IN THE NAME OF GOD

STANDARDIZATION AND COMPARISON OF DOT-ENZYME LINKED IMMUNOABSORBENT ASSAY (DOT-ELISA) WITH INDIRECT FLUORESCENT ANTIBODY TEST (IFA) FOR DIAGNOSIS OF HUMAN TOXOPLASMOSIS

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MY DEAR FAMILY

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ABSTRACT

STANDARDIZATION AND COMPARISON OF DOT-ENZYME LINKED IMMUNOABSORBENT ASSAY (DOT-ELISA) WITH INDIRECT FLUORESCENT ANTIBODY TEST (IFA) FOR DIAGNOSIS OF HUMAN TOXOPLASMOSIS

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Dot-ELISA is a solid phase diagnostic method for detection of antigen or antibody that is used widely for diagnosis of protozoan and metazoan diseases of human and animals.

To evaluate this method in diagnosis of human toxoplasmosis, the test was standardized, using golden positive and negative serum samples. Then 215 human serum samples were evaluated for IgG and IgM against *Toxoplasma gondii* by IFA and Dot-ELISA. Using statistic program, *Epiinfo 6.0* showed that these tests have a good agreement in diagnosis of toxoplasmosis with Kappa = 0.8607 for IgG and Kappa = 0.8865 for IgM

antibodies ($P<0.05$).

This study and the works carried out by the other scientists indicate that the Dot-ELISA test is rapid, simple, and cost effective, does not need expensive equipment and has a good sensitivity and specificity. However, as the results are expressed qualitatively, therefore, it is not possible to use for antibody titer determination. Yet, it is quite useful for screening test especially in the field and where there are no well-equipped laboratories.

Key words: Dot-ELISA, IFA, toxoplasmosis.

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ABBREVIATIONS

4C1N: 4-chloro-1-naphtol

AIV: avian influenza virus

BCIP: 5-bromo-4-chloro-3-indol phosphate

BRSV: Bovine Respiratory Syncytial Virus

BSA: Bovine Serum Albumin

CDC: canine distemper virus

CF: Complement Fixation Test

CPV: canine parvovirus

CSF: cerebrospinal fluid

DAB: 3,3-diaminobenzidine

DI: dot immunoperoxidase assay

ELISA: Enzyme Linked Immunoabsorbent Assay

ESA: Excretory/Secretory Antigens

HRP: Horseredish peroxidase

IFA: Indirect Fluorescent Antibody Test

IH: Indirect Hemagglutination Test

LA: Latex Agglutination Test

MAT: Modified Agglutination test

PCR: polymerase chain reaction

CHAPTER 1

INTRODUCTION

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Toxoplasma gondii is a coccidian parasite of the cat and this infection may lead to major public health problems due to its potential capacity for transmission to man. Most human infections are subclinical and even clinical infections are rarely fatal; however, in pregnant women the organism may cross the placenta and infect the fetus with serious consequences.

Man frequently becomes infected by ingesting semi cooked or row meat containing *Toxoplasma* cysts and by ingestion of oocyst from cat feces.

The serological prevalence of toxoplasmosis infection approximates 30% in adults. Infection rates differ geographically.

Clinical signs include encephalitis, hydrocephaly, lymphadenopathy, myocarditis, myositis, pneomonitis, retinochoroiditis, and uveitis.

Toxoplasmosis is a danger to immunosuppressed persons such as organ transplant patients; sever cases of disease in this group and in cancer patients have been serologically recognized and may result from

development of defects in cellular immunity, which allow reactivation of latent infection.

Since the diagnosis of clinical toxoplasmosis is often difficult, serologic procedures have been used to aid in diagnosis. The two most used assays, IFA test and ELISA, require expensive fluorescence microscopes or photometers, also IFA require highly trained technicians and is subjective.

The dot enzyme-linked immunosorbent assay (Dot-ELISA) is a highly adaptable solid-phase immunoassay for antibody or antigen detection. The test uses small amounts of reagent dotted onto solid surfaces such as nitrocellulose and other paper membranes, which avidly bind proteins. After incubation with antigen-specific antibody and enzyme-conjugated anti-antibody, the addition of a precipitable, chromogenic substrate causes the formation of a colored dot on the solid phase, which is visually read.

The Dot-ELISA has been used extensively in the detection of human and veterinary protozoan and metazoan parasitic diseases, including amebiasis, babesiosis, fascioliasis, cutaneous and visceral leishmaniasis, cysticercosis, echinococcosis, malaria, schistosomiasis, toxocariasis, toxoplasmosis, trichinosis, trypanosomiasis and even ixodid tick infestation. The technique is rapid, easy to perform, cost effective and field portable. In addition, the Dot-ELISA may be configured to detect antibodies or parasite antigen in microtiter plates either for large-batch testing or with dipsticks for small numbers of determinations. As Dot-ELISA is visually read and can be used in field condition we decided

to standardize this test for diagnosis of human toxoplasmosis.

This study is aimed to:

1. Prepare soluble antigen of RH strain of *Toxoplasma gondii*
2. Standardize Dot-ELISA for diagnosis of human toxoplasmosis
3. Comparison and evaluation of two used assays, Dot-ELISA and IFA

CHAPTER 2

SILENT FEATURE

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2.1. *Toxoplasma gondii* (Nicolle and Manceaux 1908)

Toxoplasma gondii is an intestinal coccidium of felids with a wide range of intermediate hosts. Infection by this parasite is widespread in many warm-blooded animals, including man . The name of *Toxoplasma* (toxon=arc, plasm=form, Greek) is derived from its crescent shape. Nicolle and Manceaux first discovered *Toxoplasma gondii* in 1908 in a rodent, *Ctenodactylus gundi* (21).

In 1923, Janku described *Toxoplasma gondii* in the retina of a hydrocephalic baby, but the role of the parasite as a human pathogen was not widely known until Wolf and Cowen reported congenital Toxoplasmosis in man. Their report encouraged considerable interest in human toxoplasmosis and, within five years, Sabin had characterized the clinicoparasitological aspects of congenital toxoplasmosis. Pinkerton and Weinman reported the first known cases of fatal toxoplasmosis in adult human patients (21).

Toxoplasma gondii is among the most prevalent chronic parasitic