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Development of a lateral flow immunochromatography test for the rapid detection of bovine tuberculosis

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Abstract

Detection of specific antibodies would be a useful test strategy for bovine tuberculosis (bTB) as a complement to the single skin test. We developed a lateral flow immunochromatography (LFIC) test for rapid bTB detection based on the use of a conjugate of gold nanoparticles with a recombinant G protein. After evaluating 3 *Mycobacterium bovis* (MB) antigens: ESAT-6, CFP-10 and MPB83 for the control line, we selected MPB83 given it was the most specific. The performance of the test was analyzed with 820 bovine sera: 40 sera corresponding to healthy animals, 5 sera from animals infected with *Mycobacterium avium* subsp *paratuberculosis* (MAP) and 775 sera of animals from herds with bTB. All these sera were also submitted to a validated bTB-ELISA using whole-cell antigen from MB. From the 775 sera of animals from herds with bTB, 87 sera were positive by the bTB-ELISA, 45 were positive by LFIC and only 5 animals were positives by skin test (TST). To confirm bTB infection in the group of TST (-), bTB-ELISA (+) and LFIC (+) animals, we performed postmortem examination in 15 randomly selected animals. Macroscopically, these 15 animals had numerous small and large yellow-white granulomas, characteristic of bTB, and the infection was subsequently confirmed by PCR in these tissues with lesions (gold standard).

No false positive test result was detected with the developed LFIC either with the sera from healthy animals or from animals infected with MAP demonstrating that it can be a useful technique for the rapid identification of animals infected with bTB.

Key words: Bovine tuberculosis; lateral flow immunochromatography; MPB83;

Mycobacterium bovis.

Abbreviations

ABTS, hydrogen peroxide/2,2-azino-bis 3-ethylbenzthiazoline-6 sulfonic acid; BSA, Bovine Serum Albumin; bTB, bovine tuberculosis; ELC, Enhanced ChemiLuminescence; ELISA, Enzyme-Linked Immunosorbent Assay; Igs, Immunoglobulins; LFIC, lateral flow immunochromatography; MAP, *Mycobacterium avium* subsp *paratuberculosis*; MB, *Mycobacterium bovis*; MTC, *Mycobacterium tuberculosis* complex; PBS, Phosphate-buffered saline; PBST, Tween-20 in PBS; TBS, Tris-buffered saline; TST, skin test; T-TBS, Tween-20 in TBS; WCA, Whole-Cell Antigen.

1. Introduction

Mycobacterium bovis is the etiologic agent of bovine tuberculosis (bTB) and a member of the *Mycobacterium tuberculosis* complex (MTC), which includes *M. tuberculosis* responsible for tuberculosis (TB) in humans. Although cattle are the primary host, *Mycobacterium bovis* (MB) also affects humans and wild animals, which may act as reservoirs. Despite the ongoing control efforts and extensive eradication programs based on the skin test (TST) and slaughter strategies, bTB is spread worldwide and remains an animal health concern in several countries. In reports to the OIE of their situation with bTB, 82 countries (44%) reported the presence of the disease (Wiker et al., 2009). In addition to its implication in human health, this disease causes severe economic losses in developing

countries (Olea-Popelka et al., 2017). The lack of success in eradication programs has been attributed in part to the lack of rapid and effective diagnostic techniques and appropriate control strategies.

In Latin America, about 70% of cattle are in areas with bTB and only 17% inhabit areas virtually free from this disease (De Kantor and Ritacco, 2006). A compelling revelation of the limitations of TST is the fact that, for example, in Ethiopia, where most farmers consume raw milk, MB was isolated from farmers with TB, although none of the animals from these farms developed positive test results for bTB by comparative TST (Nuru et al., 2017). Instead, several serologic tests revealed the identification of MB-infected animals that had been missed by the routine tuberculin TST, thus the cause of perpetuating the disease (Casal et al., 2014; Garbaccio et al., 2019; Griffa et al., 2020; Waters et al., 2017).

Lateral flow immunochromatography (LFIC) has been increasingly applied for point-of-care testing because of its simplicity speed. Rapid test with *M. tuberculosis* complex antigens were used to diagnosis of bTB (Chembio's CervidTB STAT-PAK and DPP VetTB tests) (Buddle et al., 2010). A recently study determined that an Animal TB Rapid Test (Lionex-test) could detect MB in milk (Kelley et al., 2020).

In the present work, we developed a rapid bTB detection test based on a LFIC using MB antigens for fast and easy detection of bTB, even in animals non-reactive to the TST.

2. Material and Methods

2.1. Development of the lateral flow device

G protein conjugation with colloidal gold nanoparticles

Different amounts of recombinant G protein (Sigma-Aldrich) were added to 500 µL of colloidal gold solution and incubated at room temperature for 10 min. Then a 10% NaCl

solution (100 µL) was added; the mixture was stirred for 10 min and the optical density at 580 nm was measured in order to determine the amount of G protein to conjugate.

