Egyptian Journal of Medical Microbiology Volume 34 / No.1 / January 2025 195-203 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Development of Rapid Home-Made Immunochromatographic Test for Diagnosis of *Cryptosporidium Parvum*

Mohamed A. Hendawy, Ibrahim R. Aly, Rabab S. Zalat, Shaimaa S. Mohamed*
Department of Parasitology, Theodor Belharz Research Institute, Giza, Egypt

ABSTRACT

Key words:
Cryptosporidiosis -, diagnostic test-, Silver nanoparticles -Sandwich ELISA-, Immunochromatography Test (ICT)

****Corresponding Author:***
Shaimaa Shaker Mohamed
Theodor Bilharz Research Institute
Tel.: 01000774456
dr.shaimaashaker@gmail.com

Background: *Immuno-chromatographic Test (ICT) devices have emerged as valuable tools for diagnosing parasitic infections. These tests are cost-effective, user-friendly, and require minimal training, making them ideal for use in settings with limited resources. Additionally, ICT offers rapid results with high accuracy, which makes it an attractive alternative to more complex diagnostic methods.* ***Objective:*** *This study aimed to develop a homemade immune-chromatographic kit utilizing nanotechnology for detecting specific antigens of Cryptosporidium parvum in human stool samples.* ***Methodology:*** *A comparative analysis was conducted between two diagnostic methods (sandwich ELISA and ICT) using 56 human stool samples confirmed to be infected with Cryptosporidium by Ziehl–Neelsen staining. The study also included 30 stool samples infected with other parasites and 20 healthy stool samples as negative controls. Silica nanoparticles were conjugated with anti-Cryptosporidium antibodies for enhanced sensitivity in both assays.* ***Results:*** *For sandwich ELISA, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 92.9%, 91%, 91.8%, and 92.6%, respectively, for the Cryptosporidium-infected group (52 out of 56 positive). Five healthy controls and other non-Cryptosporidium parasitic infections were falsely positive. In contrast, the homemade ICT detected 54 out of 56 Cryptosporidium-infected samples as positive, with two false positives from the control group.* ***Conclusion:*** *The ICT demonstrated superior sensitivity and specificity compared to sandwich ELISA, making it a more reliable, cost-effective, and accurate tool for diagnosing Cryptosporidium infections, especially in resource-limited settings.*

INTRODUCTION

Cryptosporidiosis is a diarrheal disease characterized by loose stool, nausea and vomiting, dehydration, pain in the abdomen, and fever. In immuno-competent hosts, symptoms usually subside after 2-4 weeks. Cryptosporidiosis can occasionally manifest as pulmonary or tracheal disease, causing coughing and fever. Infection is induced by the consumption of sporulated oocytes that are transferred by the faecal-oral pathway, as well as through water and air borne. In immuno-competent humans, infections usually resolve; spontaneously, In patients with compromised immune systems, such as those with AIDS or on immunosuppressive medicine, infection may not be self-limiting, causing dehydration and, in severe circumstances, death[1] .

Although there have recently been more incidences of cryptosporidiosis recorded globally (to three cases per 100,000 people), other indicators, including clinical symptoms, suggest that the true prevalence of infection is likely 100 times greater[2].In industrialized nations, the global incidence of *Cryptosporidium* infection is considerably less prevalent in undeveloped countries given that a large number of people in the latter still lack access to basic sanitation and drinking water[3]. In developing countries, cryptosporidiosis hardly affects immune-competent people, but it causes ten to fifteen per cent of instances of severe diarrheal illness, particularly in malnourished children under five[3, 4].

Assays for detecting *Cryptosporidium parvum* antigen have been found to be highly effective in the diagnosis of intestinal parasites. These reagents give patients with low parasite levels the extra sensitivity needed to confirm infections while also providing a pertinent alternative to the standard examination procedure. However, the conventional immunoassay methods like enzyme linked immuno-sorbent assay (ELISA necessitates several reagent addition, washing, and incubation procedures[5].

Biotechnology plays a main role in the area of development of many rapid diagnostic tests for detection of several parasitic infections. Immuno-chromatography (ICT) technique is one of the fastest, most accurate, and most sensitive methods for identifying certain antibodies or antigens associated with parasite infections. Since nanomaterial have a large surface region, several target-specific molecules of

interest can adhere to them for ultrasensitive detection. Conventional approaches are constrained in their ability to achieve such high sensitivity. Furthermore, nanomaterial has unique features that could enable pathogen identification in real time and relatively tiny sample quantities[5].

Compared to currently in use technologies, The usage of nanoparticles as tags or labels allows for direct, very precise, sensitive, fast, and inexpensive detection of infectious pathogens in small volumes. This improvement in early diagnosis makes treatment precise and timely[6].

The aim of our work is development of an immuno-chromatography homemade kit for detection of specific antigens of *Cryptosporidium parvum* (CPA) in human stool samples using nanotechnology.

