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Review Article

Immunochromatographic Techniques: Benefits for the Diagnosis of Parasitic Infections

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Received: August 28, 2014; **Accepted:** November 20, 2014; **Published:** November 26, 2014

Abstract

Immunochromatographic tests (ICT) were first developed in the late 1960s mainly for the detection of serum proteins. In recent decades many ICTs have been described for the diagnosis of various infectious diseases. Parasitic infections constitute a major public health problem worldwide with various diagnostic challenges. Fortunately, the new immunochromatographic technology provides additional diagnostic options for parasitic diseases which can be reviewed and compared to other traditional diagnostic methods. Several ICTs have been developed for the diagnosis of malaria, leishmaniasis, filariasis, trypanosomiasis, trichomoniasis, toxoplasmosis, intestinal parasitic protozoa, and other parasitic infections. Although ICTs have been available for use in the field for a while, the available information about their performance in various parasitic diseases is inconclusive. Nonetheless, scientists agree that ICTs are a promising tool for the diagnosis of parasitic diseases with an acceptable level of diagnostic performance. ICTs are rapid, easy to perform and interpret, save time and effort, and compare favorably to other parasitological tests as a point-of-care diagnostic method. However, some claim that ICTs for the diagnosis of parasitic infections are not as sensitive as other immunoassays and may have a high rate of false positive results. This article summarizes the findings of studies which assessed various ICTs in the diagnosis of different parasitic infections, and discusses their limitations and discrepancies. The diagnostic performances and health outcomes of ICTs for various parasitic diseases are discussed in the light of current research studies.

Keywords: Immunochromatography; Parasitic infections; Diagnosis; Malaria; Visceral leishmaniasis; Lymphatic filariasis; Intestinal parasitosis; Trichomoniasis; Chagas disease; Toxoplasmosis

Abbreviations

ICT: Immunochromatographic Test; PCR: Polymerase Chain Reaction; LDH: Lactate Dehydrogenase; PLDH: *Plasmodium* Lactate Dehydrogenase; HRP-2: Histidine-Rich Protein 2; NADH: Nicotinamide Adenine Dinucleotide; VL: Visceral Leishmaniasis; rK39; recombinant antigen K39; LF: Lymphatic Filariasis; CFA: Circulating Filarial Antigens

Introduction

Lateral flow immunochromatographic tests (ICTs) have been primarily developed for rapid field testing, but have also been incorporated in clinical laboratories. These tests usually consist of single-use, disposable cartridges or strips which generate detectable colored end products interpreted as positive or negative. Immunochromatographic tests rely principally on the capture of the target antigen (or sometimes antibodies) from various specimens. The assay utilizes antibodies mounted on a paper strip or a nitrocellulose membrane as the immobile capture antibody (test area). Capillary flow is used to move a colloidal gold or colored microparticle-labeled antibody conjugate which binds to the target antigen in the mobile phase as it moves toward the capture antibody in the immobile phase. A positive test is produced by the capture of the moving labeled antigen/antibody complex by a second immobilized anti-species antibody (typically an anti-human immunoglobulin G) in the test area, and the formation of a colored line or pattern. Another control antibody to the conjugate binds the excess colloidal dye conjugate and acts as the control line. The control line is an indicator of the validity of test [1]. Immunochromatographic tests are usually known as dipstick tests, and the most common assay of this type that is familiar to the public are over-the-counter pregnancy test kits.

Immunochromatographic test devices have been developed by many diagnostics companies for the detection of several pathogens in various infectious diseases and even for the detection of biothreat agents [1]. In parasitic diseases, the gold standard technique for diagnosis is the direct detection of the parasite or its products by microscopic examination. However, microscopic diagnosis, though sensitive and specific, requires a high level of expertise and is not considered a rapid diagnostic method. Tests to identify parasite nucleic acids with molecular techniques, principally Polymerase Chain Reaction (PCR), have increased the speed and sensitivity of diagnosis to the species level compared to conventional methods. However, PCR-based protocols are available only in research settings and in diagnostic laboratories in developed countries, but are not currently available for use in most areas where parasitic infection is endemic. Moreover, molecular techniques are not suitable for point-of-care diagnosis and require expensive equipment and reagents.

Citation: El-Moamly AA. Immunochromatographic Techniques: Benefits for the Diagnosis of Parasitic Infections. Austin Chromatogr. 2014;1(4): 8.