Subsequently, colloidal gold solution was centrifuged at 4000rpm for 40 minutes at 4°C, suspended in buffer sodium borate 2mM, sucrose 2% pH 6,4 and incubated for 20 minutes with recombinant G protein diluted to 50µg/ml in ultrapure water. The resultant conjugate was purified through centrifugation (4000rpm, 40 minutes at 4°C) and washed with buffer sodium borate 2mM, sucrose 2%, BSA 1% pH 6,4. Finally, conjugated gold nanoparticles were diluted in Conjugate Pad Solution: PBS, sucrose 5%, sodium azide 0,05%, BSA 0,5% pH 6,4 and the mixture was dispensed onto the conjugate pad.

Capture reagents

We assessed three MB antigens, ESAT-6, CFP-10, and MPB83, diluted in ultrapure water to 10µg/µl and different dilutions were prepared before use. Bovine Immunoglobulins (Igs), used as the control line capture reagent, were purified from bovine serum through saline precipitation using a saturated ammonium sulphate solution. Finally, the test and control capture reagents were dispensed using a micro-aerosolization device (Linomat V, Camag), in two parallel lines on 25 × 300mm HiFlow Plus nitrocellulose membrane (HF120, Millipore) at 1.5µl/cm.

Preparation of chromatographic strips and test procedure

The developed device was assembled as follows: nitrocellulose membrane, conjugated pad, sample pad and absorbent pad. Two hundred µl of different sera diluted to 1/50 in PBS were applied to the sample pad. Results were interpreted 10-20 minutes after adding the sample.

2.2. Dot plot

Dot blot was perfomed by placing 5 µL of each sample on a nitrocellulose membrane (Amersham GE Healthcare, England). Subsequently, the membrane was blocked overnight at 4°C with 5% non-fat dried milk/0.1% (v/v) Tween-20 in TBS (T-TBS), washed with T-TBS, and probed for 1h with bovine sera diluted 1/100 in T-TBS. A peroxidase-conjugated anti-bovine immunoglobulin G (Sigma) dilution of 1:5000 was used as a secondary antibody. The dot blot was developed by enhanced chemiluminescence (ECL) following manufacturer's instructions (Amersham GE Healthcare) using an autoradiography film.

2.3. ELISA (enzyme-linked immunosorbent assay)

ELISA was performed as described in Griffa et al., 2020. Briefly, polystyrene microtiter ELISA plates (Maxisorp, NUNC) were coated with 100 µL of carbonate buffer (0.1M sodium bicarbonate, 0.1M sodium carbonate, pH 9.6) containing 33.8 ng of the whole-cell antigen (WCA) and incubated overnight at 4 °C. Subsequently, wells were blocked, washed and 100 µL per well of sera diluted 1/100 in Phosphate-Buffered Saline (PBS) were added and incubated for 1 h at 37 °C. Then, wells were washed with Tween-20 in PBS (PBST) to finally add peroxidase-labeled affinity purified protein G (BioRad Laboratories) in a 1:4000 dilution. After incubation, the plates were washed 3 times and the reaction was developed using hydrogen peroxide/2,2-azino-bis 3-ethylbenzthiazoline-6 sulfonic acid (ABTS, Sigma-Aldrich) in citrate buffer (pH 5), as the substrate/chromogen system.

The antibody reactivity of each sample was expressed with a corrected OD, OD_{405} (OD at 405nm obtained in the sample wells minus OD at 405nm in the control). Values higher than the cut-off point, 0385, were considered positive.

2.4. Sera samples

For the initial development of the LFIC we assessed 5 sera samples from healthy bovines, 5 from bovines infected with MAP and 5 from MB experimentally infected bovines. In all cases, healthy animals were previously characterized as TST (-) conducted as the caudal fold test and confirmed negative by postmortem examinations, MAP infection was confirmed by mycobacterial isolation from feces and subsequently IS*900*-PCR amplification and, finally, MB infection was confirmed by postmortem examination, mycobacterial isolation and IS*6110*-PCR amplification.

Subsequently the performance of the LFIC was evaluated using 820 bovine sera belonging to different herds in Argentina: 40 bovine sera from healthy animals (herd 1), 5 from animals infected with MAP (herd 7) and 775 bovine sera of animals from herds with continuous presence of bTB based on prior TST results (herds 2-6). The bTB-free herd (herd 1), accredited by the National Service of Agricultural and Food Health and Quality (SENASA, Argentina), was negative to the TST for more than 10 years, and had negative herd results on all prior slaughter examinations. On the other hand, in the 5 herds with bTB (herds 2-6) animals were exposed to TST every 90 days and TST reactors were removed according to the official regulations of Argentina. However, they contained continuous TST reactors and positive results in slaughter examinations. In these herds, although veterinarians remove TST positive animals persistently, the prevalence of bTB based on TST ranges from 2 to 7%. Finally, herd 7 contains animals with PTB for more than 15 years with a prevalence approximate of 30% based on ELISA-PPA-3. In all cases, sera were obtained 15–17 days after the tuberculin test to evaluate the humoral immune response.