METHODOLOGY

Study population:

The present work was carried out on 106 individuals classified according to the parasitological examinations into three groups: Group I including 56 human stool samples infected with Cryptosporidiosis which was confirmed by Ziehl–Neelsen (ZN) stain as a gold test for diagnosis of *Cryptosporidium parvum*, goup II including 30 human stool samples infected with other parasites than Cryptosporidiosis (10 active Giardia lamblia, 10 active Entamoeba histolytica and 10 Schistosoma mansoni) in addition to 20 negative control stool samples (group III).

Sample collection and microscopic identification:

The stool samples have been obtained from patients in several regions of Egypt. All samples included negative control and other parasites than *Cryptosporidium parvum* were used for two faecal smear tests: Formalin ethyl acetate concentration method (MIFC) 7 and modified ZN acid fast stain8.

Parasitological examination:

The samples were preserved and concentrated using the MIFC method. The following steps were carried out, in a beaker, one millilitres of stock solution B (potassium iodide solution 10%) and four millilitres of stock solution A (merthiolate 0.1%, formaldehyde-glycerine solution 36–40%) were combined. A half g (1-2 ml) of stool was added and the specimen was mixed by vigorous shaking for a few seconds. This mixture was strained through domestic tea sieves into a 15 mL centrifuge tube. To the centrifuge tube, 7 ml of cold ether (stored in the refrigerator at 4°C) were added. The stopper was inserted, and the tube was shaken firmly. (If any ether remained on top after shaking, add 1 ml tap water and the tube shaken again). The stopper was removed, and the tube was let to stand for two minutes. Then the tube was centrifuged for 5 minutes at 3000 rpm. After centrifugation, the tube had four layers: an ether layer on top, a plug of faecal debris, a MIF layer, and silt containing helminthic eggs and protozoa cysts. The faecal blockage was carefully removed from the tube wall with a wooden applicator stick, and all layers except the bottom layer of silt were drained out. Following the mixing of the sediment, a drop was put on a slide, covered, and inspected under a microscope.

Acid-fast staining of faecal oocytes:

Faecal smears were stained using an acid-fast staining kit (Medical Industries Inc., Las Vegas, Nev.), following a modified Ziehl- Neelsen technique[9]. After staining, the smears were examined under a microscope, and *Cryptosporidium* oocytes were counted in 100 high-power fields. The number of oocytes per mg of sample was then calculated.

Preparation of silica nanoparticles:

Colloidal silica spheres with a variable ethanol to water ratio were produced using a modified version of Sato-Berrú[10]. The materials that were utilized were tri-distilled water, ethanol (99.99%), tetraethyl orthosilicate (TEOS) (99.99%, Aldrich), and ammonium hydroxide solution (28%, Aldrich). The silica spherical nanoparticles were prepared by mixing 3.5 ml of ammonium hydroxide solution, 1.5 ml of TEOS, (50-x) ml of ethanol, and x ml of water (x = 0,1, 5, 10, 15, 20, 30, 40, and 45). For one hour, thin stirred to create a homogenous mixture. Every experiment was conducted at room temperature. Once SiO2 nanoparticles began to form, the reaction medium began to take on a light blue tinge. The samples were characterized using dynamic light scattering and transmission electron microscopy. A JEOL JEM-200CX transmission electron microscope with a 60 kV acceleration voltage was used to conduct TEM testing. Samples were moved onto copper grids coated with carbon before being observed, and they were left to dry for 24 hours at room temperature. DLS studies using a Nano-seizer system (model ZS90) were conducted. The antibodies used as the capture antibody were put on a nitrocellulose membrane. A colloidal dye-labelled antibody conjugate is moved by capillary flow, and as it approaches the capture antibody, it attaches to the target antigen. Capturing the moving labelled antigen/antibody combination in the test region with a second anti-species antibody, together with the creation of a coloured line, indicates a positive test. As a control line, an extra control antibody to the combination attaches the excess colloidal dye conjugate. A test's validity can be determined by looking at the control line.

Synthesis of nitrocellulose membranes

Strips of 2.5 x 30 cm^2 were cut from nitrocellulose membranes (FF80HP blocked type, capillary rise 60-100 s/4 cm, total calliper 200 µm). Human immunoglobulin G (Invitrogen, A18805), human immunoglobulin M (Invitrogen, A18835), and rabbit immunoglobulin G (Merck, 12-348) antibodies were each diluted to 1 mg/mL in 0.01 M PBS, pH 7.4. And the control line, in that order.

The liquids were poured onto the membranes at a rate of 1 µL/cm, allowing a 0.3 cm space between the testing and control lines. In an oven that had been warmed to 37°C, the strips were allowed to air dry Fig (1).

