

***TOXOPLASMA GONDII* INFECTION IN DONATED BLOOD AT THE
NATIONAL BLOOD TRANSFUSION SERVICE IN KENYA.**

BY

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degree of Master of Medicine in Internal Medicine.**

DEDICATION

This book is dedicated to God Almighty for bringing me this far and to my mother Rosemarie Wambua for her continued guidance and support.

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ABBREVIATIONS:

AIDS	Acquired Immune Deficiency Syndrome
CD4	Cluster Differentiation 4
CNS	Central Nervous System
CSF	Cerebral Spinal Fluid
DNA	Deoxyribonucleic Acid
DOCMT	Department of Clinical Medicine and Therapeutics
ELISA	Enzyme Linked Immunosorbent Assay
Hep B	Hepatitis B
Hep C	Hepatitis C
HIV	Human Immunodeficiency Virus
IFAT	Immunofluorescence Antibody Test
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ISAGA	Immunosorbent Agglutination Assay
KAIS	Kenya Aids Indicator Survey
KNH /UON ERC	Kenyatta National Hospital/ University of Nairobi, Ethical Research Committee
MB. Ch. B	Bachelor of Medicine and Surgery
M.Med	Master of medicine
NHANES	National Health and Nutrition Examination Survey
NBTS	National Blood Transfusion Services
NEG	Negative

PCR	Polymerase Chain Reaction
POS	Positive
PI	Principal Investigator
RBTC	Regional Blood Transfusion Centre
<i>T. gondii</i>	<i>Toxoplasma gondii</i>
TMB	Trimethylene Blue
UNITID	University of Nairobi, Institute of Tropical and Infectious Diseases
VDRL	Venereal Disease Research Laboratory
WHO	World Health Organisation

Abstract

Introduction

Toxoplasmosis is a zoonotic disease caused by the protozoan parasite *Toxoplasma gondii*, an obligate intracellular parasite. This parasite infects a wide range of animals, humans, other mammals and various bird species.

Toxoplasmosis is of significant clinical importance in pregnant women and in immunodeficient individuals. An active infection in pregnancy portends a risk of congenital malformations and chronic carriage of infection with potential for reactivation of disease in immunosuppression. In the immunodeficient, infection is potentially lethal, most commonly manifesting as encephalitis, but may also present with chorioretinitis, pneumonitis and myocarditis amongst others. These can manifest as an acute infection or as reactivation of latent infection acquired in the past.

T. gondii is transmissible via ingestion of food contaminated with oocysts, meat containing tissue cysts, from pregnant mother to child transplacentally and via blood transfusion and organ transplants.

The purpose of this study was to assess donated blood samples for the prevalence of exposure to the infection as well as for the prevalence of recent infection which would cause infection in susceptible hosts.

Objective

To determine the seroprevalence of acute and chronic *Toxoplasma gondii* infection in donated blood at the National Blood Transfusions Service.

Methodology

This was a cross sectional observational study, with a sample size of three hundred and fifty (350). Donated blood was obtained via random selection from the NBTS in conjunction with the RBTC's. All samples were tested for both IgG and IgM via ELISA using the Human

Toxoplasma test kits. Blood samples found to be positive for IgM or that gave indeterminate values were tested further for IgG avidity using Novatec Toxoplasma test kits.

The blood donors socio-demographic data was obtained from the standard questionnaire filled in prior to blood donation. The data was entered into a computer data base from which spreadsheets were generated and transferred to a standard software system for analysis.

Results

None of the blood samples examined via IgM ELISA was found to have acute *T.gondii* infection. Latent infection was 42.3% via IgG ELISA. The highest prevalence rates of latent infection were seen in donor samples from Eldoret (52.9%) and Nakuru (50%) and the lowest prevalence was in Nairobi (22.9%). There were more blood samples from male donors (81%). Forty one percent (41%) of blood samples from male donors tested positive for latent *T.gondii* infection compared to 29.9% of samples from female donors. Majority of blood donors were between 20 and 29 years of age. There was an increase in prevalence of latent *T.gondii* infection with increasing age of blood donors, with a mean age for positivity to IgG markers for *T.gondii* at 29 years and a mean age of negative result of IgG markers at 26.7 years. This was statistically significant (p value of 0.005). The prevalence of latent infection was higher in the unemployed (57.7%) and self employed (37.7%) as compared to the employed (34.3%). This was statistically significant (p value of 0.001).

Conclusion

A large number of the donated blood samples, 42.3%, had markers of previous exposure to *T.gondii* infection. These figures are higher than previous studies done in the country and are much more reflective of the general population for the fact that blood was from healthy blood donors and that the blood was from different regions of the country. There was a noted rise in latent infection with increase in age similar to other studies elsewhere and earlier in this country.

1.0 Introduction:

Toxoplasmosis is a zoonotic disease caused by the protozoan parasite *Toxoplasma gondii*, an obligate intracellular parasite. The parasite infects a wide range of animals, humans, other mammals and various bird species. Human infection is acquired via consumption of food or water contaminated with faeces containing sporozoites, via ingestion of undercooked infected meat containing tissue cysts, transplacentally from infected mother to unborn child and via organ transplantation and blood transfusion.

Toxoplasmosis is of significant clinical importance in pregnant women and in immunodeficient individuals. An active infection in pregnancy portends a risk of congenital malformations and chronic carriage of infection with potential for reactivation of disease in immunosuppression. In the immunodeficient, infection is potentially lethal, most commonly manifesting as encephalitis, but may also present with chorioretinitis, pneumonitis and myocarditis amongst others. In this population, infection may manifest acutely or as reactivation of latent infection acquired in the past¹.

Kenya, like most of sub-Saharan Africa countries has a huge burden of HIV infection. The most recent HIV prevalence rates are from the Kenya AIDS Indicator Survey (KAIS) in 2012. HIV prevalence rates nationwide in adults 15-64 years of age were 5.6%, indicative that a large number of Kenyan adults (1,192,000) between 15-64 years of age are living with HIV. These individuals are potentially at risk of severe toxoplasma infection.

At the National Blood Transfusion Service (NBTS), blood is only screened for HIV, hepatitis B (Hep B), hepatitis C (Hep C) and syphilis. There is a risk of transmission of toxoplasmosis through transfusion of infected blood. This is of particular importance if it occurs in immunosuppressed patients such as low birth weight babies, in patients with malignancies and patients on immunosuppressive medication like post transplant patients. Acquisition of infection in this subset of patients is potentially fatal. These same patients frequently develop anaemia and need blood transfusions. Due to the protean manifestation of the disease particularly when the disease does not present with Central Nervous System (CNS) manifestations, it is possible that we have been missing a number of cases of toxoplasmosis to the detriment of the patients.

This study aimed to determine if blood considered safe for transfusion may indeed be infected with *Toxoplasma gondii*. If this be the case, screening of blood for toxoplasmosis would be

warranted prior to transfusion to the subset of patients most at risk of developing severe infection (in the pregnant and immunosuppressed). The study also provided useful information on the seroprevalence of *Toxoplasma gondii* infection in the country with blood donors serving as a surrogate for the general population.

2.0 Literature review:

2.1 Biology:

Toxoplasma gondii is an obligate intracellular coccidian parasite. Nicole and Manceau described the organism in 1908, after they observed the organism in the blood, liver and spleen of a north African rodent -*Ctenodactylus gondii*³. Alfonse Splendore in Sao Polo, Brazil described the same organism discovered in a rabbit still in the same year 1908. The parasite was named *Toxoplasma*, 'Toxo' being the greek term for bow or arc in reference to the organism's shape.