Animals in all herds were injected intradermally in the caudal fold with 0.1 ml of PPD-B and the responses were analyzed as described by Eirin et al. (2015). The PPD-B reagent was provided by SENASA.

2.5. Gross necropsy

Fifteen animals from the bTB-affected study herds that were negative in the TST were sent to slaughterhouses due to age, low milk production, infertility or other reduced production traits and a wide range of organs were collected. The serological status of these cattle was unknown to the herd operators before slaughter. Following examination of the medial retropharyngeal, submandibular, tracheobronchial, mediastinal, mesenteric, ileocolic, and hepatic lymph nodes by meat inspectors for gross lesions, fresh and fixed samples of all these tissues, with or without gross lesions, were collected for histopathology and *IS*6110 PCR analysis.

Necropsies were performed in slaughterhouses authorized by SENASA. The Institutional Animal Care and Use Committee (CICUAE) of CICVyA-INTA, whose regulations are in agreement with the European Union Laws for protection of experimental animals, authorized this study.

2.6. Molecular Identification of M. tuberculosis complex by PCR

Samples from different organs of the animals subjected to necropsy were submitted for PCR testing. The extraction of the DNA was performed following manufacturer's instructions (Inbio highway S.A). Briefly, 25mg of each tissue sample was disaggregated with protease K solution during 4 hours at 56°C in agitation. Then, 200µl of lysis buffer was added and samples were incubated during 15 minutes at 56°C. Subsequently, DNA was precipitated in ethanol and centrifuged using the microcolumns provided in the kit.

Finally, microcolumns were washed twice and DNA was eluted in 200µl ultrapure water. To identify *M. tuberculosis* complex mycobacteria, PCR was performed using specific primers for the insertion sequence IS*6110* (Forward, 5'GATCGGAAC GTCGGCTGGTCAGG3' and Reverse 5'GATCGCCTTGCTCATCGCTGCCG3') as described by Hermans et al., 1990. The amplified product is 240 bp.

3. Results and discussion

For the development of the LFIC, we conjugated commercial recombinant G protein to colloidal gold. G protein is a small globular cell surface protein that binds to the Fc fragment of bovine IgG and other animal species with high affinity. The determination of the optimal amount of protein for conjugation with colloidal gold nanoparticles was performed through flocculation curves. In Figure 1, an arrow indicates the selected concentration of G protein (~0.075 µg/ µL) used for the stabilization of gold nanoparticles surface. Subsequently, in order to confirm the complex formation, we performed a dot blot by placing 5 µL of the conjugate (G protein-gold nanoparticles), gold nanoparticles and recombinant G protein on a nitrocellulose membrane. Figure 2 shows the results obtained confirming the conjugation of the gold nanoparticles to the recombinant G protein.

Then, with the aim of determining the antigens and their optimal condition to be used as the test line (TL) in the LFIC, we sprayed 3 specific MB antigens at different concentrations (5, 2, 1, and 0.5 µg/µL) onto a nitrocellulose membrane. We evaluated the following MB purified recombinant proteins: early secretory antigen target 6 kDa (ESAT-6), culture filtrate protein 10 (CFP10) and mobility protein from MB (MPB83). We selected these proteins because their antigenic properties have been extensively studied and characterized with interesting results (Lyashchenko et al., 2020). Purified bovine immunoglobulins (0.05

µg/µL) were used as the control test (control line, CL). To evaluate the performance of the antigens, we initially assessed 5 samples of sera from healthy cattle, 5 sera from cattle infected with MAP and 5 sera from MB experimentally infected animals.

In this first analysis, MPB83 was sensitive and specific at 1 µg/µL concentration, as it was only detected by sera from MB experimentally infected animals (Figure 3). By contrast, ESAT-6 and CFP-10 antigens showed nonspecific signals. Indeed, they were detected in negative sera and in sera from animals infected with MAP at all concentrations evaluated (5, 2, 1, and 0.5 µg/µL) (data not shown). The control line of the test was easily detected in all cases. Based on these results, we selected MPB83 (1 µg/µL) for the test line and bovine immunoglobulins at 0.05 µg/µL as the control test for bTB detection in the LFIC. In the case of a positive test, the immune complex (G protein conjugated-colloidal gold nanoparticles-antibody) reacts with the immobilized MPB83 on the membrane, making the red test line visible. In the case of a negative test, only the red control line is detected; otherwise, the test has to be considered invalid.