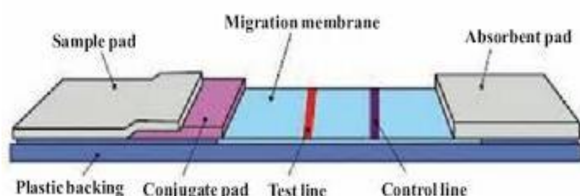

Fig. 1: The nitrocellulose membranes

Immuno-chromatographic test (ICT).
Construction of the Nano chip

The conjugate pad was positioned on a 300 x 80 mm plastic baking plate, has a 1-2 mm overlap in the membrane's bottom. The bottom of the conjugate pad was then attached to the sample pad in a similar way. The absorbent pad was attached with a 1-2 mm overlap to the upper side of the membrane. A CM 4000 Cutter (Bio-Dot, CA, USA) was used to cut the prepared master card into 3.8 mm-wide strips. After that, the strips were placed into the plastic container and sealed within the desiccant gel-filled aluminium foil bag. After that, they were stored at room temperature and kept dry until needed. Figure 1 presented a demonstration of the test strip concept. The test strips are flatwise oriented. Samples of diluted stool (80–100 µL) were put into the test strip cell's sample holder and allowed to move for five minutes. The specific anti-CPA antibody interacted with the cryptosporidium (if any were present in the samples) upon dissolving off the conjugate pad. It had a nano-graphine tag on it. As the complex moved down the membrane, more antibodies tagged with nanographine were drawn in by the BSA-CPA that was fixed on the membrane, creating red test lines. The control line was then produced by the goat anti-human IgG antibodies attracting these excess antibodies. After three to ten minutes, the test findings were assessed visually or by using a Bio-Dot TSR3000 Membrane Strip Reader (Bio-Dot, CA, USA) to scan the test lines.

Detection of CPA Using Sandwich ELISA
Assay Principle

In the current study, sandwich ELISA was created and employed to qualitatively assess stool sample *Cryptosporidium parvum* antigen. The assay uses the microplate-based enzyme immunoassay technique, in which the wall of a microtiter well is coated with highly purified antibodies [11]. A hundred microliters per well of pure paraffin wax pellets (1/100 for IgG diluted in 0.06 M carbonate buffer, pH 9.6). After applying the solution on polystyrene microtiter plates, they were left to rest at room temperature for the entire night. Three washing cycles in 0.1 M PBS/T, pH 7.4, were performed on the plate. PBS was used to individually dilute stool samples that tested positive for Cryptosporidium or other parasites, as well as free, non-infected samples, 1:3.200 µl/well of blocking solution (0.1% BSA in 0.1 M PBS/T) was used to block the free sites, and the mixture was incubated for two hours at 37°C. Three washing buffer washes were performed on the plate. Faecal supernatant samples (100µl/well) were added to each well, and the plate was washed three times with washing buffer after the samples were incubated for two hours at 37°C. Each well was filled with 100µl of peroxidase-conjugated pAb at a dilution of 1/50 for IgG, and the plates were incubated for one hour at 37°C.Washing buffer was used five times to wash the plate. One tablet of O-phenylene diamine dihydrochloride (OPD) (Sigma) should be dissolved in 25 millilitres of 0.05 M phosphate citrate buffer, pH 5, to create the substrate solution. Next, peroxidase H202 (Sigma) should be added. Following half-hour incubation in darkness at ambient temperature, 50 microliters of 8 N H2SO4 were added to each well to terminate the enzyme substrate solution. With the use of an ELISA reader (Bio- Rad micro plate reader, Richmond, CO), the absorbance was determined at 492 nm.

Preparation of antigens:

Initially identified from a calf, *Cryptosporidium parvum* oocytes had been found in the excrement of sick calves. Ethyl acetate (Sigma Chemical Co., St. Louis, Mo.) was added to excrement suspended in phosphate buffered saline (PBS) (pH 7.2) at a dilution of 1:10 before the mixture was centrifuged at 350 x g to remove faecal fat. Twice, distilled water was used to clean the pellet. The preparation was centrifuged at 450 x g for 20 minutes after being stacked over a discontinuous gradient of sucrose with three densities (1.18, 1.09, and 1.02 g/ml)[12].

To eliminate contaminating microorganisms, the oocyte slurry was placed in a stirred cell with a 3-um-pore size polycarbonate membrane filter (Nuclepore Corp., Pleasanton, Calif.). Bacteria could pass through the filter with enough agitation, and the oocyte suspension was microscopically free of both bacteria and faeces.

After centrifugation at 500 x g and discarding the supernatant, the pellet was suspended at a concentration of 2 x 107 oocytes per millilitre PBS[12].