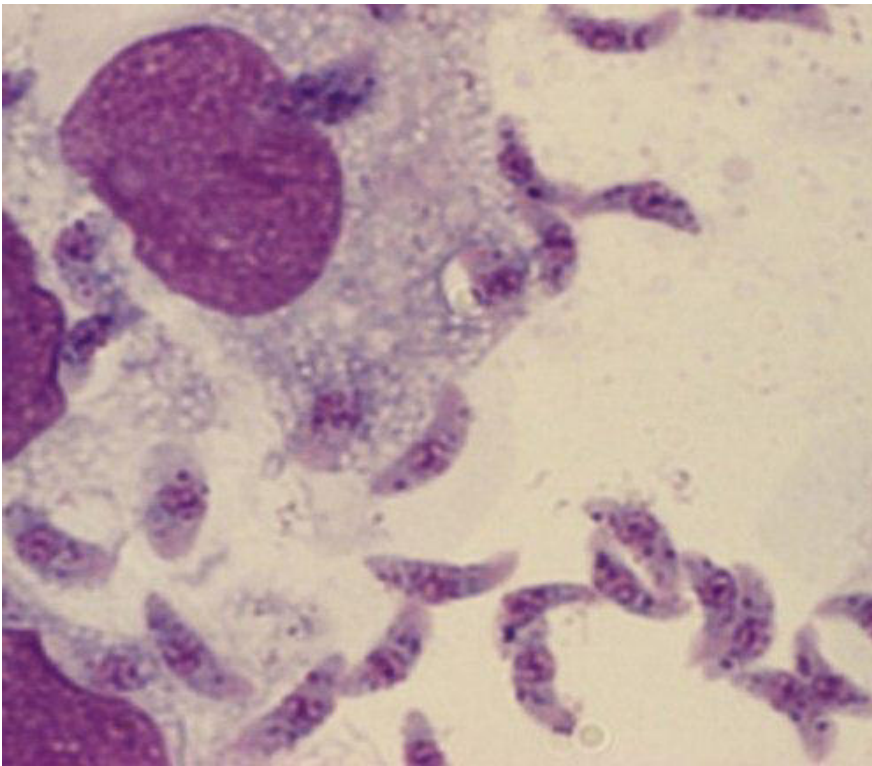


Figure 1: Light microscopy demonstrating *T. gondii*

In 1923, Janku reported parasitic cysts in the retina of an infant who had hydrocephalus, seizures and unilateral microphthalmia.

The entire lifecycle was described in 1970 following finding of oocysts in the small intestine of a cat^{4,5}.

T. gondii belongs to the phylum Apicomplexa, which consists of intracellular parasites that have a characteristically polarised cell structure and a complex organellar arrangement at their apical end.⁶

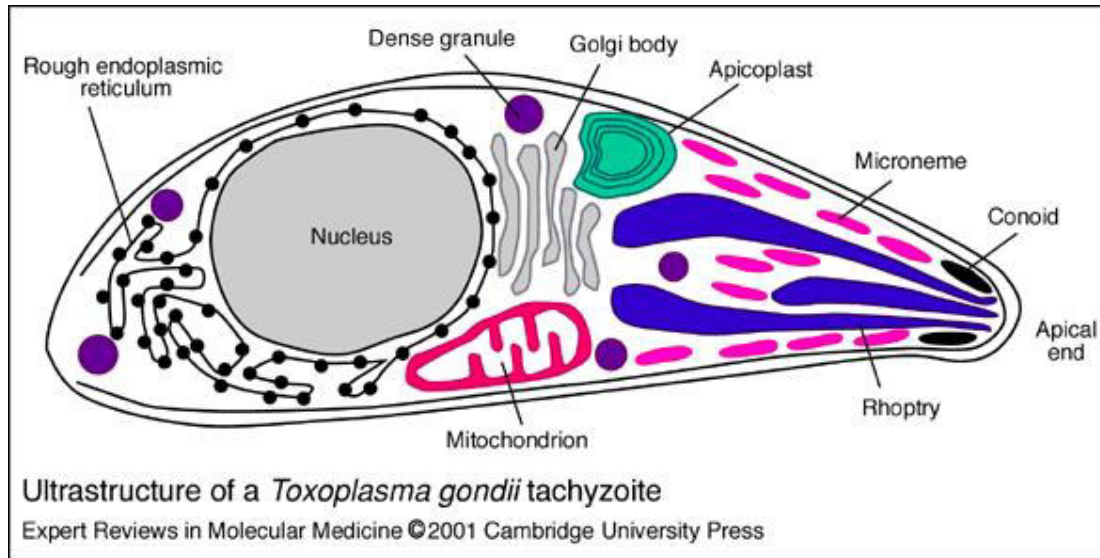


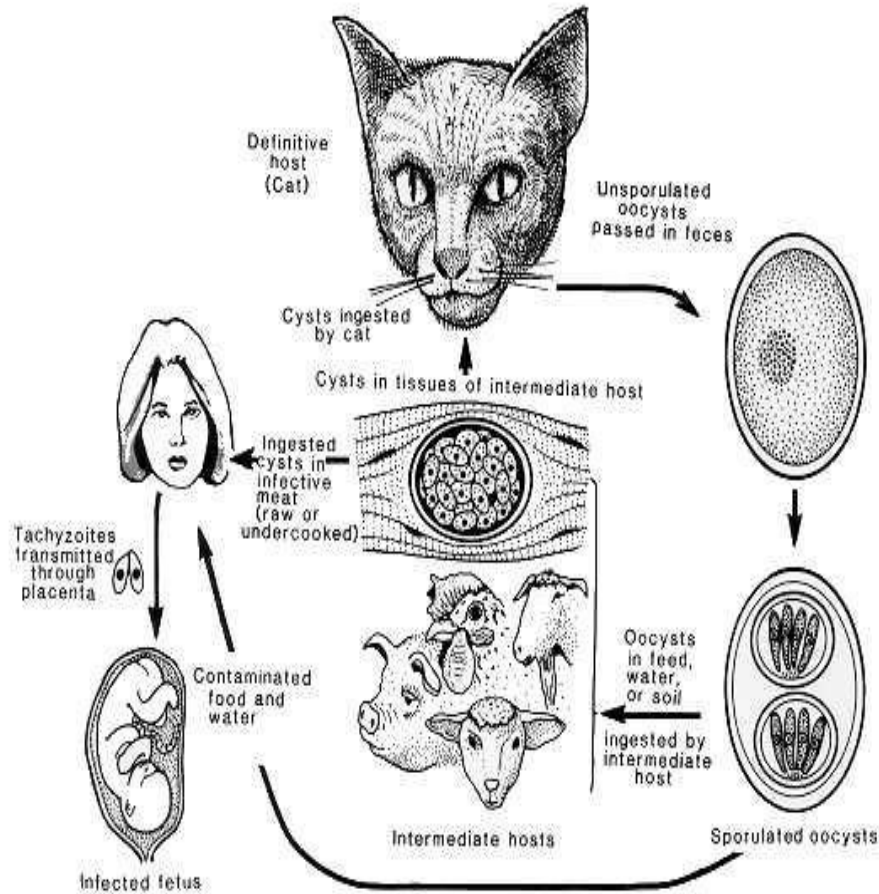
Figure 2: Ultra structure of *T. gondii* tachyzoite

There are 3 major genotypes of *T. gondii*, I, II, III⁷. These genotypes differ in virulence and epidemiology. Type I and II are more commonly isolated in congenital disease, while type III is more commonly isolated from animals¹.

2.2 Life cycle:

T. gondii, primarily exists in 3 forms, all of which are infectious stages namely i) oocysts which are oval in shape with 2 sporocysts each containing 4 sporozoites and found in the definitive host, ii) tachyzoites, which are crescent shaped and are the rapidly dividing forms in pseudocysts, and iii) bradyzoites, which are spindle shaped and dormant, and found in true tissue cysts.

The definitive host is the cat and other felines^{4, 5}. Warm blooded animals, birds and mammals serve as the intermediate hosts.



Adapted from the European Society of Clinical Microbiology and Infectious Diseases, CMI, 8, 634–640

Figure 3: Lifecycle of *T. gondii*

After ingestion of tissue cysts or oocysts by the feline, there's release of viable organisms following breakdown of the cyst wall by proteolytic enzymes which invade the epithelial cells of the small intestine. Tissue cysts release bradyzoites which penetrate the lamina propria below the epithelial cells and multiply as tachyzoites.⁸ Tachyzoites can infect and replicate only in nucleated cells.

Once attached to the host cell, the parasite penetrates the cell and forms a parasitophorous vacuole within which it divides. Replication continues until the number of parasites within the

cells approaches a critical mass and the cell ruptures, releasing parasites that infect adjoining cells.⁹

Five morphologically distinct asexual stages (A-E) of *T. gondii* develop in enterocytes before gametogony begins¹⁰. Gametes are thought to differentiate from stages D and E.