In total 820 sera samples (45 corresponding to healthy animals, 5 from animals infected with MAP, and 775 belonging to animals from herds with bTB) were tested by LFIC with MPB83, and also by an ELISA developed and validated in our laboratory, based on a whole-cell antigen (WCA) (Griffa et al., 2020). From the 775 animals belonging to herds with bTB, 87 were bTB-ELISA (+), 45 were also positive by LFIC and only 5 were TST (+) (Table 1). These herds with long history of bTB were under National Control and Eradication Programs based on TST and slaughter of positive animals. However despite this control strategy potential bTB infected animals remains in these herds. This may be due to the fact that the animals belonging to these herds become “anergic” to cellular

immune response caused by different factors such as advanced stage of the disease, lesions progression, successive applications of the TST or alterations in physiological conditions such as stress or advanced pregnancy among others (SENASA)(Torres, 2016). Therefore, this eradication program using only TST, which is the routine diagnostic test for the detection of bTB in many countries, has not been very successful in reducing or eliminating the disease. These results are consistent with those previously reported by several authors (Casal et al., 2014; Waters et al., 2015). These researchers report, on the one hand, a large number of false negatives to the TST positive by serological tests and, on the other, an anamnestic effect produced by the TST in the detection of MB antibodies (Casal et al., 2017, 2014; Griffa et al., 2020). These results, together with our results previously mentioned, showed that these herds could contain animals infected with bTB but undiagnosed by the TST (Table 1).

To confirm bTB infection in the group of TST (-), bTB-ELISA (+) and LFIC (+) animals, we performed postmortem examination in 15 randomly selected animals by the personal in charge of the herds. Macroscopically, these 15 animals had numerous small and large yellow-white granulomas, characteristic of bTB, in the pleura as well as granulomatous lesions in the peritoneum, liver and mesenteric lymph nodes. In all cases, bTB infection was subsequently confirmed by IS*6110* PCR amplification from tissue lesions (Hermans et al., 1990). IS*6110* was amplified in all samples from TST (-), bTB-ELISA (+) and LFIC (+) animals (data not shown). These results confirm that all euthanized animals were infected with MB, although bTB was undetectable by the TST previously performed in these animals. Since only TST positive animals are sent to slaughter according to the official

regulations in Argentina, we were unable to perform postmortem examination on all animals positive by serological test.

False-negative reactions to the TST can occur for different reasons, predominantly because of desensitization to PPD-B or anergy owing to overwhelming or generalized infection with MB (de la Rua-Domenech et al., 2006; Kelley et al., 2020; Klepp et al., 2019). Thus, developing a complementary detection test based on serum antibody responses could improve the detection of bTB infected animals. Previous studies have demonstrated that the intradermal injection of PPD- B in MB-infected cattle boosted the levels of antibodies specific to MB antigens such as MPB83 and MPB70 (Lyashchenko et al., 2004; Waters et al., 2015). The animals sampled in our study were tested with TST every 60 days at the dairy farms. Thus, the results obtained may indicate a remarkable boost to preexisting serum antibody responses to both antigens, WCA used in ELISA and the recombinant MPB83 antigen used in the LFIC.

On the other hand if we compare the ELISA and LFIC, the higher number of sera detected by ELISA may be due to the use of a whole-cell antigen in the ELISA vs only MPB83 antigen in LFIC. ELISA could be less specific, and may also detect animals infected with other mycobacteria, whereas the LFIC use a specific antigen of MB. Future studies will be conducted to deepen the performance of both serological tests.

One important factor in the development of serologic tests is the choice of antigens. Most of these tests use antigens shared with other mycobacteria and therefore lead to low specificity in detection of bovine tuberculosis. In our study, MPB83 was sensitive and specific. This antigen was only detected by sera from MB infected animals, and remained undetectable in sera from healthy animals and sera from animals infected with MAP.

4. Conclusions

Our study highlights the need to complement the reference TST with studies that evaluate humoral immune response, such as the LFIC developed or ELISA. Rapidity and one step analysis, low operational cost, simple instrumentation, high specificity, better sensitivity, long term stability under different set of environmental conditions are unique advantages related to LFIC strips that allow this type of test to be easily used in herds for an initial screening to reveal infected animals that have escaped to TST.

The detection test developed in this work could contribute to the improvement of bTB control programs to decrease the occurrence of positive MB infected cattle that remain in dairy farms perpetuating bovine tuberculosis.

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Declarations of conflicting interests

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article

Ethics statement

The Institutional Animal Care and Use Committee (CICUAE) of CICVyA-INTA, whose regulations are in agreement with the European Union Laws for protection of experimental animals, authorized this study. Necropsies were performed in slaughterhouses authorized by the National Service of Agricultural and Food Health and Quality (SENASA).

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References

Buddle, B.M., Wilson, T., Denis, M., Greenwald, R., Esfandiari, J., Lyashchenko, K.P., Liggett, S., MacKintosh, C.G., 2010. Sensitivity, specificity, and confounding factors of novel serological tests used for the rapid diagnosis of bovine tuberculosis in farmed red deer (Cervus elaphus). Clin. Vaccine Immunol. 17, 626–630. doi:10doi:10doi:10doi:10doi:10doi:10doi:10doi:10.1128/CVI.00010-10