Parasite antigen purification and characterization[13]:

Parasep filter faecal concentrator tubes system, was utilized to purify *Cryptosporidium parvum* antigen because it captures waste and unwanted particles from stool specimens keeping them from pushing through the centrifugation process and into the sedimentation cone[14]. Demonstration of the use of a Bio-Rad protein

assay kit (Bio-Rad, Richmond, CA, USA) allowed for the subsequent determination of the protein content. Lastly, the molecular weight of the isolated *Cryptosporidium parvum* antigen was ascertained using SDS PAGE[15].

Evaluation of Reactivity (Indirect ELISA used to purify antigen):

The original method by Engvall and Perlman[16] was modified somewhat to employ this technique.

Animal:

A male New Zealand white rabbit, about two months old and weighing around 2.0 kg, was bought from Cairo University's Faculty of Agriculture's Rabbit Research Unit (RRU). In order to produce the antibodies[17].

Manufacturing and Handling of Polyclonal Antibodies:

Before being injected, rabbit sera were examined using ELISA to check for Cryptosporidium antibodies and cross-reactivity with other parasites. Rabbits were given intramuscular (IM) injections of 1 mg of pure antigen diluted 1:1 in complete Freund adjuvant (CFA)[18].

Two weeks after the first injection, two booster doses were given, each containing 0.5 mg of antigen blended in an equivalent volume of incomplete Freund adjuvant (IFA).The rabbits' sera were collected one week following the final booster dose, and the polyclonal antibodies fraction was purified using a 50% ammonium sulphate precipitation technique[19]. Additional polyclonal antibody purification was carried out using the 7% caprylic acid technique [20].To get rid of any non-specific interaction with the bovine antigen, the refined polyclonal antibody was subsequently adsorbed with foetal calves' serum (FCS). Using indirect ELISA, the anti-Cryptosporidium IgG polyclonal antibody's reactivity against purified antigen was evaluated[18].

Labelling of rabbit anti-*Cryptosporidium* serum IgG polyclonal antibody with Horseradish Peroxidase (HRP) (Periodate Method) [21, 22]:

5 mg HRP (Sigma) were suspended again in 1.2 ml of distilled water, then added 0.3 ml of freshly produced sodium periodate and incubated at room temperature for twenty minutes. At 4°C, the HRP solution was dialyzed against a 1 mM sodium acetate buffer (pH 4), and multiple overnight adjustments were made. A pH 9.6 solution containing 5 mg/ml of IgG polyclonal antibody was produced using 0.02M carbonate buffer. After taking HRP out of the dialysis tube, 0.5 mL of antibody solution was added. For two hours, the mixture was incubated at room temperature. After adding 100 µl of sodium borohydride, the resulting mixture underwent incubation for two hours at 4°C. Several adjustments were necessary in order to dialyze the HRP conjugate polyclonal antibody in 0.01 M PBS (pH 7.2).

Ethics approval and consent to participate:

All procedures followed were in accordance with the ethical standards of the ethics committee of Theodor Bilharz Research Institute (under Federal Wide Assurance No. FWA00010609) and with the Helsinki Declaration of 1975, as revised in 2008.

Statistical analysis:

A number (%) or the mean ± standard deviation (SD) will be utilized for representing the data. To establish correlations among various elements, the Spearman's rank correlation factor will be utilized. For data analysis, the SPSS computer program (version 13 Windows) will be utilized. We'll express the data as mean ± standard deviation[23]

RESULTS

Purification and characterization by gel filtration chromatography:

Following the extraction of cryptosporidium antigen (CPA) from the pellet supernatants, gel filtration chromatography was used to successfully purify the material. Figure (2) shows the OD280 profile of the antigen fractions obtained following purification using gel filtration chromatography. The antigen that has been eluted is shown by four peaks, with the greatest OD value of 1.25 at fraction number 11, which corresponds to the section with the maximum protein concentration. SDS-PAGE was used to examine the eluted proteins (Fig. 3). The first peak contained most of the refined CPA antigen. Which was also the void volume, but there was also a small band that eluted in the second peak of the chromatogram (Fig. 3).

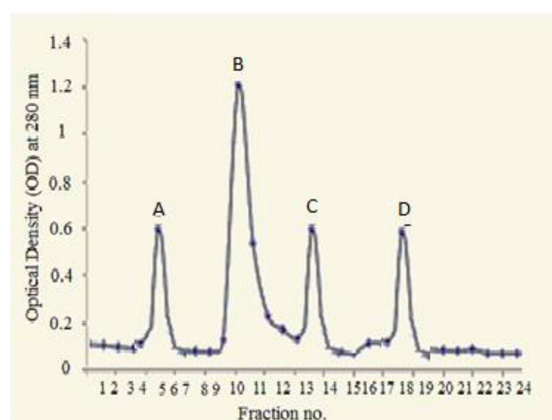

Fig. 2: Elute profile for the purified CPA antigen by gel filtration chromatography

