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Rapid and portable molecular test for *Mycobacterium* sp. based on double-tagging amplification and electrochemical readout

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Tuberculosis (TB) remains one of the leading causes of death worldwide, with *Mycobacterium tuberculosis* as the main pathogen responsible. Although several rapid molecular tests endorsed by the World Health Organization (WHO) show high accuracy, their implementation in low-resource settings is still limited by cost and infrastructure requirements. In this work, a rapid and portable molecular test was developed for *Mycobacterium* sp. detection based on double-tagging polymerase chain reaction (PCR) and electrochemical magneto-genosensing. The method enables amplification of mycobacterial DNA using primers targeting the *gyrB* and *IS6110* genes through a double-tagging PCR completed within approximately one hour, followed by electrochemical detection on a handheld, battery-operated device, yielding a measurable current signal within 15 minutes, including single-step incubation and electrochemical readout. The test achieved a limit of detection as low as 117 CFU mL^{-1} for the *gyrB* target, comparable to the commercial GeneXpert® MTB/RIF assay, while significantly reducing assay time and equipment needs. Total analysis, including DNA extraction, amplification and detection, was completed within approximately two hours. In addition, comparable electrochemical responses were obtained when PCR amplification was carried out using either a conventional benchtop thermocycler or a portable battery-operated miniPCR device, further supporting the applicability of the method in decentralized and field settings. Overall, this approach combines high sensitivity, portability, and simplicity, offering a promising solution for point-of-care TB diagnosis and adaptable to other infectious diseases by changing the primer sequences.

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Introduction

Tuberculosis (TB) remains one of the oldest and deadliest infectious diseases worldwide. According to the World Health Organization (WHO) Global Tuberculosis Report 2024, approximately 10.8 million people developed TB in 2023, corresponding to an incidence rate of 134 cases per 100 000 population.[1] Despite substantial advances in research, prevention, and treatment programmes, the global reduction in TB incidence since 2015 is only about 8.3%, far from the WHO End TB Strategy target of a 50% reduction by 2025.[1] Moreover, WHO projections indicate that, without major acceleration in innovation and investment, the 2030 goals—an 80% reduction in incidence and a 90% reduction in deaths—will not be achieved. These findings underline the urgent need for rapid, accessible, and low-cost diagnostic technologies capable of improving early detection, particularly in high-burden and resource-limited settings.[1]

To meet this challenge, modern TB diagnostics have evolved considerably, including molecular tests such as nucleic acid amplification tests (NAATs) and line-probe assays,[2,3] interferon-gamma release assays (IGRAs),[4,5] and biomarker-based tests.[6,7] However, microbiological culture remains the gold standard for confirming infection due to its high specificity and sensitivity.[8] Culture-based methods require specialized infrastructure and extended incubation times; although improvements in culture media have shortened detection times to approximately 12–14 days for *Mycobacterium tuberculosis* detection, the method remains limited to well-equipped laboratories and is not feasible in many low-resource settings.[9] Sputum smear microscopy, one

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of the most widely used diagnostic techniques in such environments, is inexpensive and rapid but highly operator-dependent, resulting in variable sensitivity and specificity.[10] Moreover, cross-reactivity with non-tuberculous mycobacteria can lead to misdiagnosis or inappropriate treatment. To enhance accuracy, traditional Ziehl–Neelsen staining has been progressively replaced by mercury vapour fluorescence or LED-based microscopy, improving detection rates but still constrained by infrastructural and personnel requirements.[8,10,11]

Due to the inherent limitations in sensitivity and specificity of these conventional techniques, the WHO recommends molecular testing as the first-line diagnostic approach for all TB-suspected patients.[12] Among these, NAATs such as GeneXpert® MTB/RIF Ultra (Cepheid, USA) have significantly improved diagnostic accuracy and rifampicin-resistance detection compared with traditional methods, although their dependence on stable electricity and high instrument cost limit their application in resource-poor settings.[13] More adaptable alternatives, such as Truenat™ MTB, MTB Plus, and MTB-RIF Dx (Molbio Diagnostics, India), provide portable, battery-operated solutions suitable for decentralized laboratories,[14,15] though they show somewhat reduced sensitivity in specific populations,[16] including children,[17] HIV-positive individuals,[18] and extrapulmonary TB cases.

Other immunodiagnostic and biomarker-based assays—such as interferon-gamma release assays (IGRAs) and lateral-flow lipoarabinomannan (LF-LAM) tests (*e.g.*, Alere Determine TB LAM Ag, Abbott, USA; Fujifilm SILVAMP TB LAM, Fujifilm, Japan)[19]—offer complementary diagnostic tools, particularly valuable for HIV-associated TB.[17] In parallel, line-probe assays (LPAs) combine PCR amplification and reverse hybridization to simultaneously identify *M. tuberculosis* and mutations conferring resistance to first-line drugs such as rifampicin and isoniazid, enabling rapid characterization of multidrug-resistant TB.