Following gametogenesis, fertilisation takes place and oocysts are formed when a wall is laid down around the fertilised zygote⁸. Oocysts are then expelled into the intestinal lumen following rupture of the enterocytes and are unsporulated when excreted in the faeces.

The oocysts sporulate within 1-5 days after excretion depending on ambient temperature, humidity and aeration by dividing into 2 sporocysts, each containing 4 sporozoites - this is the infective form.

In the intermediate host, tachyzoites are disseminated throughout the body of the host, in macrophages, lymphocytes as well as free in plasma. Sometime after infection from as early as 3 days to about 7 weeks later, tachyzoites transform into bradyzoites in tissue cysts. Signals responsible for the transformation are unknown¹¹⁻¹³. Bradyzoites differ from tachyzoites in that they multiply slowly, express stage specific molecules and are functionally different¹.

Human infection occurs through several means, it may follow consumption of food or water contaminated with cat faeces¹⁴⁻¹⁸ via ingestion of undercooked infected meat containing tissue cysts^{1, 14}, transplacentally from an infected mother to unborn child¹⁹ and via organ transplantation and blood transfusion.²⁰⁻²²

2.3 Epidemiology:

T. gondii is one of the most common parasites infecting man and other warm blooded animals. It has been found worldwide from Alaska to Australia. Approximately 1/3 of humanity has been exposed to this parasite¹⁴.

The prevalence of *T. gondii* varies depending on the age, locale and cultural habits of a population. Increased prevalence has been associated with an increase in age²³⁻²⁶. The prevalence is also lower in cold regions like Antarctica, hot dry climates and at high altitudes.¹ Eating of raw/rare meat also increases the prevalence of infection²⁷, implicated in the high prevalence of infection in France for example¹⁴. Oocyst contamination of the

environment particularly drinking water sources has been implicated as a cause of a number of outbreaks worldwide¹⁴⁻¹⁸.

Local studies have demonstrated an increase in prevalence with increase in age. Blood of preschoolers and that of school going children was assayed; there was a noted rise from 35% to 60% seropositivity for latent infection.²³

A comparative study assessing 94 HIV patients and 86 controls showed a prevalence of 54% of toxoplasma in both populations via enzyme immunoassay and latex and dye tests. 22% of the HIV-positive group had IgG levels in excess of 180 units/ml (approximating to a dye test titre of 1:1300) compared to 1% of the HIV-negative group. There was no correlation between high levels of IgG and clinical stage of HIV disease or features indicative of active toxoplasmosis. It was postulated that the high IgG titres in the absence of clinical manifestation was due to early reactivation of the disease²⁷.

Rubina Shaheen Cocker in 1991, for her M. Med dissertation, carried out an immunological survey of toxoplasmosis in a rural Kenyan population in Kiambu District (sample size 454), via ELISA and IFAT methods, which demonstrated exposure rates of 53.7% and 45.8% respectively. Higher values were seen with ELISA as it is a more sensitive test as compared to IFAT.²⁹

An unpublished study by Philip Kamau, for his M.Med dissertation in pregnant women attending the KNH ANC in 2010, demonstrated a prevalence of 23% and 30% seropositivity for acute and chronic infection respectively.³⁰

In Uganda studies in HIV positive patients, showed a prevalence of 54% for IgG seropositivity with 23% demonstrating parasites in peripheral blood³¹. In South Africa a comparative study between HIV positive and HIV negative individuals demonstrated a prevalence of IgG positivity of 9.8% and 12.8% respectively³².

A study assessing the prevalence of *T. gondii* antibodies using the Sabin Feldman dye test in newly delivered mothers in Dar es Salaam, Tanzania demonstrated 35% positivity, with only 11.5% being HIV positive³³. In South Africa in non HIV pregnant women and children was 6.4%³². In Burkina Faso, a study in pregnant women demonstrated an exposure rate of 25% with a significant increase in age from 16.3 to 49.1%²⁴.

The prevalence of infection in patients visiting Korle Bu Teaching Hospital in Ghana, was 32% and 29.7% for IgG and IgM/A respectively³⁴. In Mali assessment of blood donors demonstrated a prevalence of 22.6% from HIV positive patients and 21% from HIV negative patients. In AIDS patients the prevalence was 60%³⁵.

The national prevalence of exposure to toxoplasmosis in the USA is 22.5%, in women aged 15-44years it is 15%²⁵. In India, a nationwide survey demonstrated a prevalence of 24.3% and 2% for IgG and IgM respectively³⁶. Prevalence of exposure in Slovakia in healthy blood donors was found to be 24.2%²⁶. In France, in a rural population in Lorraine, a prevalence of 47% for IgG was demonstrable, none was IgM positive³⁷.

2.4 Clinical manifestations of toxoplasma infection:

Acute toxoplasmosis is typically asymptomatic in most individuals, being symptomatic in only about 10% of patients, where it manifests with a self limited, non specific illness. The most typical clinical manifestation is an isolated cervical or occipital lymphadenopathy; lymph nodes are not tender, do not suppurate, are usually discrete and stay enlarged for less than 4 to 6 weeks¹.

In a few, there's development of fever, headache, malaise, myalgia, lymphadenopathy, hepatosplenomegally and atypical lymphocytosis after an incubation period of 5 to 20 days¹⁵.¹⁶. Rare cases of severe disease in immunocompetent hosts has been described, they include pneumonitis, myocarditis and polymyositis^{38, 39}.

2.4.1 Congenital Toxoplasmosis:

This occurs only when a woman becomes infected during pregnancy or when a woman becomes pregnant shortly after acquiring toxoplasmosis⁴⁰. Congenital infections acquired in the first trimester are more severe than those attained in the 2nd and 3rd trimesters. If maternal infection occurs in the first trimester, risk of foetal infection is around 10% but disease is more severe. If infection occurs in the 3rd trimester, rates of congenital infection rise to 65 to 100 % at term, with disease in the infant being mild⁴¹.

Focal lesions develop in the placenta with consequent infection of the foetus as it is an incomplete barrier, 30-50 % of infections acquired during pregnancy will be transmitted to the foetus⁴². Pathologically, lesions associated with *T. gondii* infection may be due to destruction of parasitized cells by tachyzoites, tissue necrosis from rupture of cysts or coagulation necrosis secondary to vascular thrombosis⁴³.

At first there is generalised infection in the foetus which is cleared from the visceral tissues and remains localised in the CNS¹⁴. Clinical manifestations of congenital toxoplasmosis are varied, with the most serious sequelae associated with infection acquired in the first trimester of pregnancy. These include-spontaneous abortions, still births, hydrocephalus due to necrosis occurring around the ventricles and the aqueduct of sylvius, intracerebral calcifications-large areas of necrosis becoming calcified, seizures, deafness, psychomotor retardation, microcephaly, chorioretinitis, strabismus and blindness. Systemic manifestations include fever, hypothermia, jaundice, vomiting, diarrhoea, lymphadenopathy, hepatosplenomegaly, pneumonitis, myocarditis, and rash - punctate macules and/ or ecchymoses.

While the majority of infants who acquire infection late in pregnancy appear normal at birth, meticulous examination often shows abnormalities such as retinal scars or abnormal CSF^{44, 45}.

2.4.2 Ocular disease:

Toxoplasmosis may account for approximately 1/3 of cases of retinochoroiditis. It may occur as a sequelae of congenital infection or in the setting of postnatally acquired infection¹. Retinochoroiditis following acute acquired infection is more common than was previously thought and may account for more cases of disease than congenital retinochoroiditis^{46, 47}.

Active disease causes pain, photophobia and blurred vision in the absence of constitutional symptoms. Other features are macular involvement with loss of central vision and nystagmus secondary to poor fixation. Extraocular muscle involvement leads to disorders of convergence and strabismus.

Fundoscopy findings - the vitreous is hazy, elevated, pale yellow and white cotton patches may be seen in the retina. Healed scars are pale with distinct margins and prominent black

spots of choroid pigment. Recurrences occur at the border of the scars and may lead to blindness⁴⁸.