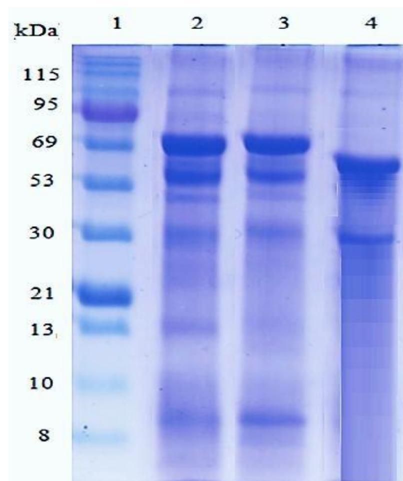

Fig. 3: SDS-PAGE of target antigens eluted from affinity chromatography columns
Lane 1: Low molecular weight standard
Lane 2: Crude CPA
Lane 3: Crude CPA after culture
Lane 4: Purified CPA antigen eluted from sephacryl S-200.

Manufacturing and Handling Polyclonal Antibodies:

Before each injection of the vaccination dose, Blood samples were collected from New Zealand white rabbits to be tested by indirect ELISA, to determine whether they had any particular anti-cryptosporidium antibodies. One week following the initial booster dose, a rising antibody level was seen. Immune sera revealed a significant titer against cryptosporidium membrane antigen three days after the second booster dosage, with an OD492 of 2.97 at 1/250 dilution (Fig. 4).

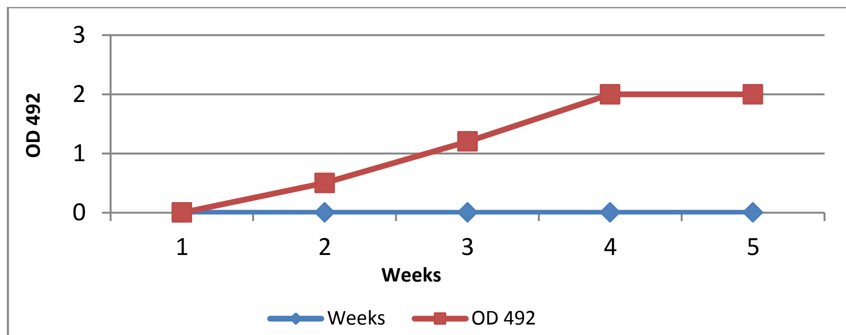

Fig. 4: Reactivity of immunized rabbit anti-*CPA* antisera by indirect ELISA

Standardization of sandwich-ELISA

The anti-CPA IgG polyclonal antibody was utilized to detect CPA in stool samples. It was used as an antigen capture agent in sandwich ELISA, with HRP polyclonal antibody as a conjugate. Before using the sandwich ELISA technique to human feces samples, it was used to standardize and optimize various material concentrations and dilutions. The optimum concentrations of the coating antibody and conjugate anti- CPA polyclonal antibody were evaluated by coating an ELISA plate with different concentrations of purified anti- CPA antibody (5, 10, 20 and 40 µg/ml) against three different concentration of CPA antigen (5, 5 and 20 µg/ml) to specify the optimum concentration of the coating antibodies to be used. By OD readings at 492 nm, the optimum concentration of coating antibodies was 20 µg/ml.

Concentrations of HRP conjugated rabbit anti-CPA IgG polyclonal antibody were also evaluated to indicate the ideal coating antibody concentration that should be applied. An ELISA plate was coated with three different concentration of CPA antigen (5, 10 and 20, 30, 40 ug/ml) and different concentrations of the conjugate antibodies (5, 10, 20, 40 and 80µg/ml) were added. By OD readings at 492 nm, 40 µg/ml was the ideal concentration of HRP-conjugated antibodies Table (1).

Table 1: Detection of CPA antigen in stool samples Using Sandwich ELISA

GROUPS	Positive cases		Negative cases	
	(n.)	OD (X+ SD)	(n.)	OD (X+ SD)
Healthy control(n= 20)	-	-	30	0.321±0.02
Cryptosporidium (n= 56)	52	0.995±0.12	4	0.109±0.05
Giardia (n= 10)	2	0.474±0.03	8	0.217±0.06
Ent. Hist (n=10)	1	0.643±0.06	9	0.188±0.04
Schistosoma (n=10)	0	-	10	0.194±0.08

Utilizing a homemade quick card to identify CPA 54 (96.4 %) cases gave positive results in *Cryptosporidium* group as shown in Figure (5), the other 2 cases gave false negative results (3.6 %), were negative in stool of patients with light infection group with *cryptosporidium* cases. Four patients were giving false positive within other parasite infection group. Every healthy control patient had a negative test result (Table 2).