Despite their benefits, access to rapid molecular tests remains limited in many regions. Globally, in 2021, only about 38% of people newly diagnosed with TB received a WHO-recommended rapid molecular test as the initial diagnostic.[20] The remaining majority were diagnosed *via* slower or less sensitive methods (*e.g.*, smear microscopy) or clinically without confirmation. This gap highlights the need for scaling up rapid molecular testing as a priority for improving TB control, allowing patients to be correctly diagnosed and started on effective treatment faster—an essential step toward controlling the TB epidemic and achieving global targets.

In this work, a rapid and portable test for tuberculosis detection is presented based on double-tagging amplification of mycobacterial nucleic acids followed by electrochemical biosensing. The assay employs primers targeting the *gyrB* and *IS6110* genes, achieving particularly high sensitivity with *gyrB* amplification. The novelty of the proposed approach lies in the integration of double-tagging PCR, magnetic capture and enzymatic labeling of the amplicons, a disposable cartridge for magnetic actuation and washing, and a handheld battery-operated electrochemical reader into a single decentralized molecular diagnostic workflow. In addition, the compatibility of the amplification step with a compact battery-operated thermocycler supports the possibility of moving PCR outside the conventional laboratory environment. The proposed method therefore represents a promising alternative for decentralized TB testing, as both amplification and electrochemical readout can be performed in portable formats suitable for primary healthcare or resource-limited settings, with minimal intervention from the end user and within a short total assay time.

Experimental section

Instrumentation

PCR tests were performed using a Veriti™ 96-well thermal cycler (Applied Biosystems™, USA) and a 16-well portable battery-powered thermal cycler (miniPCR® mini16X, miniPCR bio, USA). Electrophoresis was done in a Mini-Sub Cell GT horizontal electrophoresis system with a PowerPac Basic power supply (ref. 1640300, Bio-Rad Laboratories, USA). The photos of the electrophoresis gels were taken using a Gel Documentation System, GenoSens 2100 Touch (Clinx Science Instruments Co., Ltd, China). Electrochemical readout was achieved with a cartridge, which allows magnetic actuation and washing, and a portable electrochemical device operated with batteries (BioEclosion SL, Spain). The samples were also measured on carbon screen-printed electrodes (ref. DRP-C110) using a portable bipotentiostat DRP-STAT200 operated using DropView 200 for instrument control and data acquisition (Dropsens, Spain).

Chemicals and biochemicals

The magnetic particles streptavidin Dynabeads™ M-280 (ref. 11205D, Invitrogen, USA) and anti-digoxigenin-POD (ref. 11207733910, Roche, Switzerland) were used as a detection system for the amplicons. Different sets of buffers were used for specific procedures in the experiments, and their composition is described in S1 (SI). The reagents used for the electrochemical measurement include hydroquinone and hydrogen peroxide 30% (v/v) solution (catalogue no. H9003 and 31642 from Merck, Germany, respectively). The PCR was performed by using a Ready Mix Taq PCR Reaction Mix with $MgCl_2$, as a cofactor of the enzyme (ref. P4600, Sigma-Aldrich). For gel electrophoresis, Sybr Safe DNA gel stain (ref. S33102, Invitrogen) and a GeneRuler 100 bp DNA ladder (ref. SM0241, ThermoFisher, USA) as a molecular weight marker were used. The *Mycobacterium bovis* BCG-Pasteur strain was grown in DIFCO™ Middlebrook 7H9 broth (ref. 271310, Becton Dickinson) supplemented with Tween® 80 (ref. P4780, Sigma-Aldrich) at 0.05% (v/v), ADC and glycerol at 0.08% (v/v) for 2–3 weeks at 37 °C in a 25 cm^2 cell culture flask. For solid culture, DIFCO™ Middlebrook 7H10 agar (ref. 262710, Becton Dickinson, USA) supplemented with

glycerol at 0.5% (v/v) and OADC was used. For the specificity study, genomic DNA was also extracted from a panel of non-target bacterial strains including *Mycobacterium fortuitum* (ATCC 6841), *Streptococcus agalactiae* (ATCC 8181), *Pseudomonas aeruginosa* (ATCC 15442), *Klebsiella pneumoniae* (ATCC BAA-1705), *Escherichia coli* (ATCC 10536), *Enterobacter* spp. (used as a control by the Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica), and *Salmonella choleraesuis* (ATCC 13311). These strains were used to evaluate the analytical specificity of the PCR assays targeting the *IS6110*, *gyrB* and *rpoB* genes.