Casal, C., Díez-Guerrier, A., Álvarez, J., Rodríguez Campos, S., Mateos, A., Linscott, R., Martel, E., Lawrence, J.C., Whelan, C., Clarke, J., O'Brien, A., Domínguez, L., Aranaz, A., 2014. Strategic use of serology for the diagnosis of bovine tuberculosis after intradermal skin testing. Vet. Microbiol. 170, 342–351. doi:10doi:10doi:10doi:10doi:10doi:10doi:10doi:10.1016/j.vetmic.2014.02.036

Casal, C., Infantes, J.A., Risalde, M.A., Díez-Guerrier, A., Domínguez, M., Moreno, I., Romero, B., de Juan, L., Sáez, J.L., Juste, R., Gortázar, C., Domínguez, L., Bezos, J., 2017. Antibody detection tests improve the sensitivity of tuberculosis diagnosis in cattle. Res. Vet. Sci. 112, 214–221. doi:10doi:10doi:10doi:10doi:10doi:10doi:10doi:10.1016/j.rvsc.2017.05.012

De Kantor, I.N., Ritacco, V., 2006. An update on bovine tuberculosis programmes in Latin American and Caribbean countries. Vet. Microbiol. 112, 111–118. doi:10doi:10doi:10doi:10doi:10doi:10doi:10doi:10.1016/j.vetmic.2005.11.033

de la Rua-Domenech, R., Goodchild, A.T., Vordermeier, H.M., Hewinson, R.G., Christiansen, K.H., Clifton-Hadley, R.S., 2006. Ante mortem diagnosis of tuberculosis in cattle: A review of the tuberculin tests, γ-interferon assay and other ancillary diagnostic techniques. Res. Vet. Sci. 81, 190–210. doi:10doi:10doi:10doi:10doi:10doi:10doi:10doi:10.1016/j.rvsc.2005.11.005

Garbaccio, S.G., Garro, C.J., Delgado, F.O., Tejada, G.A., Eirin, M.E., Huertas, P.S., Leon, L.A., Zumárraga, M.J., 2019. Enzyme-linked immunosorbent assay as complement of intradermal skin test for the detection of mycobacterium bovis infection in cattle. Tuberculosis 117, 56–61. doi:10doi:10doi:10doi:10doi:10doi:10doi:10doi:10.1016/j.tube.2019.05.006

Griffa, N., Moyano, R.D., Canal, A.M., Traveria, G.E., Santangelo, M.P., Alonso, N., Romano, M.I., 2020. Development and diagnostic validation of an ELISA based on an antigenic mixture for the detection of bovine tuberculosis. Vet. J. 256, 105426. doi:10doi:10doi:10doi:10doi:10doi:10doi:10doi:10.1016/j.tvjl.2020.105426

Hermans, P.W., van Soolingen, D., Dale, J.W., Schuitema, A.R., McAdam, R.A., Catty, D., van Embden, J.D., 1990. Insertion element IS986 from Mycobacterium tuberculosis: a useful tool for diagnosis and epidemiology of tuberculosis. J. Clin. Microbiol. 28, 2051–8.

Kelley, H. V., Waibel, S.M., Sidiki, S., Tomatis-Souverbielle, C., Scordo, J.M., Hunt, W.G., Barr, N., Smith, R., Silwani, S.N., Averill, J.J., Baer, S., Hengesbach, J., Yildiz, V.O., Pan, X., Gebreyes, W.A., Balada-Llasat, J.-M., Wang, S.-H., Torrelles, J.B., 2020. Accuracy of Two Point-of-Care Tests for Rapid Diagnosis of Bovine Tuberculosis at Animal Level using Non-Invasive Specimens. Sci. Rep. 10, 5441.

doi:10doi:10doi:10doi:10doi:10doi:10doi:10doi:10.1038/s41598-020-62314-2

Klepp, L.I., Eirin, M.E., Garbaccio, S., Soria, M., Bigi, F., Blanco, F.C., 2019. Identification of bovine tuberculosis biomarkers to detect tuberculin skin test and IFNγ release assay false negative cattle. Res. Vet. Sci. 122, 7–14. doi:10doi:10doi:10doi:10doi:10doi:10doi:10doi:10.1016/j.rvsc.2018.10.016

Lyashchenko, K., Whelan, A.O., Greenwald, R., Pollock, J.M., Andersen, P., Hewinson, R.G., Vordermeier, H.M., 2004. Association of tuberculin-boosted antibody responses with pathology and cell-mediated immunity in cattle vaccinated with Mycobacterium bovis BCG and infected with M. bovis. Infect. Immun. 72, 2462–7. doi:10doi:10doi:10doi:10doi:10doi:10doi:10doi:10.1128/iai.72.5.2462-2467.2004

Lyashchenko, K.P., Sridhara, A.A., Johnathan-Lee, A., Sikar-Gang, A., Lambotte, P., Esfandiari, J., Bernitz, N., Kerr, T.J., Miller, M.A., Waters, W.R., 2020. Differential antigen recognition by serum antibodies from three bovid hosts of Mycobacterium bovis infection. Comp. Immunol. Microbiol. Infect. Dis. 69, 101424. doi:10doi:10doi:10doi:10doi:10doi:10doi:10doi:10.1016/j.cimid.2020.101424

