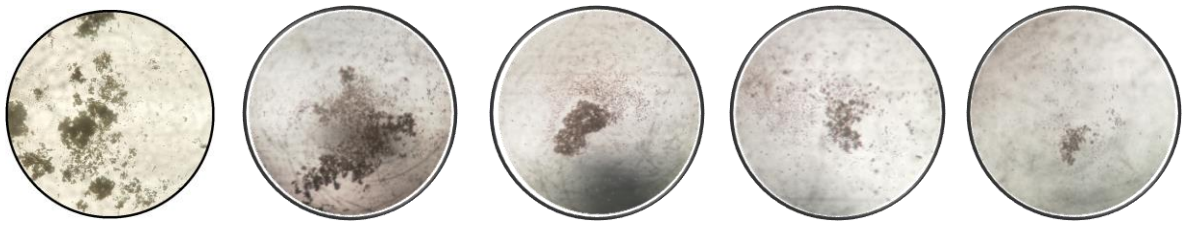

Figure 3. Comparison of *M. tuberculosis* growth in liquid culture media by microscopy evaluation. The standard MODS culture medium was considered as growth reference for the evaluation of the different evaluated dry culture media. Bacterial growth is reported as growth percentage. Microbiological culture was performed using a final dilution of 1:1000 of a stock of 3.8 x 10^8 CFU/mL. Images correspond to day 9 of incubation at 37°C.

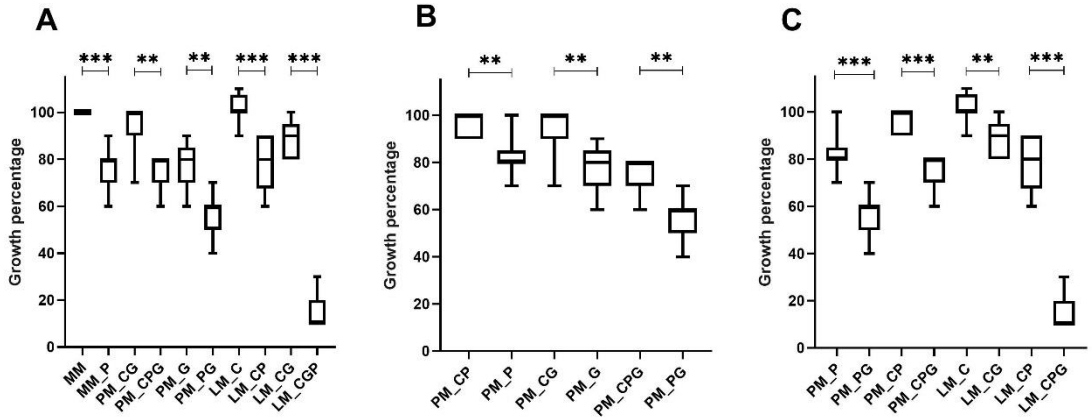

Figure 4. Culture performance comparison in different dry culture media. (A) Effect of the antibiotic mixture PANTA, (B) catalase, and (C) gamma irradiation, on the *M. tuberculosis* growth on dry culture media. Growth percentages estimated by comparison with the standard MODS medium non-supplemented with PANTA are reported in box plot graphics. Whiskers represent minimum and maximum values. Capped lines represent comparison culture media pairs.

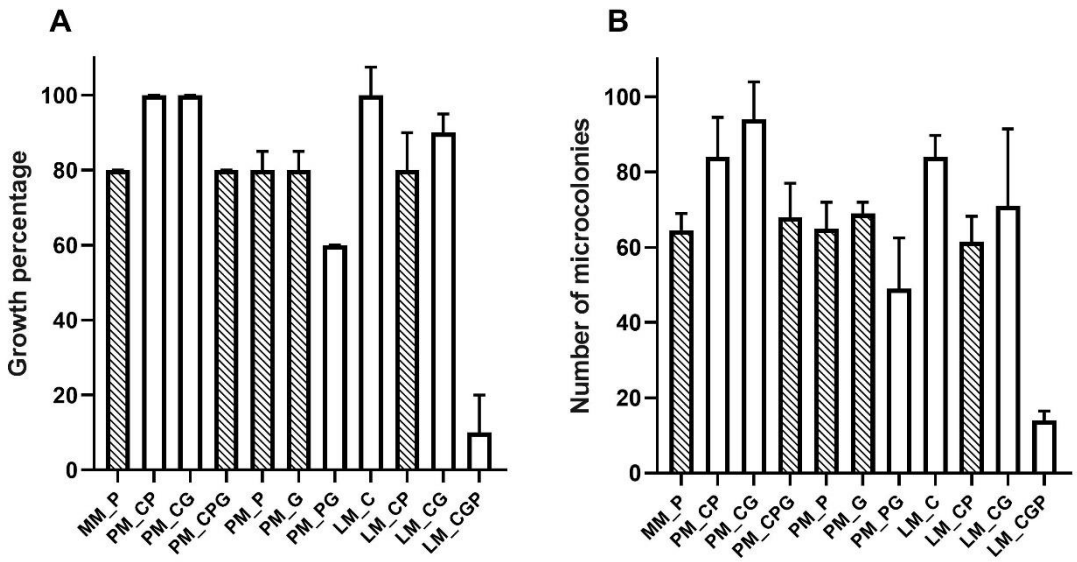

Figure 5. Comparison of culture performance of the dry culture media against the standard MODS culture medium supplemented with PANTA (MM_P). Culture performance was compared against the standard MODS medium with PANTA due to the antibiotic mixture is used to reduce contamination with clinical samples culture. **(A)** Box plots representing growth percentage, **(B)** Box plots representing number of microcolonies. Whiskers represent interquartile range. Bars filled with dashed lined represent non-significative difference compared to standard MODDS with PANTA with a P-value >0.05.

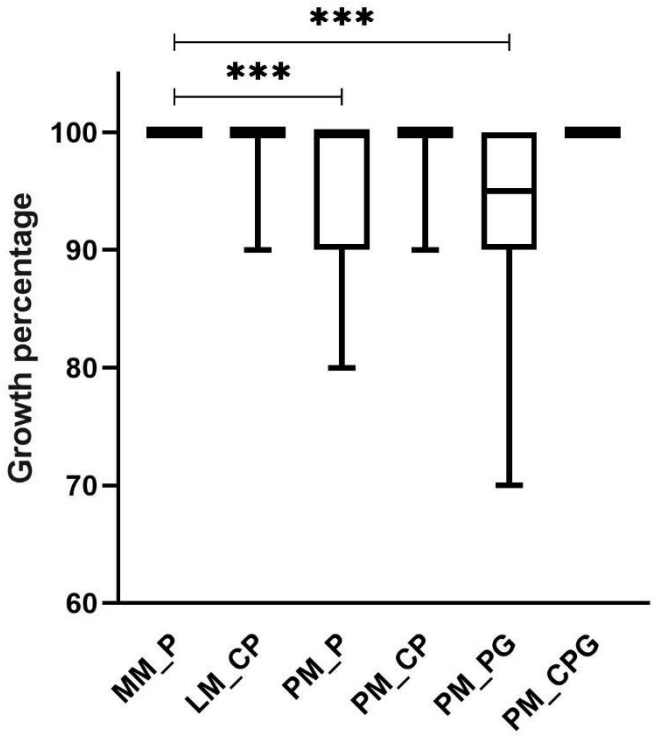

Figure 6. *M. tuberculosis* growth percentage in the control medium and the dry evaluated culture media. Median (p50) and minimum and maximum values are plotted. Culture medium that showed a significant difference in MTB growth are highlighted ($P < 0.001$).