Oligonucleotide sequences

The oligonucleotides were obtained from Sigma-Aldrich. These primers were selected for the amplification of *IS6110* (245 bp),[21] *rpoB* (351 bp), and *gyrB* (483 bp)[22] genes. The selected primer sequences were verified by BLAST to be conserved in both *Mycobacterium bovis* BCG-Pasteur and *Mycobacterium tuberculosis* strains, ensuring their applicability to TB samples. Each primer set was double-tagged at the 5′ end with biotin (BIO) and digoxigenin (DIG), as summarized in Table 1.

DNA extraction and double-tagging polymerase chain reaction

The extraction protocol was adapted from the GenoType MTBDR plus and MGIT culture (Hain Lifescience GmbH, Germany), and it was optimized as described in S2 (SI). Briefly, 1 mL of *M. bovis* BCG-Pasteur culture was transferred into a 2.0 mL tube and centrifuged at 10 000*g* for 15 min. The supernatant was discarded, and the pellet was resuspended in 150 μL of water and mixed with a vortex. Then, the sample was incubated at 95 °C for 20 min and centrifuged at maximum speed for 5 min. Finally, the supernatant was transferred to a new tube and stored at −20 °C. For the specificity study, genomic DNA from *Mycobacterium fortuitum* (ATCC 6841), *Streptococcus agalactiae* (ATCC 8181), *Pseudomonas aeruginosa* (ATCC 15442), *Klebsiella pneumoniae* (ATCC BAA-1705), *Escherichia coli* (ATCC 10536), *Enterobacter* spp., and *Salmonella choleraesuis* (ATCC 13311), using *Mycobacterium bovis* BCG as a positive control, was extracted following a rapid heat-based procedure. Briefly, 1 mL of bacterial culture was incubated at 98 °C for 10 min, followed by the addition of 200 μL of Tris/EDTA buffer and a second incubation at 98 °C for 10 min. Samples were then stored at −20 °C for 20 min and centrifuged at 10 000 rpm for 10 min, and the recovered supernatants were quantified using a Qubit™ 1X dsDNA HS assay kit (ref. Q33230, Thermo Fisher Scientific, USA). All extracted DNA samples were adjusted to 0.3 ng μL^{-1} prior to amplification.

The double-tagging polymerase chain reaction (PCR) was performed using three set of primers labelled with BIO-Fw and DIG-Rev,[23,24] for the amplification of the *IS6110*, *rpoB* and *gyrB* genes. Briefly, 1.5 μL of chromosomal DNA was added along with 10 pmol of the forward and reverse primers and 0.75 U of Taq polymerase, for a total reaction mixture of 25 μL. The reaction mixture was exposed to an initial step at 94 °C for 5 min followed by 30 cycles of 94 °C at 30 s, 62 °C for 30 s, 72 °C for 45 s, and a last step of 5 min at 72 °C, as shown in Fig. 1, panel A. Negative controls were performed without adding DNA. Further details about the double-tagging PCR procedure are also described in S2 (SI). The resulting amplicons were analyzed with conventional agarose gel electrophoresis.

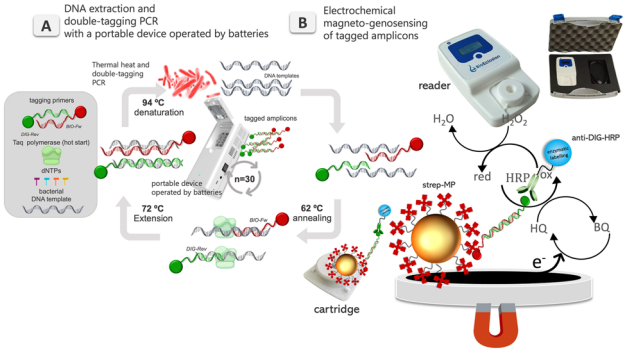

Fig. 1 Schematic representation of the portable molecular test for *Mycobacterium tuberculosis* detection. The workflow includes: (A) bacterial DNA extraction and double-tagging PCR using primers targeting the *rpoB*, *IS6110* and *gyrB* genes, followed by (B) electrochemical magneto-genosensing of the tagged amplicons using streptavidin magnetic particles (strep-MPs) and anti-digoxigenin–HRP conjugates. The reaction mixture is loaded into a disposable cartridge enabling magnetic actuation, washing, and electrochemical readout. Measurements are performed at −0.12 V for 30 s on a fully portable, battery-operated handheld device.

Portable electrochemical magneto-genosensing using a cartridge-based handheld device

With the aim of developing a rapid and portable test for tuberculosis detection, an electrochemical magneto-genosensor was established in this work, integrating a cartridge-based platform with a handheld device for

Table 1 Primer sequences, labeling, and expected amplicon sizes for the *IS6110*, *gyrB* and *rpoB* targets

Gene	Primer sequence (5′-3′)	Type	5′-Label	Size (bp)
IS6110	GCGTAGGCGTCGGTGACAAA	Forward	Biotin	245
	CGTGAGGGCATCGAGGTGGC	Reverse	Digoxigenin	
gyrB	ACATCAACCGCACCAAGAACGC	Forward	Biotin	483
	GTGCCTTACGTGCCGCGATACG	Reverse	Digoxigenin	
rpoB	CGACCACTTCGGCAACCG	Forward	Biotin	351
	TCGATCGGGCACATCCGG	Reverse	Digoxigenin	

electrochemical readout. The procedure, illustrated in Fig. 1, comprises two main steps: (A) DNA extraction by thermal heating and double-tagging PCR amplification and (B) electrochemical detection of the amplified double-tagged products in a compact, user-friendly device.