Fig. 5: ICT for diagnosis of *cryptosporidium*

Table 2: Detection of CPA Using Homemade ICT

GROUPS	Positive cases		Negative cases	
	(N)	-/+	(N)	-/+
Healthy control(n= 20)	0	-	20	--
***Cryptosporidium* (n= 56)**	54	++	2	--
***Giardia* (n= 10)**	2	+	8	--
***Ent. Hist* (n=10)**	2	+	8	--
***Schistosoma* (n=10)**	0	+	10	--

52 samples out of 56 in the group infected with cryptosporidiosis tested positive using sandwich ELISA; five samples out of 50 in the healthy control group and samples containing parasites other than cryptosporidiosis tested positive. The sandwich ELISA's sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 92.9 per cent, 90%, 91.8 per cent, and 92.6 per cent, respectively. Using novel home-made ICT 54 out of 56 were positive in cryptosporidiosis infected group, while 2 out of 50 were false positive in healthy control and other parasites than cryptosporidiosis group giving sensitivity, specificity, PPV and NPV 96.4 %, 92.0 %, 93.3 %, and 96.1%, respectively as shown in Table (3).

Table 3: Sensitivity and specificity of sandwich ELISA and ICT in stool specimens

	The sensitivity	The specificity	PPV	NPV
Sandwich ELISA	92.9 %	90 %	91.8 %	92.6 %
ICT	96.4 %	92.0 %	93.3 %	96.1%

DISCUSSION

Patients, especially those with HIV illness, pass different amounts of *Cryptosporidium parvum* oocytes on different days and weeks. Specifically, the most popular way for detecting *Cryptosporidium* in stool samples is the use of microscopic techniques; the proficiency of the microscopist plays a major role in the diagnostic accuracy of these procedures.

The diagnostic methods for parasites are not always very sensitive, especially when the patient is still in the acute phase of the illness or has a minor infection. This can lead to an inaccurate estimate of the frequency and severity of infection.

In patients suffering from severe diarrhoea, oocytes might be difficult to identify, therefore, in order to rule out a *Cryptosporidium* infection, three samples of stool collected on different days should be analysed under a microscope[24]. Furthermore, prior to microscopic inspection, to discover oocytes, the stool samples must be condensed using the formalin-ether sedimentation method[25].

Furthermore, fluid-like or soft faeces could be examined for the presence of cryptosporidiosis using a number of techniques, including the immuno-chromatographic test (ICT) and the enzyme-linked immuno-sorbent (ELISA), which have high sensitivity and specificity for the identification of Cryptosporidium antigens [26, 27].

Assays for detecting *Cryptosporidium parvum* antigens have shown to be highly effective in diagnosing intestinal parasites. These reagents give patients with low parasite levels the extra sensitivity needed to confirm infections while also providing a

pertinent alternative to the standard examination procedure.

Traditional immunoassay techniques, however, call for several reagent addition, washing, and incubation phases. Comparable to other conventional traditional methods, such as staining and microscopic approaches, current immuno-chromatographic technology offers novel diagnostic options such as modified acid-fast stains and trichrome, direct fluorescence antibody testing, and enzyme immunoassays for antigen detection.

Detection of *Cryptosporidium parvum* antigen (CPA) in stool has many advantages; It is non-invasive, can reveal anti-fecundity immunity, and its presence signals current infections. Furthermore, CPA detection may be used for both morbidity evaluation and diagnosis.

In the present study, all human samples included negative controls and other parasites than *Cryptosporidium parvum* (CP) were used for two fecal smear tests: Formalin ethyl acetate concentration method (MIFC) [28] and modified ZN acid fast stain method [8]**.** All human stool samples infected with cryptosporidiosi*s* were confirmed by Ziehl–Neelsen (ZN) stain as a gold test for diagnosis of *Cryptosporidium parvum*.

In this study, sandwich ELISA showed a sensitivity of 92.8%, detecting *Cryptosporidium* in 52 out of 56 infected individuals. However, four individuals with other parasitic infections tested falsely positive, resulting in a specificity of 90.0%, with no false positives in the healthy control group. Using ICT for *Cryptosporidium* detection, the sensitivity was higher at 96.4%, with 54 out of 56 infected patients testing positive. Two patients with mild infections gave false negative results, and four patients with other parasitic infections tested falsely positive, giving a specificity of 92.0%. All healthy controls tested negative.

These findings suggest that while both methods are effective, ICT offers slightly higher sensitivity. However, ICT may be more prone to false positives in co-infected patients, which should be considered when choosing the best diagnostic method.Compared to currently in use technologies, then used as tags or labels, nanoparticles allow the directed, very precise, sensitive, fast, and inexpensive detection of infectious pathogens in small volumes. This improvement in early diagnosis makes treatment precise [6]. Agreement to those authors, It was observed that the introduction of nanoparticles greatly improved the technique's sensitivity, specificity, and incidence of positive toward a higher detection of CPA in patients with mild and moderate infections. Detection of CPA was higher in both sensitivity and specificity than its detection using traditional sandwich ELISA.