Moreover, antigenic cross-reactivity limits the value of antibody detection in the serological diagnosis of parasitic diseases. Added to that, a positive serologic test does not distinguish between past and current infection, and so the test is unable to evaluate therapeutic response.

Recent diagnostic advances in parasitic infections have included the development of several ICT assays based mainly on the detection of various parasite antigens (and antibodies in some assays), with promising results. In recent years, researchers have assessed ICT kits for the detection of several parasitic diseases. Although ICT devices have been available for use in the field for a while, the available information about their performance in various diseases is inconclusive. Scientists agree that ICT devices are a promising tool in the diagnosis of parasitic diseases and are reasonably reliable (in terms of performance) in identifying positive and negative individuals. Moreover, they are cost-effective, easy to interpret by less experienced personnel, and can be used in large-scale surveys. They are also ideal for use under field conditions because the results can be read visually and laboratory equipment is not required. Although ICT assays are easy to perform and more rapid than traditional immunoassays, some claim that ICTs are not as sensitive as other immunoassays and may have higher rates of false positive results. However, these devices are more suitable as a point-of-care diagnostic method and can be useful for the rapid preliminary screening of samples, after which positive findings may be followed by other more specific confirmatory tests such as molecular-based techniques. The consensus is that ICTs are a potentially useful additional tool in the routine diagnosis of parasitic diseases.

This article summarizes the findings of studies which assessed ICTs for the diagnosis of different parasitic infections, and discusses the limitations and discrepancies of these studies. The diagnostic performances and health outcomes of ICTs for parasitic diseases are discussed in the light of current research.

Immunochromatography and malaria diagnosis

The WHO working group on malaria has put forward a global plan and a measurable target to achieve a 75% reduction in malaria morbidity and mortality by 2015 from the 2005 baseline level, taking many measures into consideration. Support for the intensified research program to develop new, improved and affordable diagnostic tools is one of the priorities [2]. WHO has begun a dialogue with scientific experts, clinical practitioners and diagnostics manufacturers regarding realistic possibilities for developing high-performing, cost-effective and rapid diagnostic tests for malaria? Stipulations for these rapid tests include the capability to detect 100 parasites/μL of all *Plasmodium* species, and suitability for performing semi quantitative measurements to monitor drug resistance. The microscopic detection of malaria has been the reference diagnostic test for many years. However, difficulties in maintaining the required technical expertise and infrastructure together with the time needed for this approach have accelerated the development of several non-microscopic rapid-diagnosis ICT assays for malaria based on the detection of *Plasmodium* antigens in whole blood. The preferred targets are antigens which are abundant in the asexual and sexual stages of the *Plasmodium* parasite. Immunochromatographic tests currently focus on the detection of histidine-rich protein-2 for *P. falciparum*, and *Plasmodium*-specific Lactate Dehydrogenase (LDH) and *Plasmodium* aldolase from the parasite glycolytic pathway for all malaria species. Immunochromatographic tests have been produced by different manufacturers under different brand names. Of these, the OptiMAL test was developed to detect the specific *Plasmodium* Lactate Dehydrogenase (PLDH) found in all *Plasmodium* species. The ParaSight-F, ICT *P.f* and *P.f/P.v,* and PATH Falciparum Malaria IC strip tests detect the specific *P. falciparum* histidine-rich protein 2 (HRP-2) [3].

The effectiveness of ICTs in detecting *P. falciparum* and non-*P. falciparum* species in malaria endemic areas has been evaluated by many investigators. However, there is controversy regarding the performance of ICT for malaria diagnosis. Some believe that integrating ICT for malaria diagnosis into the health care infrastructure would provide an important, accurate, and easy-to-use method with adequate diagnostic performance in terms of sensitivity and specificity, and positive and negative predictive values [4]. However, others believe that a more sensitive and specific technique such as PCR is required for accurate diagnosis [5-8].

