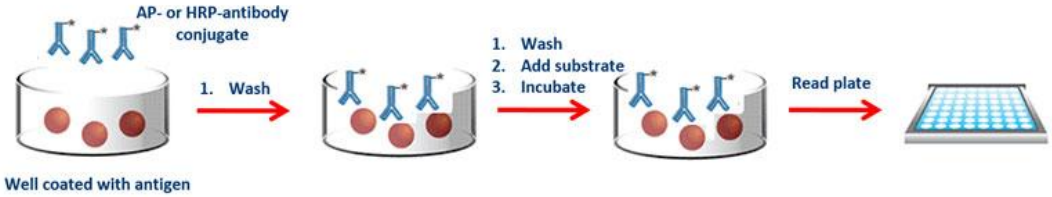
Detection of antibody in serum or antigen in stool of patients with enteric protozoa (ELAISA)

Although the ease of use and turnaround times for serologic assays are similar to microscopy, serology-based assays are more sensitive and specific. antigen-detection assays and antibody-detection assays. These include the enzyme-linked immunosorbent assay (ELISA), also called enzyme immunoassay (EIA), ELISA technique is considered as the most important diagnostic technique in biosciences nowadays, being based on the specific recognition potential of antigenic molecular design as an epitope in facing a specific antibody.

It becomes important for individuals whose blood smears do not permit identification of the parasite (e.g., differentiating between Babesia and Plasmodium) or for patients exhibiting low-parasitemia and/or who are asymptomatic (e.g., Chagasic patients) . Classifying an infected asymptomatic patient as negative could lead to transmission of the parasite during blood transfusions or organ transplants. In the case of Fasciola infection, serology tests have also been shown to be useful in the confirmation of chronic fascioliasis when egg production is low or sporadic. Finally, having these tests readily available allows for the monitoring of parasite clearance following therapy.

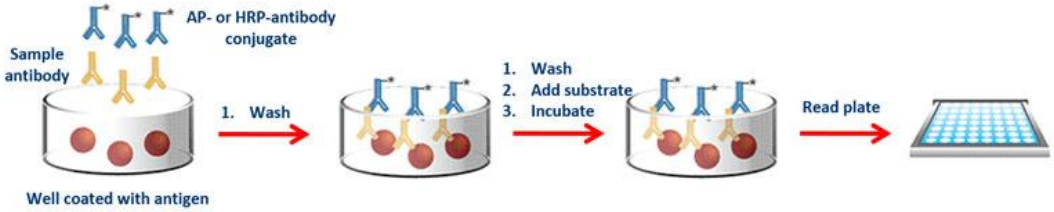
Direct ELISA Assay

This can be used to extrapolate the concentration of antigen in an unknown sample from a standard curve. Direct ELISA is suitable for determining the amount of high molecular weight antigens. Direct ELISA is considered to be the simplest type of ELISA. Fewer steps are required making it considerably faster than other types of ELISA. Cross-reactivity of the secondary antibody is also eliminated. It is, however, relatively rare as the direct labeling of primary antibodies is time-consuming, expensive, and may adversely affect the immunoreactivity of the antibody with the targeted antigen.



Indirect ELISA Assay

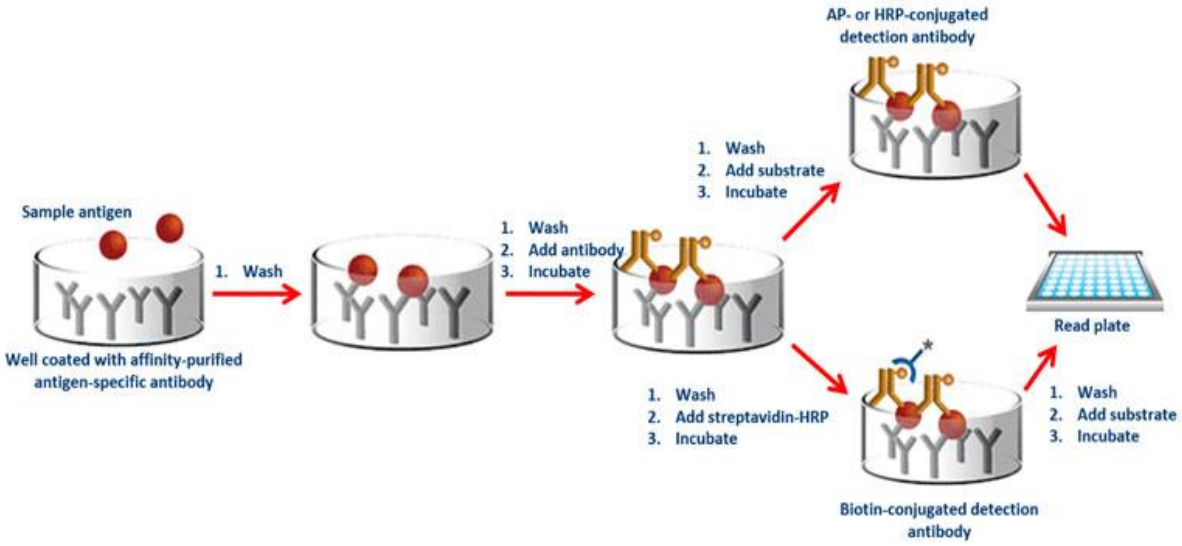
Indirect ELISA is a two-step binding process involving the use of a primary antibody and a labeled secondary antibody. This method is commonly utilized to diagnose infection by parasites and quantify antibodies against this foreign antigen. Indirect ELISA detection is versatile as different visualization markers can be used with the same primary antibody. Since more than one labeled antibody can be bound per antibody target, indirect ELISA is deemed to be highly sensitive and more flexible than direct ELISA. However, cross-reactivity and a non-specific signal may occur with the secondary antibody.