2.4.3 Toxoplasma infection in immunocompromised hosts:

Unlike in the immunocompetent where *T.gondii* infection is asymptomatic and runs a favourable course, the disease is fatal in the immunosuppressed, particularly HIV-AIDS patients with a CD4 count of less than 100. Toxoplasmosis in this population usually occurs secondary to reactivation of latent infection.

Toxoplasma encephalitis is the most common manifestation of disease in this subset of patients. It is a multi-focal necrotising encephalitis with presentation varying from a subacute gradual process and developing over weeks to an acute confusional state. Patients present with, mental status changes, seizures, focal motor deficits, cranial nerve disturbances, sensory anomalies, cerebellar signs, movement disorders and neuropsychiatric manifestations. Meningeal signs are rare¹.CSF typically shows a mononuclear pleocytosis and a mild elevation of proteins.

After the brain, the lungs and the heart are the most frequently affected organs. Pneumonitis typically occurs in those with a advanced AIDS, particularly in those not on HAART. Patients present with fever, dyspnoea and non-productive cough. Chest radiographs demonstrate reticulonodular infiltrates and tachyzoites may be identified in bronchoalveolar lavage fluid⁴⁹.

Toxoplasma myocarditis is frequently subclinical^{50, 51}.Toxoplasmosis in the immunocompromised may also present as chorioretinitis. A generalised infection may also be seen involving the liver, spleen, pancreas, intestines, thyroid, testes and spinal cord.

2.5.0 Laboratory detection

2.5.1 Direct methods

T. gondii may be demonstrated on stained specimens of tissue, blood, amniotic fluid and CSF¹⁴. The diagnosis of acute infection requires the identification of tachyzoites as tissue cysts do not distinguish between acute and chronic infection. Parasites may also be recovered from tissues and body fluids by inoculation into mice or tissue culture¹⁴.

PCR assays detect parasite in DNA in amniotic fluid, blood, CSF, aqueous humour and fluid from bronchoalveolar lavage with variable sensitivity. But this technique is not used routinely in clinical practice due to its high cost.

2.5.2 Indirect methods

These techniques make use of serology to detect IgG and IgM antibody titres hence assaying for latent and acute infection respectively.

The Sabin-Feldman dye test, tests for the presence of both IgM and IgG via the ability to lyse live *T. gondii* in the presence of human complement⁵².

Indirect fluorescence and antibody enzyme linked immunosorbent assays (ELISA) are sensitive tests for the detection of IgG antibody and correlate well with the dye test in adults^{53, 54}.

IgM capture ELISA and IgM ISAGA (Immunosorbent Agglutination assay) are sensitive for the detection of specific antibodies, though titres in infants less than 6 months old are lower than in adults making the use of IgG/IgM western blots handy⁵⁵. Western blot is a technique for identifying specific antibodies or proteins, in which proteins are separated by electrophoresis, transferred to nitrocellulose, and reacted with antibody.

A negative IgM test rules out infection, but a positive test does not necessarily imply that one has active infection as some commercially available kits have a high incidence of false positives and IgM antibodies may persist even for up to one year⁵².

IgG avidity testing helps to differentiate between past and recently acquired infection. As the immune response develops there's maturation of the IgG antibody response and the avidity

increases progressively over weeks to months. Urea or another protein denaturing agent is used to dissociate the antigen antibody complex. The results, expressed as a % of the avidity reflect the extent of antigen antibody complex dissociation caused by the denaturing agent. The test cannot be used in very early infections as the level of IgG must be high enough to allow accurate measurement of the denaturing agent^{55, 56}.

2.6.0 Treatment:

Guidelines for the management of toxoplasmosis vary from institution to institution.

Standard treatment makes use of pyrimethamine and sulfadiazine. Folinic acid is normally added to counter the bone marrow suppressive effects of pyrimethamine. Alternative drugs for those with hypersensitivity to sulphur based drugs include clarithromycin, atovaquone, azithromycin and dapson. Spiramycin is used in pregnancy as it accumulates in the placenta inhibiting transfer of the infection to the foetus.

3.0 Justification for the study

T.gondii infection is asymptomatic and of little consequence in immunocompetent individuals and those who are not pregnant. It is significant though, in the immunosuppressed where it causes disease by reactivating latent cysts or following acute infection and in the pregnant regardless of immune status where it may cause foetal loss or any of a number of congenital malformations.

T. gondii is transmissible via blood transfusion. Blood in the NBTS is only screened for HIV, Hep B, Hep C and Syphilis. Many patients in the populations at risk, -the immunodeficient (AIDS patients, those with malignancies, post renal transplant patients, low birth weight children) and pregnant women are frequently anaemic and require blood transfusions. Information on the prevalence of acute infection will therefore be useful as it will determine whether there is need for screening of blood prior to blood transfusion in these vulnerable groups.

There is paucity of data regarding the prevalence of toxoplasmosis in the general population.

4.0 Methodology:

4.1 Overall objective:

To determine the presence of *Toxoplasma gondii* infection in donated blood samples at the Kenya National Blood Transfusion Services.

4.2 Specific objectives:

To determine the seroprevalence of acute and latent *T.gondii* infection in donated blood samples in relation to age and gender.

4.3 Study design:

This was a cross sectional observation study.

4.4 Study area:

The study was carried out at the NBTS in Nairobi. The laboratory assay was done at UNITID laboratory, situated at the UON in KNH.

Blood samples were from the National Blood Transfusion Service (NBTS) in Nairobi. The NBTS was established in Kenya by the Ministry of Health, and entrusted with the responsibility of managing the blood collection and transfusion in Kenya. The NBTS is situated in Nairobi near KNH with six Regional Blood Transfusion Centres in Nakuru, Mombasa, Eldoret, Kisumu, Embu and Nairobi (Nairobi is also a RBTC) and nine Satellite Centres around the country in Machakos, Voi, Garissa, Meru, Kericho, Kakamega, Kisii, Naivasha and Nyeri . The satellite centres augment the work of the RBTC's.

4.5 Sample population:

The study samples included donated blood at the NBTS within the study period. The NBTS receives blood from RBTC's, Nairobi, Nakuru, Kisumu, Eldoret, Embu and Mombasa. Samples were from all the RBTC's with the exception of Embu.

4.6 Sample size:

The sample size used in the study was 350. The minimum sample size required for the study was 255, derived using Fisher's formula. This was based on a similar study carried out in blood donors in Mali which demonstrated a prevalence of *T. gondii* infection of 21%³³.

$$n = \frac{z^2 p (1 - p) e^2}{e^2}$$

p=prevalence as extracted (see Mali study)

e=0.05 (confidence limit)

z=1.96

z²=alpha and beta errors.

4.7 Sampling method:

The NBTS receives blood from the following RBTC's, Nairobi, Mombasa, Nakuru, Embu, Eldoret and Kisumu. Blood is obtained from voluntary non remunerated blood donors from low risk populations who meet the NBTS guidelines. The bulk of blood in the NBTS is obtained from donors in learning institutions, High Schools 5%, Colleges 60% and the remaining 25% from individuals in corporate institutions and outreach centres in common areas like particular city streets and market centres. Approximately 2000 blood samples are

received per month. About 1-2% of this is HIV infected. This information was derived from the data collected at the NBTS.

Only 70 samples from each RBTC were required. Cluster sampling was used, where samples were derived from blood donated by individuals from one particular outreach centre, either a corporate institution or designated market centre or street, (from the remaining 25% of the donor population mentioned above). This was to give a broader representation of the local population rather than skewing the data towards a relatively younger population.

Blood from the RBTC's already tested for HIV, hep B, hep C and syphilis was collected, stored in cool boxes and transported via courier to the NBTS in Nairobi. The blood samples were then moved from the NBTS to the UNITID lab, situated at the UON, KNH. The samples were then tested for the presence of *T. gondii* infection.

Flow chart of procedures for sampled blood.