Laboratory trials of the ParaSight-F ICT assay, which detects HRP-2 for the diagnosis of *P. falciparum* in blood, have reported an overall average sensitivity of 77% to 98% when >100 parasites/μL are present, with a specificity of 83% to 98% for *P. falciparum* compared to thick blood film microscopy [9-13]. However, according to other researchers, some manufacturers' brands of the ICT based on HRP-2 detection (ParaHIT-*f*, Span Diagnostic Ltd, Udhna, Surat, India) has shown very low sensitivity (11%) [14]. The OptiMAL test, which detects parasite-specific LDH, proved specific for the diagnosis of *P. falciparum* (>95%) and somewhat less specific for *P. vivax*, and was unreliable for *P. ovale* [15]. Work done by El-Moamly [16] showed that OptiMAL ICT had low sensitivity (85%, 95% CI, 79-92%) in detecting overall malaria infection, and sensitivity further decreased to 50% when the parasitic level was below 200 parasites/μL. The sensitivity of OptiMAL for the diagnosis of *P. falciparum* was 87% (95% CI, 79-94%), and sensitivity for *P. vivax* was 81% (95% CI, 66-96%); the test was unable to detect *P. malariae* species [16]. This may be explained by the lower affinity of the panspecific monoclonal antibodies developed from *P. falciparum* for *P. malarie* and *P. ovale* antigens than for *P. vivax* [17]. With OptiMAL, the PLDH enzyme remains in the blood for at least 7 to 10 days after the initiation of antimalarial therapy, resulting in a high rate of false positive findings [16]. Others found that the presence of possible cross-reactions may increase false positive rates. Rubino *et al.* [18] reported that the high number of false positive results (44.4%) with the OptiMAL ICT was due to autoantibodies such as rheumatoid factor. Previously, Grobusch *et al.* [19] found a 72% rate of pretreatment false positives with ICT in non-malaria patients with rheumatoid factor. The low sensitivity of OptiMAL for malaria species may be explained by the ability of ICT to detect only viable parasites capable of secreting the LDH enzyme, whereas it is unable to detect nonviable organisms observable with microscopy [16]. Moreover, the presence of antigen-blocking factors or the formation of immune complexes might also produce false negative results. For example, chloroquine, a regimen used for some patients with malaria, can compete with nicotinamide adenine dinucleotide (NADH) for binding to LDH, and can interact with the parasitic enzyme in the transformation of pyruvate. This

might lead to an increase or decrease in circulating levels of PLDH and thus cause erroneous results [15,20]. Some studies showed that the decline in PLDH activity paralleled a decline in the parasite burden. The PLDH assay may therefore be useful to monitor the patient's progress during therapy [21,22]. However, ICTs are not yet quantitative or semiquantitative assays, and it is not yet possible to obtain visual readings or to look for correlations with the intensity of infection without adding a reader for the gold-conjugated antibody.

The consensus of many scientists regarding the role of ICTs in the diagnosis of malaria is that these tests, at present, cannot replace microscopy. Microscopic examination is able to detect all *Plasmodium* species, allows visualization of the parasite growth stages, and can be used to evaluate parasitemic levels – all of which are essential for making therapeutic decisions. The sensitivity of ICTs for malaria remains a problem, particularly with low parasite densities, in nonimmune populations and for cases of *P. malarie* and *P. ovale* infection. Immunochromatographic tests can nonetheless be useful under certain circumstances to enhance the accuracy of the diagnosis of malignant *P. falciparum* malaria, especially when nonspecialized laboratories are involved, or to monitor the patient's progress during therapy [23,24]. However, a negative ICT result should be confirmed with other reliable methods such as microscopic examination or PCR. Immunochromatographic tests for malaria diagnosis of are easy-to-use, rapid non-microscopic tests that can save time and effort, and that may be more suitable under field conditions.

Immunochromatography and visceral leishmaniasis diagnosis

Visceral Leishmaniasis (VL) is one of the most neglected tropical diseases, and requires a global policy for integrated control programs because of its substantial health and economic burden on the poorest of the poor [25]. The disease is caused by *Leishmania donovani* in Asia and Africa, and by *L. infantum* or *L. chagasi* spp. in the Mediterranean region, South, Western and Central Asia, and South America. Visceral leishmaniasis is fatal if left untreated; however, treatment with pentavalent antimonials requires painful injections for 30 days, and is expensive and carries a risk of toxicity [26]. Therefore, a sensitive and specific diagnosis of VL is crucial to avoid under- or over diagnosis. Parasite detection in tissue smears or cultures is still the recommended reference method of diagnosis, but this method has variable sensitivity rates depending upon tissue type, is sometimes invasive, and requires experience. PCR-based protocols to detect kinetoplast DNA have increased the speed and sensitivity of species-specific diagnosis. However, PCR methods still require standardization and are not suitable for use in poor endemic areas or in the field; moreover, these methods require expensive equipment and reagents and highly trained staff. The choice of an appropriate serological diagnostic test is a controversial issue. None of the available serological tests is sufficiently specific as a stand-alone test, and none is useful for follow-up purposes. Fortunately, the new immunochromatographic technology provides additional diagnostic options which can be reviewed and compared to more traditional methods.

