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Qualitative and quantitative detection of T7 bacteriophages using paper based sandwich ELISA

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ABSTRACT

Viruses cause many infectious diseases and consequently epidemic health threats. Paper based diagnostics and filters can offer attractive options for detecting and deactivating pathogens. However, due to their infectious characteristics, virus detection using paper diagnostics is more challenging compared to the detection of bacteria, enzymes, DNA or antigens. The major objective of this study was to prepare reliable, degradable and low cost paper diagnostics to detect viruses, without using sophisticated optical or microfluidic analytical instruments. T7 bacteriophage was used as a model virus. A paper based sandwich ELISA technique was developed to detect and quantify the T7 phages in solution. The paper based sandwich ELISA detected T7 phage concentrations as low as 100 pfu/mL to as high as 10^9 pfu/mL. The compatibility of paper based sandwich ELISA with the conventional titre count was tested using T7 phage solutions of unknown concentrations. The paper based sandwich ELISA technique is faster and economical compared to the traditional detection techniques. Therefore, with proper calibration and right reagents, and by following the biosafety regulations, the paper based technique can be said to be compatible and economical to the sophisticated laboratory diagnostic techniques applied to detect pathogenic viruses and other microorganisms.

1. Introduction

Different biotechnological and biomedical applications are commonly restricted by the high cost and limited availability of tests and materials. In the detection of health conditions through pathological diagnosis, the outsourcing of blood, urine and other biofluid samples to an analytical laboratory is a standard practice. Reliable instantaneous diagnoses without recourse to sophisticated laboratory and analytical instrumentation would be a significant innovation that promises to improve health outcomes in underprivileged and remote areas [1–4]. In addition, access to simple, dependable and low cost water and air filters that are able to capture and deactivate waterborne and airborne pathogens would be equally invaluable for preventing epidemic health threats through provision of safe water and air [4–6].

Paper based diagnostics and filters offer attractive options for detecting and deactivating pathogens. Bioassays made of disposable materials can be used for regular tests to detect blood typing, cancers, generic conditions, and epidemic diseases such as hepatitis and influenza [4,5,7–10]. They can also be used to identify and filter heavy metals, chemical compounds and microbial activities in water. Successful commercialization requires bioassays to be low cost, which is best achieved through a high volume manufacturing process and with available raw materials. Paper has the potential to meet these criteria. Researchers demonstrated that antibody and enzyme active papers can detect ABO blood typing [1], bacteria [11], DNA [12], alcohol content in the breath [13], seafood freshness [14], and pathogens in biofluids [15–20]. However, simplicity, sensitivity, and amount of biofluid volume required for the test are ongoing challenges for developing paper based devices to detect pathogens, such as viruses [17–21].

Every year throughout the world, millions of people get infected by different waterborne and airborne viral diseases, such as: hepatitis, SARS, AIDS, diarrhoea, polio, chickenpox, smallpox and influenza, which are transmitted through drinking water, sexual

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contact, blood, saliva, breast milk and other body fluids [22–28]. Right diagnosis and treatment of these diseases cost billions of dollars, and still several millions of the infected people die every year [22–25,27,28]. Right diagnosis and early detection of those diseases and corresponding viruses can prevent epidemic health threats in many developing countries.

Viruses are small in size, often infectious and therefore, requires sophisticated higher level biosafety laboratory to work with. This study aims to develop paper bioassays to detect viruses. T7 bacteriophage was used as a model virus. T7 bacteriophages are not infectious to human, and therefore, experiments with T7 phages can be designed and conducted at biosafety level 1 laboratories [29]. T7 bacteriophages are lytic bacteriophages capable to infect bacterial cells such as *Escherichia Coli*. The other major advantage of using T7 phages is the availability of T7 specific primary and secondary antibodies [30,31].

In this study, a paper based *sandwich ELISA* technique was developed and demonstrated to detect and quantify the T7 phages in solution. Two monoclonal antibodies (IgM) specific to T7 phage, were used as the primary [30] and secondary antibodies [31]. The secondary antibody [31] was conjugated with horseradish peroxidase (HRP) enzymes to give a colorimetric indication in the presence of T7 phage in solution.

2. Paper based Sandwich ELISA

ELISA, enzyme-linked immunosorbent assay, is an enzyme immunoassay technique which is used to detect and report the presence of antigens, antibodies, proteins and pathogens using enzyme linked antibodies. ELISA techniques involve several incubation steps to coat antibodies, antigens and/or blocking agents, followed by a series of washing steps. Protein coating steps may require an incubation period between four hours to overnight; hence, performing an ELISA test to detect an analyte may take from several hours to as long as a day [32]. Elisa techniques can be broadly classified as direct ELISA, indirect ELISA, and *Sandwich ELISA* [32,33]. *In the sandwich ELISA* technique the analyte to be measured is bound between two primary antibodies – the capture antibody and the detection antibody, and offers higher sensitivity than *direct ELISA* or *indirect ELISA* techniques [32–34].

