Lateral Flow Immunoassay for Ultrasensitive and Affordable Naked Eye Detection of Tuberculosis

Nazifah Ariffin[a], Nor Azah Yusof[a,b], Jaafar Abdullah[b,] Nurul Hanun Raston[a], Norzila Kusnin[a]

[a]*Institute of Advanced Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[b]*Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
**Corresponding Author's Email: azah1973@gmail.com*

Abstract: Lateral flow immunoassays (LFIAs) are advantageous over conventional detection methods in terms of their simplicity and rapidity. These assays have been reported using various types of labels but colloidal gold nanoparticles are still the preferred choice as a label because of their easy synthesis, visual detection and stability. Tuberculosis, or TB, is an infectious bacterial disease caused by *Mycobacterium tuberculosis*. İt remains one of the deadliest diseases in the world. The detection of *Mycobacterium tuberculosis* using LFIAs was developed and analyzed using gold nanoparticle.

Keywords*: Lateral flow immunoassay, Mycobacterium tuberculosis, nanoparticles, colloidal gold, bacterial*

INTRODUCTION

In this study, Lateral Flow Immunoassay was used in detection of active tuberculosis (TB) (Global tuberculosis control: WHO report 2013). Tuberculosis (TB) is still one of the greatest health care problems in the world. The development of a rapid and accurate test for the diagnosis of TB is a priority for TB control. Traditional diagnostic techniques based on the isolation of the *tuberculosis* bacillus in culture media are time consuming, and it is necessary to wait for several weeks to obtain a result (Shu-Lin Zhang, 2009). In this industrialized world many complex and expensive techniques are available, but these techniques cannot be used because of their high cost and lack of trained personnel. Therefore, possible biosensor that easy to use and cheap is the lateral flow immunoassay (LFIA), which is affordable, sensitive, specific, and user-friendly (Geertruida A. Posthuma-Trumpie, 2008). LFIA is based on recognition of one or more analyte, mainly proteins, by using antibodies. The antibodies are fixed onto a nitrocellulose membrane and they interact with the analyte either in sandwich or competitive formats using a proper label

MATERIALS AND METHODS

Gold nanoparticles (AuNPs) were prepared through reduction of Aurum (III) Chloride with trisodium citrate. The prepared AuNPs were further conjugated with antibody (Rabbit anti *Mycobacterium Tuberculosis*). The LFIA strips were prepared which the conjugate pad was soaked with antibody conjugated with AuNPs and drying at 37˚c for 1 h. The antibodies IgG diluted in the antibody buffer solution at a concentration of 1 mg/mL were spotted onto the detection pad to form the detection and control lines respectively. The detection pad was dried at 37˚C for 1 h. The different pads were subsequently laminated onto the baking card with an overlap between them of around 2 mm, in order to allow the sample (*Mycobacterium Tuberculosis* antigen) to flow. Finally, they were cut 3mm wide and stored in dry conditions. The sample was then dropped on the sample pad and flowed along the LFIA strips.

RESULTS AND DISCUSSION

The conjugate pad was soaked with antibody (Rabbit anti *Mycobacterium Tuberculosis*) conjugated with AuNPs and let to dry when preparing the LFIA strip. The 1 mg/ml antibody was spotted onto the Test Line and 1mg/ml antibody IgG on the control line. The control line on the nitrocellulose membrane were optimized so that a clear red signal could be observed by naked eye. The test strips were tested by lysis buffer (no antigen) and the buffer was let to flow laterally on the nitrocellulose membrane. The red signal on the control line were observed as shown in Fig. 1 (a) by naked eye after 5-10 min buffer loading. The red signal on the control line is shown (Figure 1) when the sample flowed along the lateral flow strips. Upon testing the lateral flow strips with a sputum sample of patients with positive Tuberculosis (TB), 100µl of sputum sample was dispensed on the sample pad. As shown in Fig (b), red signal could be observed on the test line and control line. This result indicates that the antibody on the test line was successfully bound with the antigen from the sputum sample.

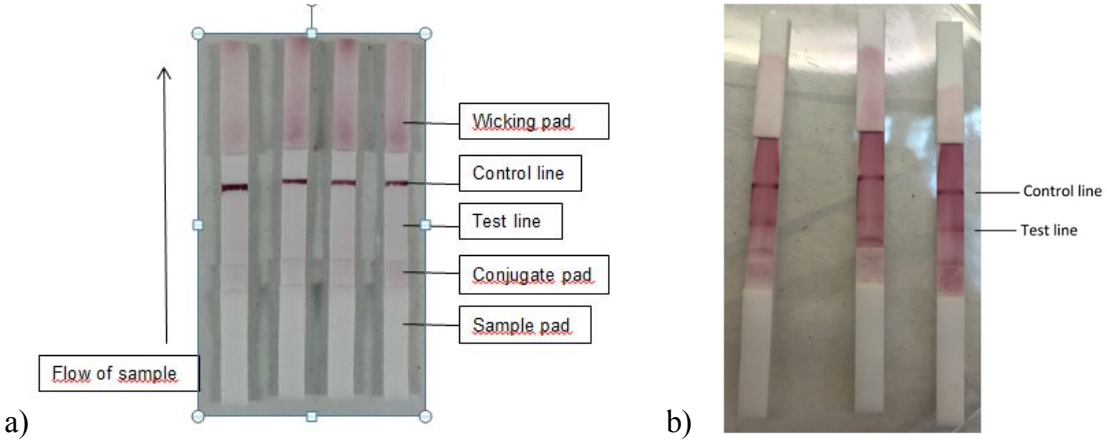

Fig. 1: (a) The LFIA strips shows red signal in control line when control sample (buffer solution without antigen) is load for 5-10 minutes. b) Red signals present on test line and control line which indicates a positive signal when sputum sample of positive *tuberculosis* patient is dispensed on the sample pad.

CONCLUSIONS

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis.* Traditional diagnostic techniques based on the isolation of the *tuberculosis* bacillus in culture media are time consuming, and it is necessary to wait for several weeks to obtain a result. Possible biosensor that easy to use and cheap is the lateral flow immunoassay (LFIA), which is affordable, sensitive, specific, and user-friendly.

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