The identification and production of the *L. chagasi* recombinant antigen K39 (rK39) is considered a major advance. When used in ELISA, it was more sensitive and specific than soluble antigens for the diagnosis of active VL. Later, the rK39 antigen was used in an immunochromatographic strip test with promising results [27-30]. The rK39-based ICT uses the *L. chagasi* rK39 antigen to detect serum antibodies to *L. donovani* membrane. This antigen comprises a 39-amino-acid repeat section in the 230-kDa LcKin protein (protein A), and is a product of a gene cloned from *L. chagasi* containing the 39-amino-acid repeat. *Leishmania chagasi, L. donovani* and *L. infantum* all contain the gene that encodes LcKin protein. The test strip membrane is coated with a band of rK39 antigen in the test area, and with immobilized anti-protein A antibody above the band in the control area, both in the immobile phase. Protein A–gold conjugate is used as the detection reagent in the mobile phase. Anti-K39 IgG in the patient's serum reacts with the protein A–gold conjugate and the mixture moves up the strip by capillary action to react with the K39 antigen in the immobile phase, giving rise to a colored band in the test area. Another band appears in the control area with either negative or positive samples. Several authors recommended the rK39 ICT as one of the most suitable serological tests for the diagnosis of VL [31,32]. However, substantial variability between regions was reported, and for reasons that remain unclear, contradictory performance results have been obtained in the Indian subcontinent compared to East Africa [33-35]. Although the results were moderately encouraging, studies which evaluated the performances of the rK39 ICT reported some variability. The reported sensitivities of the rK39 ICT ranged from 79% to 97%, and specificities ranged from 85% to 97% [36-39]. Possible reasons for this variability among studies include the use of different diagnostic approaches for comparison, and different reference standard tests to estimate the rates of true negatives and true positives. Also, the rK39 ICT kits used in different studies were produced by different manufacturers such as InBios, Arista, Amrad and Diamed. Diagnostic performance has been shown to vary across different brands or even different generations of the same brand [33]. Variations in antigen reactivity across batches and inter observer reading inconsistencies may also be responsible for the discrepant results of these studies.

The available rK39 ICT is a qualitative test for the detection of leishmania antibodies. Hence, as with other antibody detection tests, the rK39 ICT is not useful for early diagnosis and can expected to be positive in patients with subclinical infection, past infection, or those who have been cured. Furthermore, it is useless for diagnosing relapses or for monitoring therapeutic success in VL. Because of the limitation posed by prolonged antigen detectability for years after cure and by positive results due to past asymptomatic infection in healthy persons, the rK39 ICT must always be used in combination with a standardized clinical case definition for VL diagnosis. The rK39 ICT is nonetheless a cost-effective test, so it can be used in large-scale surveys in which antibody titers are not required. Also, it is easy to interpret by less experienced personnel and is ideal for use under field conditions because it can be read visually and does not require laboratory equipment, electricity or refrigeration [27]. Moreover, a minimal amount of serum is required for the strip test, and a finger-prick blood sample can also be used with some brands. This is more convenient than using serum, and ensures flexibility in that the test can be done under any field conditions. Individually-packed tests provide an additional measure of convenience with the rK39 ICT in addition to its low cost (approximately US$ 1) and long shelf life (approximately 1.5 years).

In summary, the ICT assay for the diagnosis of VL has shown a fair level of diagnostic accuracy which renders it useful as an additional tool to facilitate the global drive to eliminate VL. The judicious use of the rK39 ICT can be expected to reduce VL misdiagnosis in many of the settings where available expertise is limited as well as in rural and underdeveloped areas. The test is more rapid and easier to perform, and more suitable as a point-of-care diagnostic method, than other comparable parasitological and serological tests.