Immunometric/Sandwich ELISA Assay

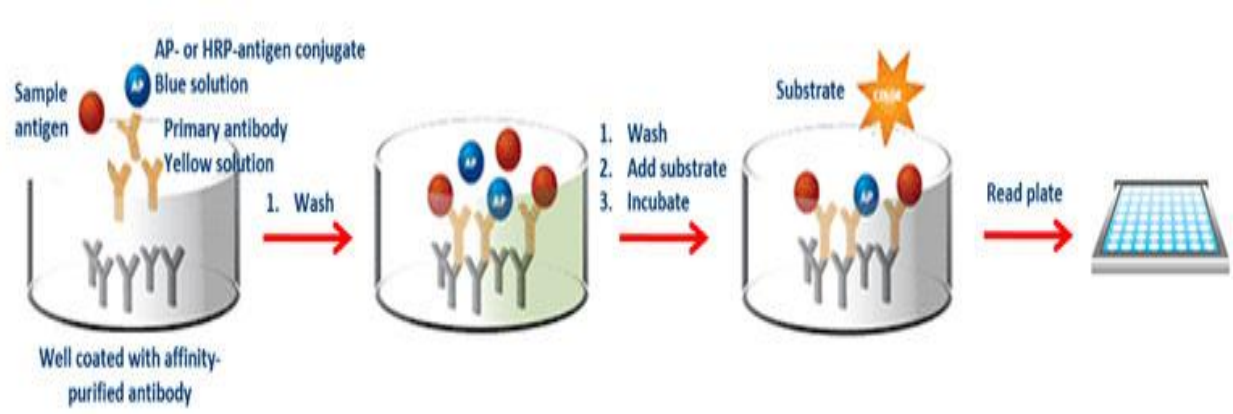
Immunometric assays, also known as sandwich ELISAs, use two antibodies specific to the antigen to capture or "sandwich" antigens in the well for detection. Immunometric assays exhibit a direct correlation between antigen concentration and

substrate response.



Competitive ELISA

In competitive enzyme immunoassays, the antigen in a sample competes for limited antibody binding sites with antigen conjugated to a reporter enzyme. This produces an inverse relationship between antigen concentration and substrate turnover.



Falcon Assay Screening Test ELISA (FAST-ELISA)

The Falcon assay screening test ELISA (FAST-ELISA) consists of using synthetic and recombinant peptides to evaluate antibody responses to an antigen . In the past, the method has been applied to the study of malaria , fasciolosis , schistosomiasis (reviewed in), and taeniasis . However, this technique is subjected to the same drawbacks as most serology-based tests. Antibodies raised against a peptide from one parasite protein may cross-react with proteins from other species. Moreover, antibodies raised against a peptide may react in some assays but not in others and some regions of a peptide may be more immunogenic than others. No recent studies have been published on the use of the FAST-ELISA for the diagnosis of parasitic infections.

3.2. Dot-ELISA

The main difference between the regular ELISA and the dot-ELISA lies in the surface used to bind the antigen of choice. In the dot-ELISA, the plastic plate is replaced by a nitrocellulose or other paper membrane onto which a small amount of sample volume is applied. The choice of binding matrix greatly improved the specificity and sensitivity of the assay by reducing the binding of nonspecific proteins usually observed when plastic binding matrixes are used. The principle is similar to the immunoblot. The dotted membrane is incubated first with an antigen-specific antibody followed by an enzyme-conjugated anti-antibody. The addition of a precipitable, chromogenic substrate causes the formation of a colored dot on the membrane which can be visually read . The benefits

of this technique include its ease of use, its rapidity, and the ease of result interpretation. It is fast, and cost-effective and more importantly can be used in the field (e.g., as a dipstick). For all these reasons, the Dot-ELISA has been and still is extensively used in the detection of human and animal parasitic diseases, including amebiasis, babesiosis, fascioliasis, cutaneous and visceral leishmaniasis, cysticercosis, echinococcosis, malaria, schistosomiasis, toxocariasis, toxoplasmosis, trichinosis, and trypanosomiasis , *Fasciola gigantica*.

Luciferase Immunoprecipitation System (LIPS)

The luciferase immunoprecipitation system (LIPS) is a modified ELISA-based assay in which serum containing antigen-specific antibodies can be identified by measuring light production. Basically, an antigen of choice is fused to the enzyme reporter Renilla luciferase (Ruc) and expressed as a Ruc-fusion in mammalian cells to allow for mammalian-specific posttranslational modifications.