To optimize the protocol, an attenuated strain of *Mycobacterium bovis* BCG-Pasteur was employed, and BLAST analysis confirmed that the selected primer sequences were conserved in both *M. bovis* BCG-Pasteur and *M. tuberculosis* genomes, the latter being the primary causative agent of tuberculosis in humans.

In the electrochemical genosensing step, streptavidin magnetic particles (strep-MPs) and horseradish peroxidase–conjugated anti-digoxigenin antibodies (anti-DIG–HRP) were used to specifically recognize and bind the biotin and digoxigenin moieties at the opposite ends of the amplified DNA products. More information about the optimization of the reagents is described in S3 (SI). In a 1.5 mL tube, 10 μL of amplified sample was incubated simultaneously with 10 μL of strep-MPs and 100 μL of anti-DIG–HRP at 500 mU mL^{-1} at room temperature, in a one-step incubation procedure for 15 min. Afterwards, the reaction mixture was directly dispensed into the disposable cartridge. This cartridge integrates (i) a dedicated loading chamber for the bead–amplicon–enzyme complexes and (ii) a microfluidic compartment housing the screen-printed electrodes, where magnetic actuation and electrochemical readout take place. Once the cartridge is inserted into the battery-operated reader, an external magnet drives and concentrates the magnetic complexes onto the electrode surface, while the microfluidic architecture facilitates the removal of excess sample and unbound reagents. A single washing step was carried out directly inside the cartridge by adding the buffer, discarding the liquid, and then adding fresh buffer to establish electrical contact for the amperometric measurement. The same measurement buffer (100 mmol L^{-1} Na_2HPO_4, 100 mmol L^{-1} KCl, pH 7.0, plus 2 mmol L^{-1} hydroquinone, 4 mmol L^{-1} H_2O_2) was used for both washing and signal generation to streamline handling and minimize user steps. The reader then performs the amperometric acquisition in a short run (30 seconds), providing a direct quantitative output on-screen and optionally enabling wireless data transfer (Bluetooth) to a mobile application.

In order to study the analytical performance of the procedure for each set of primers, calibration curves were obtained using *M. bovis* BCG-Pasteur samples ranging from 0 to 2.6×10^7 CFU mL^{-1}.

Comparison of mycobacteria detection using the handheld device and commercial electrochemical systems

Four samples of *M. bovis* BCG-Pasteur at different concentrations of 2.6×10^7, 2.6×10^5 and 2.6×10^3 CFU mL^{-1}, including a negative control (0 CFU mL^{-1}), were processed following the procedure described before (Fig. 1). The samples were amplified using the primer set targeting the *IS6110* gene in the double-tagging PCR and subsequently analyzed by electrochemical genosensing with the portable handheld device developed by BioEclosion SL, to evaluate the robustness and reproducibility of the measurements. The same samples were also tested using the cartridge adapter connected to a commercial potentiostat (DropSens, Spain), which served as the reference method. All assays were performed in triplicate for each readout system to ensure statistical reliability and comparability of results.

Comparison of mycobacteria detection using a benchtop and handheld thermocycler operated by batteries

To evaluate the feasibility of integrating a portable battery-operated thermocycler into the proposed molecular workflow, the amplification of mycobacterial DNA was compared using a conventional benchtop thermal cycler and a handheld battery-operated thermocycler (miniPCR® mini16X, miniPCR bio, USA). Samples of *M. bovis* BCG-Pasteur at 2.6×10^5 CFU mL^{-1} were subjected to double-tagging PCR using the primer sets targeting the *IS6110* and *gyrB* genes, following the same reaction mixture and thermal cycling protocol described above. Negative controls were performed in parallel in the absence of template DNA. The resulting amplicons were first analyzed by agarose gel electrophoresis in order to compare the amplification performance of both thermocyclers for each target.

After amplification, the PCR products obtained with both thermocyclers were analyzed using the same electrochemical magneto-genosensing procedure described in Fig. 1. Briefly, 10 μL of the amplified sample was incubated with streptavidin magnetic particles and anti-DIG–HRP for 15 min at room temperature, and the resulting complexes were transferred into the disposable cartridge for magnetic actuation, washing, and amperometric readout using the handheld electrochemical device. All measurements were performed in triplicate ($n = 3$). The comparison was established on the basis of the cathodic current responses obtained for each target and amplification system, including the corresponding negative controls.