Immunochromatography and lymphatic filariasis diagnosis

Lymphatic Filariasis (LF) is another important neglected tropical disease. It constitutes a major cause of permanent disability and is a major obstacle to socioeconomic development in more than seventy countries. One hundred and twenty million persons are infected and more than one billion persons are at risk for the disease worldwide [40]. The urgent need to eliminate LF requires increased efforts to develop a high-performing, rapid diagnostic test. The most common manifestations of LF are acute adenolymphangitis accompanied by attacks of fever, while lymphedema is the most common chronic manifestation, which may progress to elephantiasis and genitourinary deformities. In endemic areas, most affected people have asymptomatic clinical infection but harbor microfilariae in their blood. Identification of the microfilaria by microscopic examination of blood samples, either directly or after concentration, is the most practical diagnostic procedure. However, this parasitological detection method has low sensitivity because microfilaria may disappear from the peripheral blood once lymphedema develops. Moreover, microscopic examination is inconvenient due to the timing of blood sampling (midnight for microfilaria with nocturnal periodicity) [41]. Antibody detection has also been used, but cross-reactivity with other helminths and the inability to distinguish between past and current infections limit the value of this method for diagnostic purposes. Recent diagnostic advances include the development of sensitive immunoassays to detect Circulating Filarial Antigens (CFA) and parasite DNA in human blood. Rapid ICTs that specifically detect *Wuchereria bancrofti* CFA in whole blood have recently been developed, with promising results. The reported sensitivities range from 73% to 100% [42-46]. The AD12-ICT card test for the detection of circulating *W. bancrofti* antigens uses paired polyclonal and monoclonal antibodies specific for *W. bancrofti* antigen (AD12.1) conjugated to visible colloidal gold particles and immobilized in the test area. The test can use blood, serum or plasma, and a positive result is obtained from the capture of the moving labeled antigen–antibody complex by the second immobilized anti-species antibody in the test area (sandwich principle). Another control antibody to the conjugate binds the excess colloidal gold conjugate and acts as a control line. In recent years, researchers have assessed the accuracy of the AD12-ICT for the detection of circulating *W. bancrofti* antigens in comparison to various conventional techniques such as the TropBio Og4C3 ELISA, which detects CFA, the nucleopore membrane filtration method, and microscopic examination. They agreed that the ICT card test offered a promising diagnostic tool for detecting CFA with a reasonable level of performance in identifying positive and negative individuals. The sensitivity ranged from 94% to 100%, and specificity ranged from 84% to100% in different populations and with different gold standards for comparison [47-51]. The results also showed that although the ICT card assay generally detected all positive samples with antigen levels between 512 and >8000 units, it failed to detect many samples with antigen levels between 32 and 512 units. The higher sensitivity of the ICT compared to microscopy can be explained by the fact that ICT detects antigens, which may persist after the microfilaria have disappeared from the peripheral blood because of their nocturnal periodicity or progression of the disease. With respect to the use of ICTs in large control programs, scientists concluded that the ICT card test is a suitable and practical tool because of its ease of use, because no special equipment is needed to perform the test, and because the results are obtained within 10 min. Moreover, antigen tests can be performed with blood collected during the day or night. The results with the ICT card test are comparable to those with ELISA, and further advantages of the former are that it is faster and less complicated, and has potential for point-of-care testing and field work.

For *Brugia malayi & timori* species, an ICT dipstick has been developed to detect specific anti-filarial antibodies using a goat anti-mouse antibody in the control line and a *B. malayi* recombinant antigen in the test line. Serum samples from six centers in three countries (India, Indonesia, and Malaysia) were used to evaluate the ability of the Brugia Rapid™ ICT test to diagnose *B. malayi* infection. The overall sensitivity was 97% and specificity was 99%, and this study concluded that the Brugia Rapid ICT is thus a promising diagnostic tool for the detection of *B. malayi* infection. It may be especially useful in the brugian filariasis elimination program [52].

To conclude, ICT assays for the detection of LF are a potentially useful additional test to support proposed strategies for the control and elimination of LF. These tests are sensitive, rapid, cost-effective, simple to perform, can be done with blood collected during the day or night. Moreover, ICTs do not require special equipment or highly trained personnel, and may therefore be most appropriate for screening programs, in less-prepared settings, and to monitor the possible risk of introducing LF to nonendemic countries.