In *sandwich ELISA*, a surface is prepared using a primary antibody specific to the target molecule. Once the target molecule is applied, the primary antibody interacts with the target molecule to form an antibody-target molecule complex. In order to detect and report the target molecule attached to the primary antibody, an enzyme conjugated secondary antibody specific to the molecule is added to the system. This secondary antibody binds the other end of the target molecule leading to a sandwich structure encompassing the primary antibody, target molecule and secondary antibody complex. The enzyme conjugated to the secondary antibody reports the presence of the target molecule by reacting with the enzyme substrate and forming a colour product. In the paper based sandwich ELISA technique, a paper surface was prepared with the primary antibody. T7 bacteriophage was used as the target molecule. Fig. 1 indicates different steps of paper based sandwich ELISA test to detect T7 bacteriophages. Anti-T7 tag monoclonal IgM antibodies (mouse IgM isotype), specific to T7 tag sequence MASMTGGQQMG-K, were used as the primary and secondary antibodies [30,31]; the secondary antibody was conjugated with horseradish peroxidase (HRP) and recognized the T7 tag sequence on T7 bacteriophage. The presence of the secondary antibody could be found from the colour product produced from the enzyme-substrate reaction. Horseradish peroxidase (HRP) is a single chain polypeptide enzyme which readily combines with hydrogen peroxide (H_2O_2) and the resultant, HRP-H_2O_2 complex, can oxidize a wide variety of chromogenic hydrogen

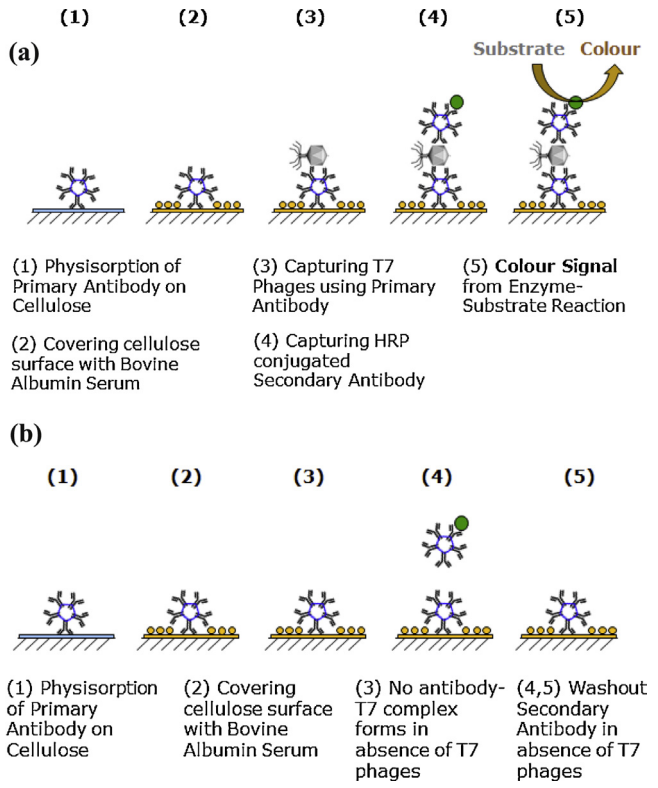

Fig. 1. Schematic of paper based sandwich ELISA. (a) Antibody (primary) – T7 phage – antibody (secondary) complex formation on paper surface in presence of T7 phages; (b) no antibody-T7 phage-antibody complex forms in absence of T7 phage.

donors [35]. Most reactions catalyzed by HRP can be expressed by the following equation [36]:

$$H_2O_2 + 2AH_2 \xrightarrow{HRP} 2H_2O + 2AH^{\bullet} \quad (1)$$

where, AH_2 and $AH^{\bullet}$ represent the reducing substrate and its radical product, respectively.

3,3′-Diaminobenzidine (DAB) is an organic compound that is used as a substrate for horseradish peroxidase (HRP) enzyme. In the presence of HRP, DAB is oxidized by hydrogen peroxide, producing a strong brownish colour complex that is stable. This can be observed visually and does not fade upon exposure to light. The HRP-DAB reaction can be expressed by the following equations [37]:

$$H_2O_2 + DAB \xrightarrow{HRP} \text{insoluble, brown-black precipitate} \quad (2)$$

Fig. 1a indicates the two layers of anti-T7 tagged antibodies which form the antibody-virus-antibody sandwich complex, in the presence of a T7 bacteriophage. The HRP conjugated with secondary antibody reacts with the enzyme substrate. The colour product formed on the paper surface from the enzyme-substrate reaction reports the presence of T7 phage in the system. In the absence of T7 phages, the primary and secondary antibodies do not bind with each other and consequently do not form a sandwich complex.

3. Experimental

3.1. Materials

T7 bacteriophages were cultured in the laboratory using *Escherichia coli* ATTC® BAA-1025 (American Type Culture Collection (ATCC), Manassas, USA) as the host and *Escherichia coli* bacteriophage T7 ATTC® BAA-1025-B2 (American Type Culture Collection (ATCC), Manassas, USA) as the seed. LB broth (Sigma L3152; (Sigma–Aldrich, Missouri, USA) and agar (Fluka 05039;