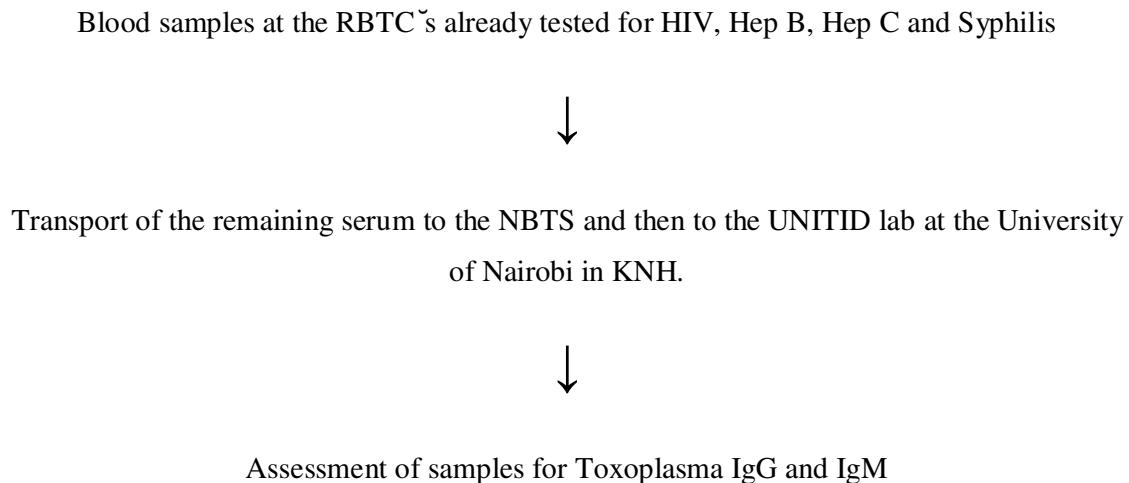


Figure 4: Flow chart of procedures for sampled blood.

4.8 Inclusion criteria:

All blood samples from individuals who qualified to donate blood as per the NBTS guidelines during the study period.

4.9 Exclusion criteria:

Blood samples from individuals who did not qualify to donate blood as per the NBTS guidelines also did not qualify to be in the study.

4.10.0 Laboratory procedures:

4.10.1 Specimen collection, transport and storage:

The remaining blood sample, from which serum for determination of HIV, Hep B, Hep C and Syphilis infection was collected, was taken from the blood transfusion services, placed in cool boxes and transported to the NBTS via courier services and from there to the UNITID lab.

Specimens were stored at between 2 and 8°C for 7 days or longer at -20°C. After freezing of the samples thawing was only done once.

4.10.2 Assay procedure:

Procedure was done exactly as per manufacturer's instructions (see appendices 3 and 4).

Reagents and specimens were stored at room temperature before use.

The calibrators for controls, both positive and negative controls were run in duplicates as per the kit manufacturer's instructions.

Table 1: Interpretation of test results:

<u>IgG</u>	<u>IgM</u>	<u>Interpretation</u>
Negative	Negative	No serological evidence of toxoplasma infection.
Negative	Positive	Acute infection.
Positive	Negative	Infected with T. gondii for more than a year.
Positive	Positive	Possible recent infection within the last 12 months.*
Positive	Equivocal	Infected with Toxoplasma gondii for probably more than one year or false-positive IgM reaction.*
Negative	Equivocal	Possible early acute infection or false-positive IgM reaction. Indeterminate*
Equivocal	Negative	Indeterminate*
Equivocal	Equivocal	Indeterminate*
Equivocal	Positive	Possible acute infection with Toxoplasma gondii.*

* Underwent IgG avidity testing using the Novatec Toxoplasma IgG avidity test kit (see appendix 6).

Principle of the assay (IgG avidity testing)

Microtiter strip wells coated with Toxoplasma antigen are incubated with diluted serum specimen (dual pipetting). After washing one well is incubated with avidity reagent and the

corresponding well with washing buffer. In this step the low avidity antibodies are removed from the antigens whereas the high avidity ones are still bound to the specific antigens.

Anti human IgG labelled with peroxidase is added. The immunocomplex is visualized with trimethylene blue (TMB) to give a blue reaction product.

Stop solution is added to stop the reaction and changes the colour of the reaction product into yellow. Absorbance at 450 nm is read using an ELISA microwell plate reader.

Interpretation of Results

Avidity of more than 40% is high and is indicative of past infection while avidity of less than 40% is in keeping with acute infection.

4.10.3 Quality assurance:

Strict measures in quality control and assurance were followed at all levels to ensure that the results are a true and valid representation of toxoplasma infection in the blood donors.

4.11 Ethical considerations:

Approval was sought from the NBTS, the DOCMT as well as from the KNH/UON ERC before the study commenced.

All information attained from the participants was treated with the utmost confidentiality.

Consenting was not required for the study. Policy guidelines on blood transfusion in Kenya allow for the use of donated blood in research regarding all aspects of blood transfusion⁵⁹.

The data was unlinked as donor identities were unknown to the PI. Samples were only identifiable via a code number. Information on the donors was acquired from a proforma designed by the PI which was in keeping with some of the information filled out in the blood donation questionnaire. Information in the proforma included: RBTC code, venue site, district, date, donor reference/code number, age, gender, number of previous donations, level of education, occupation and results of blood group, HIV, hep B, hep C and Syphilis

(Appendix 3). This information was filled out by the staff in the respective RBTC's before sending the samples to the PI for analysis of *T.gondii* infection.

Information attained from the study will be shared with the NBTS, the DOCMT and KNH/UON ERC.

Donors were advised to get back in touch with their RBTC's if they wished to know the results of any of the tests carried out following blood donation and this included results of the toxoplasmosis tests as the results will have been shared with the NBTS and the respective RBTC's. This is in keeping with standard procedures and practices. The onus is upon the donor to get in touch with their RBTC if they wish to get the results of any of the tests carried out.

4.12 Data management:

The donors socio-demographic data was obtained from the proforma sent to the RBTC's filled by the staff in the respective centres in liaison with the PI. This proforma also included results of the tests routinely carried out prior to blood transfusion (Appendix 3).

Data was kept in a lockable cabinet in the PI's office or the Statistician's office. This data was then entered into a password protected Microsoft Access Database to prevent unauthorized access to participant records. Data quality checking was performed by counterchecking the entered data with the hard copy forms. This was done by the PI and findings forwarded to the statistician for corrections.

Exploratory data analysis was then carried out to summarize the study population and identify inconsistent data and outliers. To determine the seroprevalence of acute and latent *T.gondii* infection among blood donors, simple frequencies and proportions were used with the number of participants with acute and latent *T.gondii* divided by the sample size.

Nominal variables were summarized using counts and percentages while continuous variables were summarized using measures of central tendency and dispersion. Factors associated with *T. gondii* infection were evaluated with Chi-squared tests being used for nominal variables such as gender and T-Tests being used for continuous variables such as age. Logistic regression was used to determine independent factors associated with the infection.

6.0 Results.

6.1 Socio-demographics:

A total of 350 blood samples were assayed from 5 different RBTC's. (Nairobi, Mombasa, Eldoret, Kisumu and Nakuru). The mode of sampling was cluster sampling where blood was collected from one or two sites within the area, for example in Nairobi, the 2 venues were Bomb blast (Co-op bank) near the Railways bus terminus and a site on Biashara Street.

6.1.1 Socio-demographic profile of donor population of samples used in the study.

Majority of the blood donors were male (81%). Nairobi and Nakuru had a slightly higher female donor population of 21% and 27% respectively.

Sixty six percent (66%) of blood donors were educated to tertiary level. Nakuru and Nairobi had the highest number of individuals educated to tertiary level at 77%. Kisumu had the lowest number of people educated to tertiary level at 50% and the highest number of people having attained primary education only, at 14%.

Thirty one percent (31%) of all blood donors were employed, 29% were self employed and a majority, 39% were unemployed.

Table 2: Socio-demographic profile of donor population of samples used in the study.