Immunochromatography and the diagnosis of intestinal parasites

Intestinal parasitic infections represent a major health concern not only in developing countries but also in more industrialized countries. Routine diagnostic methods may be insufficient to demonstrate the presence of intestinal parasites. Staining and microscopic examinations of stool specimens have their own limitations and require a high level of expertise. To achieve greater sensitivity and specificity, antigen-detection immunoassay methods have been developed. However, these tests require multiple reagent addition, washing and incubation steps. The newer immunochromatographic technology provides additional diagnostic options which can be compared to other traditional methods. Several coproantigen detection ICT kits have been developed and have shown adequate results in the diagnosis of intestinal parasitizes such as giardiasis, cryptosporidiosis and *Entameba histoytica* infection [53-55]. These tests may be useful additional tools, but cannot replace microscopic methods. In recent years, researchers have assessed the usefulness of ICTs for the diagnosis of intestinal parasitic infections and compared them to other routine and standard methods including conventional microscopic and staining techniques such as trichrome and modified Kinyoun acid-fast stains, direct fluorescence antibody

tests and antigen-detection enzyme immunoassays. For example, ImmunoCard STAT! An antigen-detection immuno-cartridge ICT was developed for cryptosporidiosis and giardiasis. This ICT is a qualitative test to detect *Cryptosporidium parvum*- and *Giardia lamblia*-specific antigens in stool specimens. One study assessed the diagnostic accuracy of the ImmunoCard STAT! ICT compared to the combination of the modified Kinyoun acid-fast technique with confirmation by microplate enzyme immunoassay (as the gold standard) for the detection of *Cryptosporidium* in fecal specimens [56]. Specimens from 315 symptomatic and asymptomatic patients were tested with the reference and index methods. Agreement among all three tests was shown in 22 positive and 288 samples that were negative for *Cryptosporidium*. The sensitivity of the ICT was 96%, specificity was 98%, and total accuracy of the test was 97%. No cross-reaction with *G. lamblia* or with other common intestinal helminths or protozoa was reported. The ICT was more sensitive than microscopy; this may reflect the fact that microscopy detects intact oocysts whereas the ICT detects the antigen, which persists in recently-cured cases after oocyst shedding has stopped. Oocyst detection by microscopy is an unreliable method because oocysts vary in number from day to day and from week to week, and their numbers decrease dramatically as acute infection wanes. Further disadvantages of microscopic detection are variability in the staining products and the oocyst ghost phenomenon. On the other hand, although enzyme immunoassay also detect antigens, the cost and multiple-step procedure are limiting factors for its use in less-prepared settings.

Other authors assessed different brands of ICT kits for the diagnosis of *Cryptosporidium* and *Giardia*, the two intestinal protozoan parasites incriminated in many food- and water-borne outbreaks. The reported sensitivity and specificity ranged respectively from 68% to 98% and 99% to 100% for *Cryptosporidium* and from 81% to 97% and 99% to 100% for *Giardia* [57-61]. In summary, the ICT for giardiasis and cryptosporidiosis is a potentially useful test because of its specificity; however, sensitivity was low with some brands. The test can be incorporated into routine diagnosis and screening procedures, especially where rapid, point-of-care testing is needed as in isolated or rural areas or where other reliable tests are unfeasible. Immunochromatographic tests for screening or to investigate outbreaks can be conducted close to the site of patient care (clinics, small laboratories, inpatient wards and community settings). Moreover, ICTs can be done by non-laboratory staff or by less-experienced laboratory personnel. The test is rapid and can be performed in 10 min rather than the 1-2 hours needed to complete direct fluorescence assays or enzyme immunoassays. However, in low-prevalence populations, rapid ICT tests for giardiasis and cryptosporidiosis should not be used as a screening test or as the sole method of diagnosis due to their low sensitivity. More sensitive methods such as direct fluorescence antibody tests should be used to confirm negative results.

For the detection of *Entamoeba histolytica/dispar* infection, the Biosite Triage ICT kit has been tested. Assays with fresh-frozen stool specimens from 71 patients with gastrointestinal symptoms yielded 68.3% sensitivity and 100% specificity for the detection of *E. histolytica/dispar* compared to the Alexon-Trend ProSpecT ELISA as a reference standard. Neither test was able to distinguish *E. histolytica* from *E. dispar*. For the detection of *G. lamblia*, the Triage ICT yielded 83.3% sensitivity and 100% specificity compared to microscopy with formal/ether concentration and permanent iron hematoxylin staining techniques [62]. The authors concluded that the Triage ICT strip is highly specific for the detection of *E. histolytica/dispar* complex. However, it is less sensitive than the ProSpecT ELISA. The lower sensitivity of the ICT may be due to its limited ability to detect *E. histolytica/dispar* antigen levels ≤1000 trophozoites per milliliter of stool concentrate.