Biosafety considerations

The experiments regarding the bacterial culture were performed in a Biosafety class 2 environment required for the handling of *M. bovis* BCG-Pasteur. All biological waste generated from the experiments was disposed of in accordance with the local regulations for handling biohazards.

Results and discussion

Double-tagging polymerase chain reaction

The optimization of DNA extraction and amplification conditions is detailed in section S2 and Fig. S1 (SI). Four extraction protocols were evaluated by varying two optional steps —ultrasonic pretreatment and sodium acetate addition—to simplify and accelerate the thermal heat extraction.

Electrophoretic analysis revealed that the inclusion of sodium acetate led to reduced amplification efficiency, while ultrasonication provided no significant improvement. Consequently, both steps were omitted in the final protocol, yielding high-quality DNA suitable for amplification by thermal heating alone at 95 °C for 20 min, which can be easily integrated into the PCR workflow. PCR optimization was also performed using untagged primers targeting the *IS6110*, *rpoB*, and *gyrB* genes, with annealing temperatures ranging from 60 °C to 65 °C. All primer sets produced visible amplicons under these conditions. However, *rpoB* amplification generated high background signals due to primer–dimer formation. The optimized thermal cycling profile consisted of an initial denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 45 s, with a final extension at 72 °C for 5 min, achieving high specificity and yield for downstream electrochemical detection.

The specificity of the three primer sets was further evaluated against a panel of non-target bacterial species. As shown in Fig. 2, the *rpoB* primer set produced a clear band for both *Mycobacterium bovis* BCG-Pasteur and *Mycobacterium fortuitum*, revealing limited specificity within the tested panel. The *IS6110* primer set showed improved discrimination, although faint nonspecific bands or low-intensity background signals were still observed in some non-target lanes. Among the three targets evaluated, *gyrB* provided the cleanest electrophoretic profile. Considering these results together with the primer–dimer formation previously observed for *rpoB*, only *IS6110* (245 bp) and *gyrB* (483 bp) were retained for the subsequent double-tagging PCR experiments.

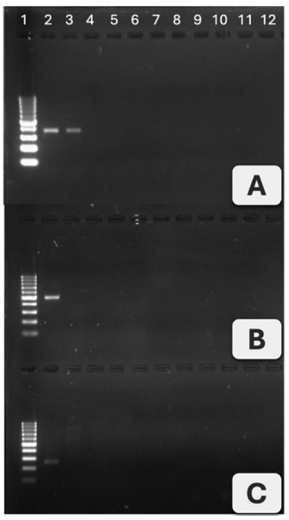

Fig. 2 Specificity study of the PCR assays targeting *rpoB* (A), *gyrB* (B) and *IS6110* (C), and using DNA extracted from non-target bacterial species (lanes 3 to 9) and *M. bovis* BCG-Pasteur (lane 2). The non-target bacterial species are lane 3: *Mycobacterium fortuitum* (ATCC 6841); lane 4: *Streptococcus agalactiae* (ATCC 8181); lane 5: *Pseudomonas aeruginosa* (ATCC 15442); lane 6: *Klebsiella pneumoniae* (ATCC BAA-1705); lane 7: *Escherichia coli* (ATCC 10536); lane 8: *Enterobacter* spp.; lane 9: *Salmonella choleraesuis* (ATCC 13311); lanes 10–12: negative controls without template DNA. PCR products were analyzed by agarose gel electrophoresis to evaluate the specificity of each primer set against non-target bacterial DNA. A molecular weight marker (GeneRuler, Thermo Fisher Scientific) was loaded in lane 1 of each panel.

The end-point PCR products obtained according to the procedure described in Fig. 1 were analyzed by agarose gel electrophoresis to confirm amplification performance across a tenfold dilution series of *M. bovis* BCG-Pasteur cultures ranging from 0 to 2.6×10^7 CFU mL^{-1} (Fig. 3). Panels A and B show the amplicons corresponding to the *IS6110* (245 bp) and *gyrB* (483 bp) genes, respectively. In both cases, the band intensity increased proportionally with bacterial concentration, with strong and well-defined bands observed for concentrations from 2.6×10^7 to 2.6×10^4 CFU mL^{-1} (lanes 2–5). A single, specific DNA band of the expected size was obtained for each target, indicating high amplification specificity. Negative controls without template DNA (lanes 12–14) showed no visible bands, confirming the absence of nonspecific amplification or primer–dimer artifacts. These results demonstrate that the double-tagging PCR provides consistent and specific amplification across a wide dynamic range, establishing a robust basis for subsequent electrochemical detection.[25] Based on the electrophoretic profiles, the visual limit of detection (LOD) was estimated at approximately 2.6×10^4 CFU mL^{-1} for both *IS6110* and *gyrB*, demonstrating reliable detection sensitivity for both targets.