		TOWN											
		Eldoret		Kisumu		Mombas		Nairobi		Nakuru		Total	
		n	%	n	%	n	%	n	%	n	%	n	%
GENDER	Male	59	84.3	59	84.3	59	84.3	55	78.6	51	72.9	283	80.9
	Female	11	15.7	11	15.7	15	21.4	19	27.1	67	19.1		
LEVEL OF EDUCATION	Primary	6	8.6	10	14.3	4	5.7	1	1.4	4	5.7	25	7.1
	Secondary	24	34.3	25	35.7	18	25.7	15	21.4	12	17.1	94	26.9
	Tertiary	40	57.1	35	50.0	48	68.6	54	77.1	54	77.1	231	66.0
EMPLOYMENT	Employed	14	20.0	18	25.7	34	48.6	29	41.4	13	18.6	108	30.9
	Self-employed	29	41.4	26	37.1	17	24.3	13	18.6	19	27.1	104	29.7
	Unemployed	27	38.6	26	37.1	19	27.1	28	40.0	38	54.3	138	39.4

6.1.2 Age distribution profile of the donor population.

Majority of blood donors were between 20 and 29 years. The youngest donor was 16 years which is the lower limit of age for blood donation and the oldest donor was 69 years. There's no upper limit of age for blood donation, so long as one is deemed fit, one can donate.

Table 3: Age distribution profile of the donor population.

			GROUPED AGE					
			16-19	20-29	30-39	40-49	50-59	60-69
			years	years	years	years	years	years
TOWN	Eldoret	n	9	38	14	7	2	0
		%	12.9%	54.3%	20.0%	10.0%	2.9%	0.0%
	Kisumu	n	12	28	20	9	0	1
		%	17.1%	40.0%	28.6%	12.9%	0.0%	1.4%
	Mombasa	n	8	31	15	15	1	0
		%	11.4%	44.3%	21.4%	21.4%	1.4%	0.0%
	Nairobi	n	1	49	17	3	0	0
		%	1.4%	70.0%	24.3%	4.3%	0.0%	0.0%
	Nakuru	n	15	46	8	1	0	0
		%	21.4%	65.7%	11.4%	1.4%	0.0%	0.0%

	TOTAL	n	45	192	74	35	3	1
		%	12.9%	54.9%	21.1%	10.0%	0.9%	0.3%

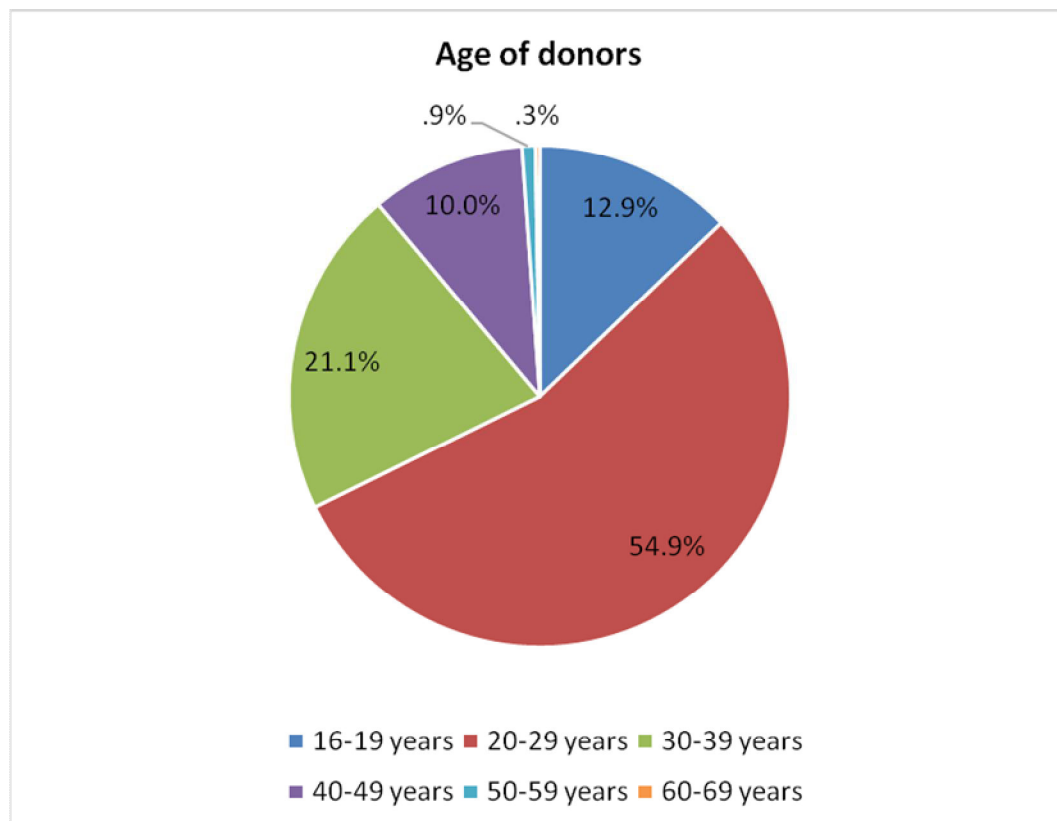


Figure 5: Age distribution profile of the donor population.

6.1.3 Blood group pattern of donated blood samples by region.

Majority of blood donors were blood group O positive 46.3%. 23.1% were A positive and 22.0 % were B positive.

Table 4: Blood group pattern of donated blood samples by region.

		Eldoret		Kisumu		Mombasa		Nairobi		Nakuru		Total	
		n	%	n	%	n	%	n	%	n	%	n	%
B L O O D G R O U P	A NEG	2	2.9%	0	0.0%	0	0.0%	1	1.4%	1	1.4%	4	1.1%
	A POS	13	18.6%	15	21.4%	10	14.3%	23	32.9%	20	28.6%	81	23.1%
	AB POS	6	8.6%	2	2.9%	1	1.4%	6	8.6%	4	5.7%	19	5.4%
	B NEG	0	0.0%	0	0.0%	1	1.4%	1	1.4%	1	1.4%	3	0.9%
	B POS	16	22.9%	21	30.0%	20	28.6%	9	12.9%	11	15.7%	77	22.0%
	O NEG	0	0.0%	1	1.4%	0	0.0%	2	2.9%	1	1.4%	4	1.1%
	O POS	33	47.1%	31	44.3%	38	54.3%	28	40.0%	32	45.7%	162	46.3%

6.1.4 ‘Standard’ serology of transfusion transmissible infections in the donated blood samples.

Transfusion transmissible infections were few, 1.4% of all donated blood for HIV, hep B and hep C each. None of the samples was infected with syphilis.

Table 5: ‘Standard’ serology of transfusion transmissible infections (TTI’s) in the donated blood samples.

TTI		Eldoret		Kisumu		Mombasa		Nairobi		Nakuru		Total	
		n	%	n	%	n	%	n	%	n	%	n	%
HIV	Negative	70	100.0	68	97.1	67	95.7	70	100.0	70	100.0	345	98.6
	Positive	0	0.0	2	2.9	3	4.3	0	0.0	0	0.0	5	1.4
Hep B	Negative	69	98.6	69	98.6	68	97.1	70	100.0	69	98.6	345	98.6
	Positive	1	1.4	1	1.4	2	2.9	0	0.0	1	1.4	5	1.4
Hep C	Negative	70	100.0	68	97.1	67	95.7	70	100.0	70	100.0	345	98.6
	Positive	0	0.0	2	2.9	3	4.3	0	0.0	0	0.0	5	1.4
Syp-hilis	Negative	70	100.0	70	100.0	70	100.0	70	100.0	70	100.0	350	100.0
	Positive	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

6.2.0 *T. gondii* sero-status in relation to the different RBTC’s.

Two (2) blood samples, 1 from Mombasa and 1 from Nakuru tested positive for IgM. Both also tested positive for IgG, but on avidity testing both had high avidity indicative of chronic infection and hence both were false positives. Two (2) samples from Nakuru were equivocal for IgM but on avidity testing, they also had high avidity indicative of old infection.

All the samples tested were not acutely infected with *T.gondii*.

IgG is a marker of latent *T. gondii* infection. The highest prevalence of latent *T.gondii* infection was seen in Eldoret at 55.7%, followed by Nakuru at 50%, the prevalence of latent infection was lowest in Nairobi at 22.9%. The prevalence of acute *T. gondii* infection was low as not a single sample had acute infection.