Immunochromatography for the diagnosis of other parasitic infections

Trichomoniasis is the most widely prevalent nonviral sexually transmitted disease. The number of adults infected annually is estimated to be 180 to 200 million, which is higher than all reported cases of gonorrhea, syphilis and chlamydial infection combined [63]. Despite its association with many adverse health outcomes, this treatable disease is ignored as a public health issue. Clinicians often rely upon insensitive diagnostic methods such as direct microscopic examination of vaginal smears, and culture is time-consuming and expensive. Immunochromatographic antigen-detection tests for *Trichomonas vaginalis* have been developed and evaluated against culture, microscopic examination of direct wet mounts, and PCR. The ICT kits were more sensitive than vaginal wet mount examination in detecting *T. vaginalis*. Compared to culture, sensitivity of the OSOM ICT kit was 84% and specificity was 98% [64]. The sensitivity and specificity of the Xenostrip-Tv®, an ICT-antigen detection *T. vaginalis* assay, were 66.6% and 100% respectively compared to PCR, versus 48.1% and 100% for wet mount examination [65]. In summary, ICT assays for *T. vaginalis* proved to be more effective as a screening test than wet vaginal preparations. The ICT was also able to detect both symptomatic and asymptomatic infections, and is rapid, objective, easily to interpret, and can identify *T. vaginalis* without microscopy. Thus the ICT appears to have a place in routine diagnosis and screening for *T. vaginalis* infection alongside other routinely-used techniques.

Another immunochromatographic kit was developed for the diagnosis of Chagas disease, an important parasitic disease caused by *Trypanosoma cruzi* infection. The Chagas Stat Pak test, produced with recombinant protein, showed 99.6% sensitivity and 99.9% specificity in comparison to ELISA in 5998 serum samples [66]. Other ICT kits were developed for *Toxoplasma gondii* infection. A recombinant truncated surface antigen was used to detect *T. gondii* antibodies in mice and cats. The test was validated and shown to be accurate and suitable for use under field conditions [67]. Also for veterinary use, ICT antibody-detection kits have been developed for the diagnosis of *Babesia equi* and *Babesia caballi* in horses, *Babesia gibsoni* in dogs, and *Babesia bovis* for bovine babesiosis; all showed high diagnostic accuracy [68-71].

Conclusion

In recent decades many immunochromatographic approaches have been produced for the diagnosis of various parasitic infections such as malaria, leishmaniasis, filariasis, trypanosomiasis, trichomoniasis, toxoplasmosis, intestinal parasitic protozoa, and others. Evaluations of these assays showed that ICTs are a promising tool for the diagnosis of different parasitic diseases with a reasonably reliable level of diagnostic performance [4,27,28,47-52,56,64,66,68-

71]. Moreover, ICTs are simple and rapid assays that can be completed in 10-15 min. They reduce the need for trained examiners and costly equipment. They are suitable as point-of-care diagnostic methods and can be used under harsh field conditions. However, some believe that ICT assays are not as sensitive as other immunoassays, and note that the results should be confirmed by other reference tests [5-8,14,33-35,62,65]. The consensus seems to be that ICT assays for the detection of parasitic diseases are potentially useful additional diagnostic tools that can support currently proposed strategies for the control and eradication of parasitic diseases. The judicious use of ICT assays would reduce the misdiagnosis of parasitic infections in many settings where available expertise is limited, as well as in rural and underdeveloped areas.

It is hoped that new and improved immunochromatographic assays will be developed in the near future evading the draw backs of the currently available ICTs. The most critical component of improvement lies in the affinity, specificity, and mass production of the monoclonal antibody involved [1]. Quantitative or semi quantitative ICT models are also required to measure the intensity of infection and monitor therapeutic success. Continued developments and improvements are anticipated with an increasing range of ICT applications for other parasitic diseases as well as for different diagnostic and therapeutic purposes.

Acknowledgment

I thank K. Shashok (Author AID in the Eastern Mediterranean) for improving the use of English in the manuscript.

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Austin Chromatogr - Volume 1 Issue 4 - 2014
ISSN 2379-7975 | www.austinpublishinggroup.com

Citation: El-Moamly AA. Immunochromatographic Techniques: Benefits for the Diagnosis of Parasitic Infections. Austin Chromatogr. 2014;1(4): 8.