The present study showed that the sensitivity and the specificity values of ICT were 96.4% and 92.0 %. Respectively compared with sandwich ELISA results where the sensitivity and the specificity values were 92.8% and 90.0% respectively.

Our findings corroborated those of [29] who assessed a novel marked immuno-chromatographic technique for Cryptosporidium identification. According to their findings, this test may offer a quick and trustworthy substitute for the modified Ziehl- Neelsen staining, which has a sensitivity and specificity of 86.7% and 100%, respectively, in the diagnosis of cryptosporidiosis. By eliminating the need for wet mount microscopy and concentration processes, this tactic would also save time. It is especially suitable for labs lacking in molecular diagnostic capabilities or with little microscopy experience.

Although diagnostic technologies for the identification of intestinal protozoa have advanced significantly (for example, multiplex PCR assays are now available), some labs have limited access to this molecular technology, while others do not have it at all. Additionally, their use has been restricted because to their expense and the requirement for technical expertise, particularly in high-prevalence areas like developing nations [30].

Immunocompromised individuals can develop life-threatening, long-lasting infections from the parasite. Therefore, cryptosporidiosis is one of the most serious opportunistic infections for people with acquired immunodeficiency syndrome. Implementing sensitive and reliable diagnostic tests for epidemiological surveillance and morbidity reduction is the best strategy to control the infection in these patients [30].

Kits for antigen detection are commercially available for enzyme-linked immuno-sorbent assay (ELISA) and immuno-chromatographic (IC) formats. However, the kit's sensitivity may be low in some circumstances, depending on the antibody employed. On the other hand, it can analyse a large number of samples quickly and has a good specificity of 98%–100% [31]. The ICT assay is a widely used technique with quick findings that is used in many laboratories. The sensitivity is said to be somewhat low, but the specificity is said to be excellent (98%–100%) [31], which agreed with our results.

In addition to being sensitive, the usage of nanoparticles in ICT for CPA detection can lessen the potential for cross-reactivity among samples from cases that have additional parasite infections, making the sample more valuable for determining treatment efficacy.

CONCLUSION

Compared to existing technologies, the use of nanoparticles as labels or tags allows for the direct, accurate, sensitive, and quick identification of infectious microorganisms in tiny volumes at a lower cost. They

can also be used in large-scale surveys to diagnose parasite infection.

Acknowledgment:. The work is based upon an internal research project (grant No. 108 D) supported by Theodor Bilharz Research Institute (TBRI).

Authors' contributions: All authors contributed to the article and approved the submitted version. The authors contributed equally to this work; it was a group effort.

Competing interests: All authors report no conflict of interest. “No financial or non-financial benefits have been received or will be received from any party related directly or indirectly to the subject of this manuscript.

REFERENCES

1. Sponseller JK, Griffiths JK, Tzipori S. The evolution of respiratory cryptosporidiosis: evidence for transmission by inhalation. Clinical microbiology reviews. 2014; 27(3), 575-58.
2. Shrivastava AK, Kumar S, Smith WA, Sahu PS. Revisiting the global problem of cryptosporidiosis and recommendations. Tropical parasitology. 2017; 7(1), 8-17.
3. Bouzid M, Kintz E, Hunter PR. Risk factors for *Cryptosporidium* infection in low and middle income countries: A systematic review and meta-analysis. PLoS neglected tropical diseases. 2018; 12(6), e0006553.
4. Shoultz DA, de Hostos EL, & Choy RK. Addressing *Cryptosporidium* infection among young children in low-income settings: the crucial role of new and existing drugs for reducing morbidity and mortality. PLoS Neglected Tropical Diseases. 2016; 10(1), e0004242.
5. Aly I, Zalat R, El Aswad BEDW, Moharm IM, Masoud BM, Diab T. Novel nanomagnetic beads based-latex agglutination assay for rapid diagnosis of human schistosomiasis *haematobium*. International Journal of Medical and Health Sciences. 2014; 7(12), 977-983.
6. Banerjee R, Jaiswal A. Recent advances in nanoparticle-based lateral flow immunoassay as a point-of-care diagnostic tool for infectious agents and diseases. Analyst. 2018; 143(9), 1970-1996.
7. Blagg W, Schloegel EL, Mansour NS, Khalaf GI. A new concentration technic for the demonstration of protozoa and helminth eggs in feces. American Journal of Tropical Medicine and Hygiene. 1955; 4(1), 23-8.
8. Stenzel DJ, Boreham P. *Blastocystis hominis* revisited. Clinical microbiology reviews. 1996; 9(4), 563-584.
9. Garcia LS, Shimizu RY., Novak, S., Carroll, M., & Chan, F. Commercial assay for detection of *Giardia lamblia* and *Cryptosporidium parvum* antigens in human fecal specimens by rapid solid-phase qualitative immunochromatography. Journal of clinical microbiology. 2003; 41(1), 209-212.
10. Sato-Berrú, R, Saniger JM, Flores-Flores J, Sanchez-Espíndola M. Simple method for the controlled growth of SiO2 spheres. Journal of Materials Science and Engineering. A. 2013; 3(4A), 237.
11. Espino AM, Finlay C. Sandwich enzyme-linked immunosorbent assay for detection of excretory secretory antigens in humans with fascioliasis. Journal of Clinical Microbiology.1994; 32(1), 190-193.
12. Arrowood MJ, Donaldson KIMBERLEY. Improved purification methods for calf-derived *Cryptosporidium parvum* oocysts using discontinuous sucrose and cesium chloride gradients. J Eukaryot Microbiol. 1996; 43(5):89S–89S.
13. Zeeshan M, Zafar A, Saeed Z, Irfan S, Sobani ZA, Shakoor S, et al. Use of “Parasep filter fecal concentrator tubes” for the detection of intestinal parasites in stool samples under routine conditions. Indian Journal of Pathology and Microbiology. 2011; 54(1), 121-123.
14. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry. 1976; 72(1-2), 248-254.
15. Thaumaturgo N, Vilar MM, Edelenyi R, Tendler M. Characterization of Sm14 related components in different helminths by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and Western blotting analysis. Memórias do Instituto Oswaldo Cruz. 2002; 97, 115-116.
16. Engevall E, Perlmann P. ELISA, quantitative assay for IgG. J. Immunohistochem. 1971; 8, 871–874.
17. Abdel-Rahman EH, Abdel-Megeed KN. *Fasciola gigantica*: immunization of rabbits with proteins isolated from coproantigen. J Egypt Soc Parasitol. 2004; 34(2):631-642.
18. Fagbemi BO, Obarisiagbon IO, Mbuh JV. Detection of circulating antigen in sera of *Fasciola gigantica*-infected cattle with antibodies reactive with a Fasciola-specific 88-kDa antigen. Veterinary parasitology. 1995; 58(3), 235-246.
19. Nowotny A, Nowotny A. "Ultramicrodetermination of Reducing Carbohydrates." (1979): 173-175.
20. McKinney MM, Parkinson A. A simple, non-chromatographic procedure to purify immunoglobulins from serum and ascites fluid.