Portable electrochemical magneto-genosensing using a cartridge-based handheld device

Before evaluating the analytical performance of the electrochemical magneto-genosensor for tuberculosis

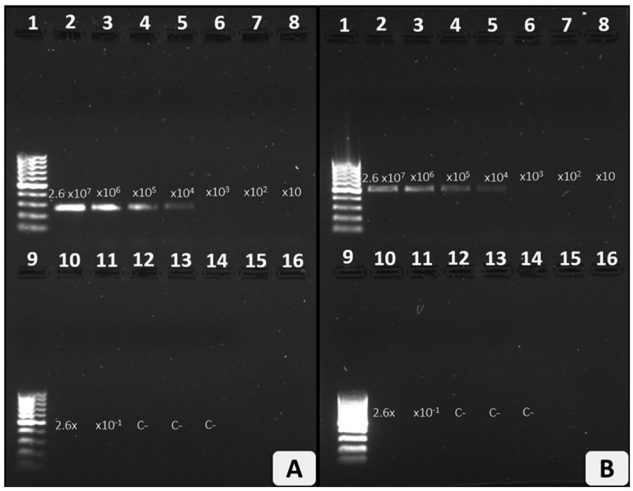

Fig. 3 Agarose gel electrophoresis of double-tagged PCR amplicons obtained from *M. bovis* BCG-Pasteur cultures ranging from 2.6×10^7 to 0 CFU mL^{-1} using primers targeting *IS6110* (panel A) and *gyrB* (panel B). In both panels, lanes 2–11 correspond to serial dilutions of bacterial samples (2.6×10^7, 2.6×10^6, 2.6×10^5, 2.6×10^4, 2.6×10^3, 2.6×10^2, 2.6×10^1, 2.6, and 2.6×10^{-1} CFU mL^{-1}), while lanes 12–14 represent negative controls without template DNA (0 CFU mL^{-1}). A molecular weight marker (GeneRuler, Thermo Fisher Scientific) was loaded in lane 1 of each panel.

detection, the concentrations of the main recognition reagents were optimized to maximize the signal-to-noise ratio. Specifically, the dilution of the streptavidin magnetic particles (strep-MPs) and the concentration of the anti-digoxigenin–HRP conjugate (anti-DIG–HRP) were assessed using double-tagged amplicons obtained with the *IS6110* primer set as a model target. As shown in Fig. S2 (SI), the best performance was obtained using strep-MPs at 1/2 dilution from the stock suspension and anti-DIG–HRP at 500 mU mL^{-1}, which were therefore selected for all subsequent electrochemical magneto-genosensing experiments.

To evaluate the analytical performance of the electrochemical magneto-genosensor for tuberculosis detection, attenuated *M. bovis* BCG-Pasteur samples with concentrations ranging from 0 to 2.6×10^7 CFU mL^{-1} were analyzed following the optimized double-tagging PCR and electrochemical procedure (Fig. 1). The corresponding calibration curves for the *IS6110* (blue) and *gyrB* (red) targets are shown in Fig. 4 (panel A), while the raw amperometric signals recorded during the measurements are displayed in Fig. 4 (panel B). The data were fitted using a four-parameter logistic model (Sigmoidal 4-PL, GraphPad Prism v8.0), yielding correlation coefficients of $R^2 = 0.9909$ for *IS6110* and $R^2 = 0.9815$ for *gyrB*.

The limit of detection (LOD) for each target was determined from twenty independent measurements of the PCR negative control ($n = 20$), containing all assay reagents except the target DNA. The cut-off signal was calculated as the mean cathodic current of the negative controls plus $t \times$ SD, where t corresponds to one-tailed Student's t value at 95% confidence and 19 degrees of freedom ($t = 1.729$). The corresponding concentration was then interpolated from the fitted calibration curve. Based on this approach, the estimated LOD values were 518 CFU mL^{-1} for *IS6110* and 117 CFU mL^{-1} for *gyrB*.

These values confirm the high analytical sensitivity of the developed biosensing platform, particularly for the *gyrB* target, which showed a lower detection threshold and a broader dynamic range. The results were consistent with agarose gel electrophoresis analysis (Fig. 2 and 3), which confirmed the absence of amplification in negative controls and demonstrated that signal intensity increased proportionally with bacterial load. Altogether, these results validate the robustness, reproducibility, and sensitivity of the electrochemical magneto-genosensor for the detection of *Mycobacterium tuberculosis*-related DNA.

Comparison of mycobacteria detection using the handheld device and commercial electrochemical systems

The analytical performance of the BioEclosion handheld electrochemical platform was evaluated by comparing its response to that of a commercial potentiostat (DropSens, Spain) (Fig. S3, SI) used as a reference system. Both devices were used to perform the electrochemical magneto-genosensing of *M. bovis* BCG-Pasteur samples at different bacterial concentrations ranging from 0 to 2.6×10^7 CFU mL^{-1}, following the same analytical protocol described previously.