Table 6: *T. gondii* sero-status in relation to the different RBTC's

		TOWN											
		Eldoret		Kisumu		Mombasa		Nairobi		Nakuru		Total	
		n	%	n	%	n	%	n	%	n	%	n	%
Acute <i>T. gondii</i> infection (IgM)	Negative	68	97.1%	70	100.0%	69	98.6%	70	100.0%	69	98.6%	346	98.8%
	Positive	2	2.9%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	2	0.6%
	Equivocal	0	0.0%	0	0.0%	1	1.4%	0	0.0%	1	1.4%	2	0.6%
Avidity	>40%	2	2.9%	0	0.0%	1	1.4%	0	0.0%	1	1.4%	4	1.1%
Latent <i>T.gondii</i> infection (IgG)	Negative	33	47.1%	41	58.6%	39	55.7%	54	77.1%	35	50.0%	202	57.7%
	Positive	37	52.9%	29	41.4%	31	44.3%	16	22.9%	35	50.0%	148	42.3%

6.2.1 Sero-prevalence of latent *T.gondii* infection in relation to age of blood donors:

There is a higher prevalence of latent infection with increase in age as demonstrated by table 7.

Table 7: Sero-prevalence of latent *T.gondii* infection in relation to age of blood donors (i).

IgG results	n	Mean Age	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum	P value
				Lower Bound	Upper Bound			
Negative	201	26.72	7.021	25.74	27.70	16	47	0.005
Positive	149	29.17	9.114	27.69	30.64	16	69	
Total	350	27.76	8.058	26.92	28.61	16	69	

Once the figures are further stratified into the different donor groups, this significance however is lost.

Table 8: Sero-prevalence of latent *T.gondii* infection in relation to age of blood donors (ii).

	Latent <i>T. gondii</i> infection				P value
	Negative		Positive		
	n	%	n	%	

Age group	16 - 19 years	29	64.4%	16	35.6%	0.085
	20 - 29 years	113	58.9%	79	41.1%	
	30 - 39 years	44	59.5%	30	40.5%	
	40 - 49 years	15	42.9%	20	57.1%	
	50 - 59 years	0	0.0%	3	100.0%	
	60 - 69 years	0	0.0%	1	100.0%	

6.2.2 Sero-prevalence of latent *T.gondii* infection in relation to gender of blood donors.

Total male donors were more than the female donors, 81% versus 19%. 29.9% of blood donated from females was positive for *T.gondii* infection as compared to 41% of blood samples donated from males. More male donors in Kisumu were positive for *T. gondii* infection than females. There was also a significant difference in the overall number of blood donors testing positive for *T.gondii* infection between males and females (p-value of 0.022)

Table 9: Sero-prevalence of latent *T.gondii* infection in relation to gender of donors.

Town	Gender	Toxo IgG					
		Negative		Positive		Total	P value
		n	%	n	%		
Eldoret	F	7	63.6%	4	36.4%	11	0.324
	M	28	47.5%	31	52.5%	59	
Kisumu	F	10	90.9%	1	9.1%	11	0.018
	M	31	52.5%	28	47.5%	59	
Mombasa	F	7	63.6%	4	36.4%	11	0.789
	M	35	59.3%	24	40.7%	59	

Nakuru	F	12	63.2%	7	36.8%	19	0.742
	M	30	58.8%	21	41.2%	51	
Nairobi	F	11	73.3%	4	26.7%	15	0.792
	M	43	78.2%	12	21.8%	55	
Total	F	47	70.1%	20	29.9%	67	0.022
	M	167	59.0%	116	41.0%	283	

6.2.3 Socio-demographic characteristics of blood donors with latent *T. gondii* infection.

There was no correlation of latent infection with level of education. Toxoplasmosis was more prevalent in the unemployed and self-employed as compared to the employed, p value of 0.001.

Table 10: Socio-demographic characteristics of blood donors with latent *T. gondii* infection.

		Latent <i>T. gondii</i> infection				P value
		Negative		Positive		
		n	%	n	%	
LEVEL OF EDUCATION	Primary	13	52.0%	12	48.0%	0.099
	Secondary	46	48.9%	48	51.1%	
	Tertiary	142	61.5%	89	38.5%	
EMPLOYMENT	Employed	71	65.7%	37	34.3%	0.001
	Self-employed	44	42.3%	60	57.7%	
	Unemployed	86	62.3%	52	37.7%	

6.2.4 Correlation of other TTI's to latent *T. gondii* infection:

There was no correlation between other TTI's tested for in the NBTS with the prevalence of latent *T.gondii* infection.

Table 11: Correlation of other TTI's to latent *T. gondii* infection.

TTI's		Latent <i>T. gondii</i> infection				P value
		Negative		Positive		
		n	%	n	%	
HIV	Negative	198	57.4%	147	42.6%	0.907
	Positive	3	60.0%	2	40.0%	
Hep B	Negative	198	57.4%	147	42.6%	0.907
	Positive	3	60.0%	2	40.0%	
Hep C	Negative	199	57.7%	146	42.3%	0.427

	Positive	2	40.0%	3	60.0%	
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7.0 Discussion

There were more male donors than female (81 versus 19%) and majority of the blood donors were within the 20-29 age bracket, despite the mode of sampling. This is due to the fact that many females may not meet donor requirements as regards haemoglobin levels, due to menstrual losses, for example or may be pregnant or lactating which are contraindications to blood donation. According to the KNBS the majority of the population in Kenya are under 15 years (42.9%) of the general population⁵⁹. There's a noted steady decline in the population with increase in age. It therefore is in keeping with the demographics of our country that the majority of blood donors were in the younger age group of 20-29 years (54.9%) not the 16-19 year group as this has a narrower grouping, though a notably significant figure (12.9%) , after the 30-39 year age group (21.1%). The profile of blood donors in the country is similar to that in the rest of the world as seen in data provided by the WHO⁶⁰. As humans grow older, there is an increase in lifestyle diseases like diabetes and hypertension necessitating use of medication which is also a contraindication to blood donation, another reason why younger blood donors make up the majority.

Majority of blood donors were educated to tertiary level 66%, followed by secondary level 26.9% and least 7.1%, were educated only to primary level. Increase in level of education is associated with a greater capability to voluntarily donate blood. The NBTS tends to target high schools and colleges for blood donation, regular blood donors may have started this practise then, hence exposure to the idea of donating blood occurring in people with secondary and tertiary level of education.

The total prevalence of latent *T.gondii* infection in the 5 different RBTC's was 42.6%. The highest prevalence was noted in Eldoret 54.3%, followed by Nakuru at 50% and the lowest prevalence was in Nairobi at 22.9%. The prevalence of acute infection was 0%. Eldoret and Nakuru are both farming towns; the higher prevalence of latent *T. gondii* may be associated with closer contact with pet cats than with the other more urbanised towns where the nature of housing does not quite allow for the keeping of pets. The higher prevalence of latent infection may also be associated with greater contamination of drinking water sources with infective sporocysts.

There was a noted rise in the prevalence of latent infection with increase in age. Stratification of the age groups did not show the same probably due to the fact that a much larger number of donated blood samples were from within one age group (20-29 years, 54.9%). Bowry et al in a study of preschool children and early school age children in 1986, showed a rise from 35% to 60% respectively²³. A rise in prevalence of infection has also been demonstrated elsewhere, for example in Burkina Faso, Slovakia and the USA²⁴⁻²⁶.

The prevalence of latent *T.gondii* infection was higher in the unemployed and self employed as compared to the employed, p value of 0.001. The prevalence was 34.3% for the employed, as compared to 57.7% and 37.7% for the self-employed and unemployed respectively. This could be related to socioeconomic status, as the employed have a steady source of income as compared to the unemployed and self-employed, hence may have access to cleaner drinking water and vegetables uncontaminated with sporocysts. The self employed may largely be farmers and may be in greater contact with domestic felines which are a source of infection. Employment status does not necessarily have to go hand in hand with the level of education in Kenya, as a large number of individuals may be employed in the informal sector which does not need high levels of education.