Journal of immunological methods. 1987; 96(2), 271-278.

21. Nakane PK, Kawaoi A. Peroxidase-labeled antibody a new method of conjugation. Journal of Histochemistry & Cytochemistry.1974; 22(12), 1084-1091.
22. Tijssen P, Kurstak E. Highly efficient and simple methods for the preparation of peroxidase and active peroxidase-antibody conjugates for enzyme immunoassays. Analytical biochemistry.1984, 136(2), 451-457.
23. Zane HD. Laboratory safety and test quality assurance. Immunology: theoretical and practical concepts in laboratory medicine. Saunders WB, Philadelphia. 2001; 193-207.
24. Khurana S, Chaudhary P. Laboratory diagnosis of cryptosporidiosis. Tropical parasitology. 2018; 8(1), 2-7.
25. Pacheco FT, Silva RK, Martins AS, Oliveira RR, Alcântara-Neves NM, et al. Differences in the detection of Cryptosporidium and Isospora (Cystoisospora) oocysts according to the fecal concentration or staining method used in a clinical laboratory. The Journal of parasitology. 2013; 99(6):1002-8.
26. Agnamey P, Sarfati C, Pinel C, Rabodoniriina M, Kapel N, Dutoit E, et al. Cryptosporidium National Network. Evaluation of four commercial rapid immune-chromatographic assays for detection of Cryptosporidium antigens in stool samples: a blind multicenter trial. Journal of Clinical Microbiology. 2011; 49(4), 1605-1607.
27. Hawash Y. Evaluation of an immunoassay-based algorithm for screening and identification of *Giardia* and *Cryptosporidium* antigens in human faecal specimens from Saudi Arabia. Journal of parasitology research, 2014.
28. Young KH, Bullock SL, Elvin DM, Spruill CL. Ethyl acetate as a substitute for diethyl ether in the formalin-ether sedimentation technique. J. clin. Microbiol. 1979; 10: 852-853.
29. Goudal A, Laude A, Valot S, Desoubeaux G, Argy N, Nourrisson C, et al. Rapid diagnostic tests relying on antigen detection from stool as an efficient point of care testing strategy for giardiasis and cryptosporidiosis? Evaluation of a new immune-chromatographic duplex assay. Diagnostic microbiology and infectious disease. 2019; 93(1): 33-36.
30. Gerace E, Presti VDML, Biondo C. *Cryptosporidium* infection: epidemiology, pathogenesis, and differential diagnosis. European Journal of Microbiology and Immunology. 2019; 9(4), 119-123.
31. Khurana S, Chaudhary P. Laboratory diagnosis of cryptosporidiosis. Trop Parasitol. 2018; 8(1):2-7.