As shown in Fig. 5, the cathodic current responses obtained with the handheld electrochemical reader (battery-operated) and the benchtop potentiostat were in excellent agreement across the entire concentration range tested. The current intensities increased proportionally with bacterial load, demonstrating consistent electrochemical behavior between both systems. Importantly, no significant differences were observed in the low-concentration region or in negative controls, confirming the reliability of the portable device at clinically relevant detection levels.

The comparable signal profiles resulted in very similar limits of detection (LODs) for both readers, validating the robustness and analytical equivalence of the handheld system. These findings confirm that the portable device maintains the same analytical performance as a conventional laboratory potentiostat, while providing the advantages of portability, autonomous operation, and suitability for point-of-care testing in resource-limited environments.

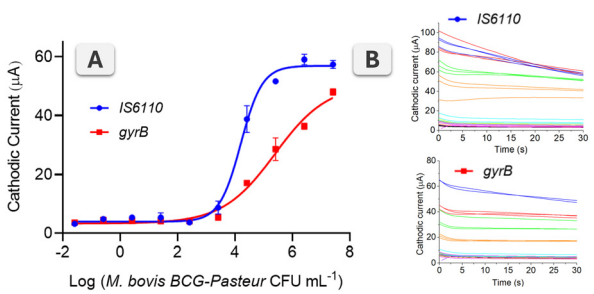

Fig. 4 Calibration curves and raw amperometric responses of the electrochemical magneto-genosensor for the detection of *M. bovis* BCG-Pasteur. (A) Calibration plots obtained from the double-tagged PCR amplicons using primers targeting *IS6110* (blue) and *gyrB* (red) genes, in samples ranging from 0 to 2.6×10^7 CFU mL^{-1}. (B) Corresponding amperometric signals recorded during the measurements, showing consistent current increases with bacterial concentration. The potential was set at −0.12 V, with a sampling interval of 0.5 s for a total acquisition time of 30 s, during which a steady state is reached. Error bars represent the standard deviation for $n = 3$ replicates.

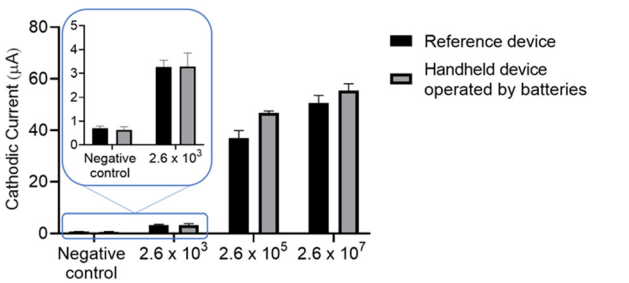

Fig. 5 Comparison of electrochemical magneto-genosensing responses obtained for *M. bovis* BCG-Pasteur samples at concentrations ranging from 0 to 2.6×10^7 CFU mL^{-1}, measured using the BioEclosion handheld reader and a commercial reference potentiostat. Both systems exhibited comparable cathodic current intensities across the tested range, confirming equivalent analytical performance. Error bars represent the standard deviation for $n = 3$ replicates.

Comparison of mycobacteria detection using benchtop and battery-operated handheld thermocyclers

The compatibility of the developed electrochemical magneto-genosensing platform with portable amplification was assessed by comparing PCR products generated with a conventional benchtop thermocycler and with a battery-operated miniPCR® device. For this purpose, *M. bovis* BCG-Pasteur samples were amplified using the double-tagged primer sets targeting *IS6110* and *gyrB*, and the resulting amplicons were subsequently analyzed by agarose gel electrophoresis and electrochemical genosensing with the portable reader. As shown in the gel image in Fig. 6, both *IS6110* and *gyrB* yielded clear amplicons of the expected size when the positive *M. bovis* BCG-Pasteur sample was processed using either thermocycler, whereas no visible bands were observed in the negative controls. These results confirm that the portable miniPCR® provides amplification performance comparable to that of the benchtop system for both targets, supporting its use as a suitable upstream step for subsequent electrochemical genosensing in decentralized settings.