Nuru, A., Mamo, G., Zewude, A., Mulat, Y., Yitayew, G., Admasu, A., Medhin, G., Pieper, R., Ameni, G., 2017. Preliminary investigation of the transmission of tuberculosis between farmers and their cattle in smallholder farms in northwestern Ethiopia: a cross-sectional study. BMC Res. Notes 10, 31. doi:10doi:10doi:10doi:10doi:10doi:10doi:10doi:10.1186/s13104-016-2349-z

Olea-Popelka, F., Muwonge, A., Perera, A., Dean, A.S., Mumford, E., Erlacher-Vindel, E., Forcella, S., Silk, B.J., Ditiu, L., El Idrissi, A., Raviglione, M., Cosivi, O., LoBue, P., Fujiwara, P.I., 2017. Zoonotic tuberculosis in human beings caused by Mycobacterium

bovis—a call for action. Lancet Infect. Dis. doi:10doi:10doi:10doi:10doi:10doi:10doi:10doi:10.1016/S1473-3099(16)30139-6

Torres, P.M., 2016. Situación de la tuberculosis bovina en la Republica Argentina. Servicio de Sanidad y Calidad Agroalimientaria (SENASA). Argentina.

Waters, W.R., Palmer, M. V., Stafne, M.R., Bass, K.E., Maggioli, M.F., Thacker, T.C., Linscott, R., Lawrence, J.C., Nelson, J.T., Esfandiari, J., Greenwald, R., Lyashchenko, K.P., 2015. Effects of Serial Skin Testing with Purified Protein Derivative on the Level and Quality of Antibodies to Complex and Defined Antigens in Mycobacterium bovis-Infected Cattle. Clin. Vaccine Immunol. 22, 641–649. doi:10doi:10doi:10doi:10doi:10doi:10doi:10doi:10.1128/CVI.00119-15

Waters, W.R., Vordermeier, H.M., Rhodes, S., Khatri, B., Palmer, M. V., Maggioli, M.F., Thacker, T.C., Nelson, J.T., Thomsen, B. V., Robbe-Austerman, S., Bravo Garcia, D.M., Schoenbaum, M.A., Camacho, M.S., Ray, J.S., Esfandiari, J., Lambotte, P., Greenwald, R., Grandison, A., Sikar-Gang, A., Lyashchenko, K.P., 2017. Potential for rapid antibody detection to identify tuberculous cattle with non-reactive tuberculin skin test results. BMC Vet. Res. 13, 164. doi:10doi:10doi:10doi:10doi:10doi:10doi:10doi:10.1186/s12917-017-1085-5

Table 1. Results of testing cattle for bovine tuberculosis by TST, ELISA, and lateral flow immunochromatography

Dairy farm	*n° sera tested*	*n° TST +*	*n° ELISA+*	*n° LFIC+*
1: herd with healthy animals	40	0	0	0
2: herd with bTB	104	1	15	11

3: herd with bTB	148	2	20	8
4: herd with bTB	118	0	6	3
5: herd with bTB	169	1	23	13
6: herd with bTB	236	1	23	10
7: animals infected with MAP	5	0	0	0
Total	820	5	87	45

n°: number; TST: Tuberculin skin test; ELISA: Enzyme-Linked ImmunoSorbent Assay; LFIC: lateral flow immunochromatography.

Figure 1. Flocculation curves showing the interaction of recombinant protein G with colloidal gold. Different amounts of recombinant G protein were added to 500 µL of colloidal gold solution and incubated at room temperature for 10 min. Then a 10% NaCl solution (100 µL) was added; the mixture was stirred for 10 min and the optical density at 580 nm was measured. The arrow indicates the selected concentration of G protein used for the stabilization of gold nanoparticles surface

Figure 2. G protein conjugation confirmed by dot plot. Five µL of the conjugate (G protein-gold nanoparticles), gold nanoparticles and recombinant G protein were placed on a nitrocellulose membrane. The membrane was blocked and probed for 1h with a bovine sera diluted 1/100 T-TBS as a primary antibody, then washed with T-TBS, and incubated for 1h with a peroxidase-conjugated anti-bovine immunoglobulin G dilution of 1:5000. The dot blot was developed by enhanced chemiluminescence following the manufacturer's instructions using an autoradiography film.

Figure 3. Bovine tuberculosis lateral flow immunochromatography. A representative figure of the developed device is shown. The assay was carried out by applying 200 µL of the serum sample diluted 1/50 in PBS. Lane 1: serum from bTB-free bovine, lane 2: serum from paratuberculosis infected bovine and lane 3: serum from bTB infected bovine. Igs = immunoglobulin control line; MPB83 = test line.

We developed a lateral flow immunochromatography (LFIC) test for rapid bovine Tuberculosis detection

MPB83 antigen was found to be sensitive and specific for *M. bovis* detection

The LFIC recognizes infected animals that have not been detected by the reference technique

The use of the LFIC could contribute to the control of bTB in herds

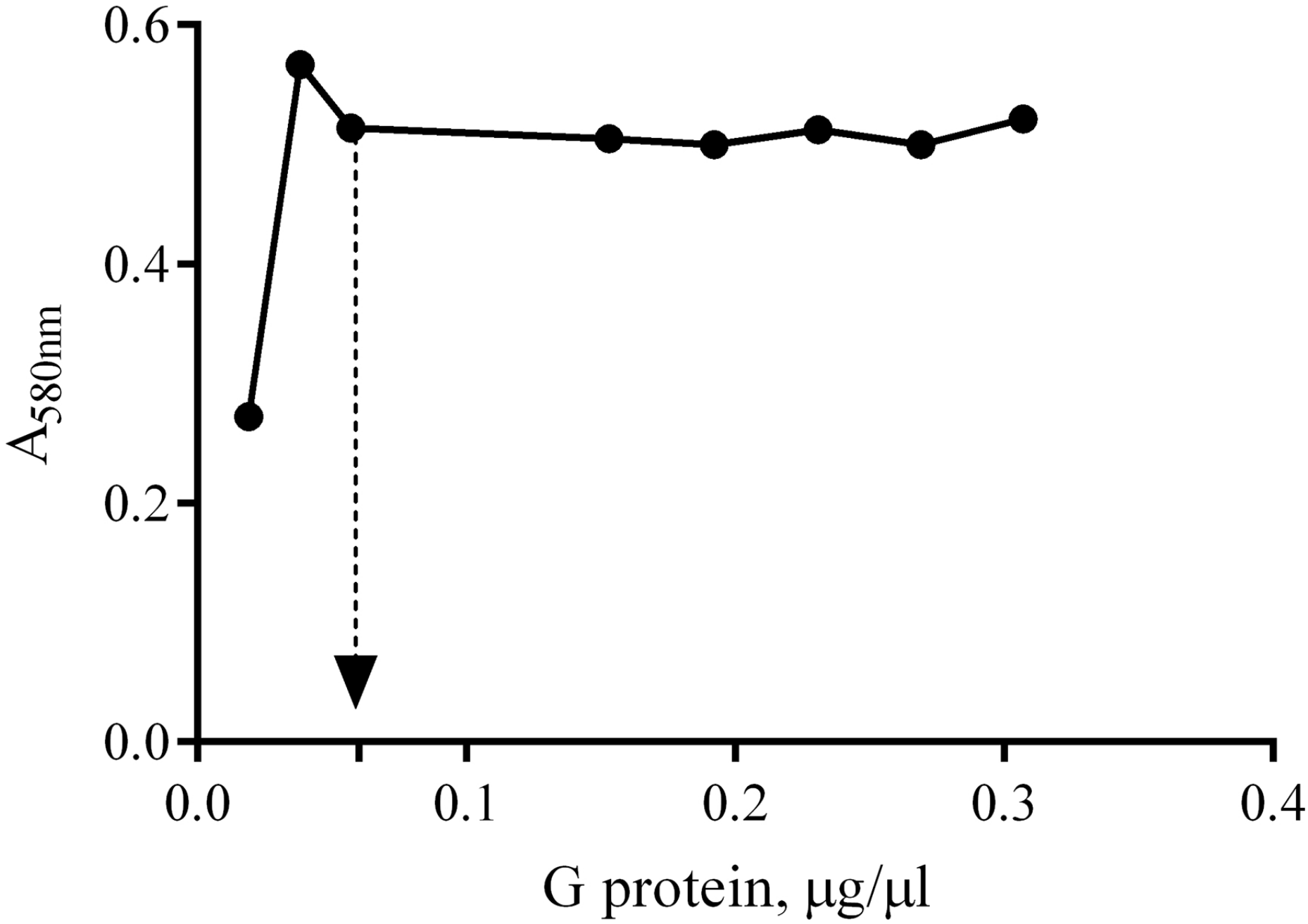

Figure 1

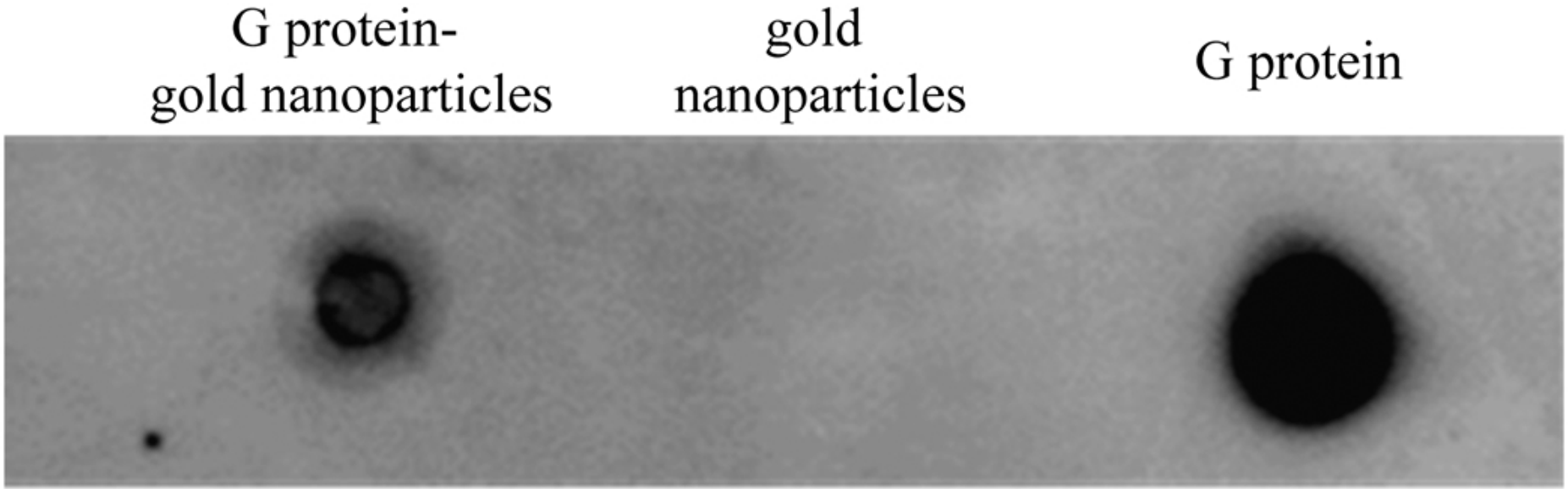
G protein-
gold nanoparticles
gold
nanoparticles
G protein

Figure 2

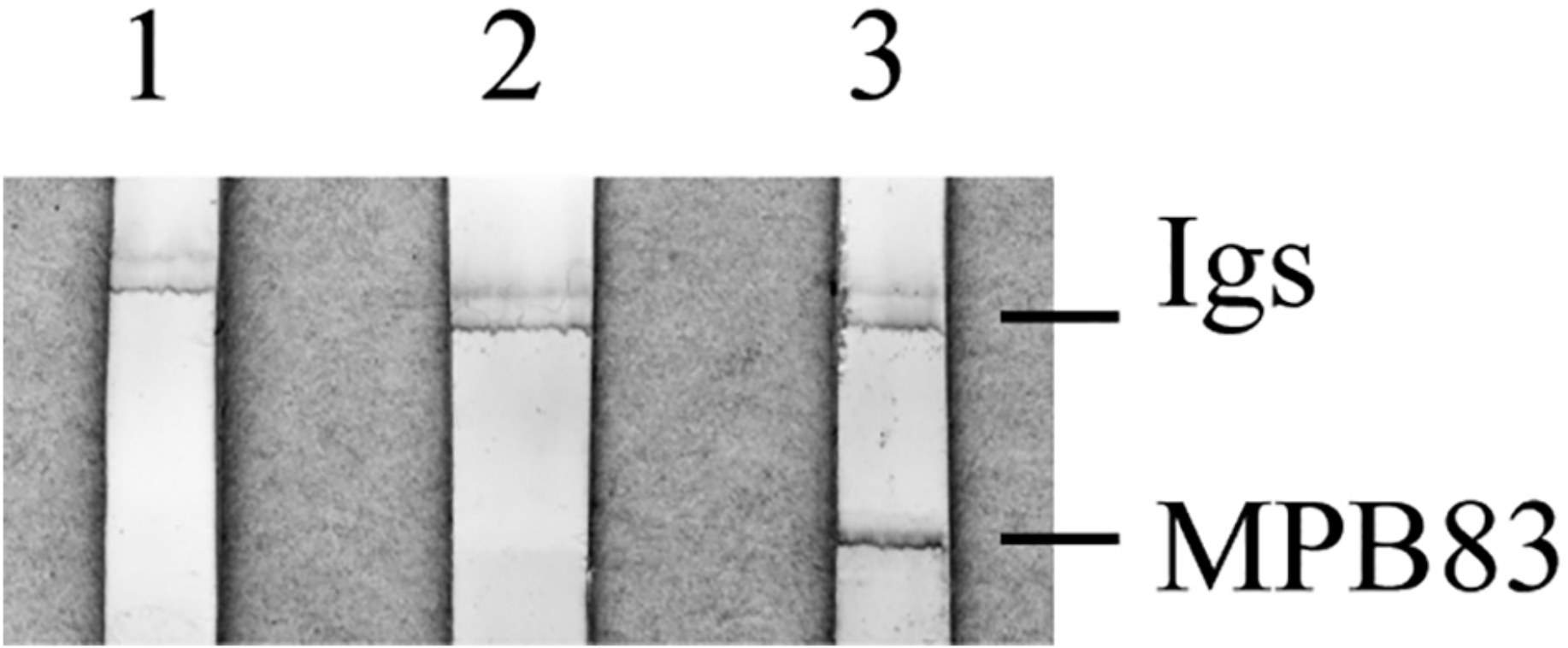

Figure 3