The same amplicons were then analyzed by electrochemical genosensing with the portable reader. As shown in Fig. 7, comparable cathodic current responses were obtained for both thermocyclers for the two gene targets evaluated. In all cases, the positive samples produced clear electrochemical signals well above those of the negative controls, while the controls without template DNA remained at background levels. No relevant differences were observed between the benchtop and handheld thermocyclers, indicating that the portable battery-operated PCR device provides amplification products fully compatible with the downstream electrochemical detection step. These results

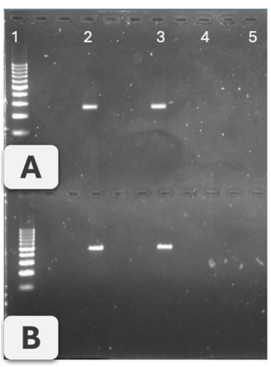

Fig. 6 Agarose gel electrophoresis of double-tagged PCR amplicons obtained using a conventional benchtop thermocycler and a handheld battery-operated thermocycler (miniPCR®). Panel A shows amplification of the *IS6110* target and panel B the *gyrB* target. In both panels, lane 1 corresponds to the molecular weight marker, lane 2 to the positive sample amplified with the benchtop thermocycler, lane 3 to the positive sample amplified with the miniPCR®, lane 4 to the negative control processed with the benchtop thermocycler, and lane 5 to the negative control processed with the miniPCR®. DNA from *M. bovis* BCG-Pasteur at 2.6×10^7 CFU mL^{-1} was used as a positive sample, whereas negative controls were processed in parallel in the absence of template DNA.

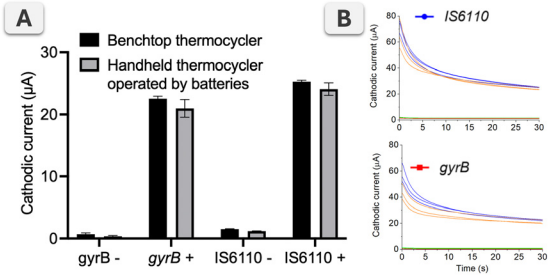

Fig. 7 Comparison of electrochemical magneto-genosensing responses obtained after double-tagging PCR amplification performed with a conventional benchtop thermocycler and a handheld battery-operated thermocycler (miniPCR®). Amplification was carried out using primer sets targeting the *IS6110* and *gyrB* genes from *M. bovis* BCG-Pasteur samples at 2.6×10^5 CFU mL^{-1}. Negative controls were processed in parallel in the absence of template DNA. (A) Cathodic current responses obtained for positive and negative samples after amplification with each thermocycler. (B) Corresponding amperometric signals recorded during the measurements. Error bars represent the standard deviation for $n = 3$ replicates.

demonstrate that the analytical workflow can be successfully transferred from a conventional laboratory thermocycler to a compact portable PCR platform without compromising assay performance. This constitutes an important step toward the implementation of a fully decentralized molecular diagnostic strategy, in which both nucleic acid amplification and electrochemical readout can be performed using portable, battery-operated instrumentation.

Conclusions

In this study, we report the development of an electrochemical magneto-genosensor for the rapid detection of TB, integrating molecular specificity with electrochemical simplicity in a compact and user-friendly format.

The analytical process comprises a double-tagging PCR followed by electrochemical genosensing. The amplification step is completed within approximately one hour, and the subsequent electrochemical detection requires only a 15 minute one-step incubation and a 30 second electrochemical readout. Overall, the entire analytical sequence can be completed in approximately two hours, highlighting the suitability of the platform for rapid testing in decentralized environments. Although polymerase chain reaction (PCR) and other amplification-based methods offer excellent analytical sensitivity, their use at the point of care has traditionally been limited by the complexity and cost of instrumentation. The emergence of compact, battery-powered PCR systems—such as miniPCR[26,27]—demonstrates the feasibility of performing amplification reactions outside centralized facilities. In this context, the double-tagging PCR described here was successfully implemented using a compact battery-operated thermocycler, providing amplification products fully compatible with the downstream electrochemical detection step. This result further supports the feasibility of a decentralized molecular diagnostic workflow combining portable amplification and electrochemical readout.

The test achieved a limit of detection as low as 117 CFU mL^{-1} when using primers targeting the *gyrB* gene, a performance comparable to the WHO-endorsed GeneXpert® MTB/RIF assay (114 CFU mL^{-1}). This level of sensitivity, combined with the compact design and low power consumption of the system, positions the platform as a promising alternative for tuberculosis screening, particularly in low-resource or field environments.

Overall, this work represents a significant step toward the development of a fully portable, amplification-based diagnostic platform that bridges molecular precision with point-of-care accessibility.

Author contributions

Melania Mesas Gómez: data curation, investigation, validation, formal analysis, writing – original draft. Daniel Ropero Gil: investigation and validation. Bárbara Molina-Moya: investigation, validation. Arnau Pallarès-Rusiñol: validation. Jofre Ferrer-Dalmau: validation. Esther Julián: conceptualization. José Domínguez: conceptualization, supervision, funding acquisition. María Isabel Pividori: conceptualization, methodology, supervision, funding acquisition.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the supplementary information (SI), which includes the composition of buffers and solutions, optimization of the DNA extraction and double-tagging PCR conditions, optimization of the electrochemical magneto-genosensing procedure, and additional information on the electrochemical platforms used for comparison. See DOI: https://doi.org/10.1039/d6sd00037a.

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