These figures vary from the most recent study on *T.gondii* infection done on pregnant women attending the ANC at KNH, by Kamau et al in 2011³⁰. In this study, the pregnant women were used as a surrogate for the population of Nairobi. In that study, the prevalence of latent infection was 30%, while that of acute infection was 23%. The high numbers of acute infection may have been due to false positive tests as the test kit used was not the immuno-capture type and neither were these positive IgM tests validated with IgG avidity testing. The immuno-capture IgM test kit is able to avoid false positives brought about by rheumatoid factor and antinuclear antibody both of which tend to be more common in females than in

males and this study was only assessing females. The IgM test kit used in this study was immune-capture and is perhaps the reason there were fewer positive results. The use of toxoplasma IgG avidity testing further reduced the number of false positive results in this case.

In comparison to other similar studies done in blood donors around the world, this study was most similar to that done in Abha, in Saudi Arabia where positive IgG (haemagglutination technique) was 52.1% and positive IgM was 4.1%⁶¹. In urban Karnataka, India, the prevalence of IgG was 20.3% and IgM was 3.6%⁶². In Durango, Mexico, 7.4% were positive for IgG and 1.9% was positive for IgM⁶³. None of these studies carried out IgG avidity testing to confirm the positive IgM tests.

8.0 Study limitations

1. The gold standard for determination of *T.gondii* infection was not done as this requires use of live *T.gondii* and hence is only done in reference labs.
2. The cost of this study limited the sample size. A bigger sample size would have been better for characterisation of the infection across the different geographical areas and may have given a better comparison when compared with other TTI's especially HIV.
3. There was a lack of in depth socio-demographic detail, which limited the ability to make inferences on the risk factors for *T.gondii*.

9.0 Conclusion.

The prevalence of latent *T. gondii* infection in the country is high, with certain areas in the country having at least half of the population infected (Eldoret and Nakuru). The prevalence of acute *T. gondii* infection is low.

There was a noted rise in latent infection with increase in age, similar to other studies elsewhere and earlier in the country.

10. 0 Recommendations

1. Sentinel surveys of blood samples for toxoplasmosis from high risk areas. This may give more information on the variables affecting the disease in these regions.

REFERENCES

1. Montoya J G, Liesenfeld O *Lancet* (2004) 363 (9425) p. 1965-1976 .
2. Kenya Demographic Health Survey *KDHS 2008-2009*.
3. Dubey, J P, The history of *Toxoplasma gondii* - the first 100 years. *J. Euk. Microbiol.* 2008; 55, 467-475.
4. Frenkel, J K, Pursuing *Toxoplasma*. *J. Infect. Dis.* 1970; 122, 553-559.
5. Dubey, J P, Miller, N L., Frenkel J K, Characterization of the new fecal form of *Toxoplasma gondii*. *J. Parasitol.* 1970b;56, 447-456.
6. Dubey J P, Lindsay D S, Speer C A, Structures of *Toxoplasma gondii* tachyzoites, bradyzoites and sporozoites and biology and development of tissue cysts. *Clinical microbiology reviews* 1998; 11 (2) p. 267-299.
7. Howe D K, Sibley L D, *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. *J Infect Dis.* 1995; 172(6):1561-1566.
8. Petersen E and Dubey J P 0521019427 - *Toxoplasmosis: A Comprehensive Clinical Guide*.
9. Harrison's Principles of Internal medicine, 18th edition
10. Dubey J P, Frenkel J K, Cyst-induced toxoplasmosis in cats. *J. Protozool.* 1972; 19:155-177.

11. Dubey J P, Frenkel J K, Feline toxoplasmosis from acutely infected mice and the development of *Toxoplasma* cysts. *J. Protozool.* 1976; 23:537-546
12. Derouin F, Garin Y J, *Toxoplasma gondii*: blood and tissue kinetics during acute and chronic infections in mice. *Experimental parasitology* 1991;73 (4) p. 460 – 468.
13. Dubey J P, Refinement of pepsin digestion method for isolation of *Toxoplasma gondii* from infected tissues. *Vet. Parasitol.*1998; 74:75-77.
14. Hill and Dubey, *T.gondii* transmission, diagnosis and prevention. *Clin Microbiol Infect* 2002; 8: 634–640.
15. Teutsch S M, Juranek D D, Sulzer A, et al, Epidemic Toxoplasmosis Associated with Infected Cats, *N Engl J Med* 1979; 300:695-699.
16. Benenson M W, Takafuji E T, Lemon S M, et al. Oocyst-transmitted toxoplasmosis associated with ingestion of contaminated water. *N Engl J Med* 1982;307:666–669
17. De Moura L, Bahia-Oliveira L M, Wada M Y, Jones J L, et al, Waterborne toxoplasmosis, Brazil, from field to gene. *Emerg Infect Dis.* 2006;12:326–329
18. Magalie et al, Fatal Outbreak of Human Toxoplasmosis along the Maroni River: Epidemiological, Clinical, and Parasitological Aspects, *Clinical Infectious Diseases* 2007; 45:e88–95.
19. Frenkel J K, Pathology and pathogenesis of congenital toxoplasmosis, *Bull. N. Y. Acad. Med.* 1974;Vol. 50, No. 2.
20. Nelson J C, Kauffmann D J, Ciavarella D, et al, Acquired toxoplasmic retinochoroiditis after platelet transfusions. *Ann Ophthalmol.* 1989 Jul;21(7):253-254
21. Renoult E, Georges E, Biava M et al Toxoplasmosis in Kidney Transplant Recipients: Report of Six Cases and Review, *Clin Inf Dis* 1997;24:625-634
22. Siegel S E, Lunde M N, Gelderman A H, et al. *Blood.* 1971;37(4):388-394.

23. Bowry T R, Camargo M E, Kinyanjui M, Sero-epidemiology of *Toxoplasma gondii* infection in young children in Nairobi, Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1986; 80 (3) p. 439-441.
24. Simapore et al. *J of medical virology* 2006, 78: 730-733.
25. Jones, Kruszon-Moran, Wilson, et al *Toxoplasma gondii* Infection in the United States: Seroprevalence and Risk Factors. *Am J Epidemiol* 2001; Vol. 154, No. 4.
26. Studenicova et al, *European Journal of Internal Medicine* 17 (2006) 470–473.
27. Cook A J C, Gilbert R E, Buffolano W et al on behalf of the European Research Network on Congenital Toxoplasmosis Sources of toxoplasma infection in pregnant women: European multicentre case-control study, *BMJ* 2000;321:142
28. Brindle R, Holliman R, Gilks C, Waiyaki P 1991, *Transactions of Royal Society of Tropical Medicine and Hygiene*.1991; 85:750-1.
29. Rubina Shaheed Cocker. An immunological survey of toxoplasmosis in a rural Kenyan population. *M.Med dissertation in Human Pathology*, 1991. UON library reference QR 201. T53C6
30. Kamau P, M.med Dissertation, 2011.
31. Lindstrom L, Deogratias H, Kaddu-Mulindwa, et al, *Acta Trop.* 2006 Dec;100(3):218-22. *Epub* 2006 Dec 8.
32. Kistiah K et al, *SA J,Epidem, Inf* 2011;26 (4) Part 1 (225-228).
33. Doehring E, Reiter-Owona E, Bauer O et al, *Toxoplasma gondii* antibodies in pregnant women and their newborns in Dar es Salaam, Tanzania *Am J Trop. Med. Hyg* June 1995; 52(6): 546-8.
34. Ayeh-Kumi P. F, Opoku A.G, Kwakye-Nuako G et al, Sero-prevalence of toxoplasmosis among patients visiting the Korle-Bu Teaching Hospital, Accra, Ghana. *RIF* 1(3):147-150 (2010)

35. Maiga I, Kiemtore P, Tounkara A. Prevalence of antitoxoplasma antibodies in patients with acquired immunodeficiency syndrome and blood donors in Bamako. *Bull Soc Pathol Exot* 2001; 94:268–270.
36. Dhumne M, Sengupta C, Kadival G et al (2007) National Seroprevalence of *Toxoplasma gondii* in India. *Journal of Parasitology*; 2007, Vol. 93, No. 6, pp. 1520-1521.
37. Fromont, Riche, Rabilloud *Toxoplasma* seroprevalence in a rural population in France: detection of a household effect. *BMC Infectious Diseases* 2009, 9:76
38. Montoya J G, Jordan R, Lingamneni S, et al Toxoplasmic myocarditis and polymyositis in patients with acute acquired toxoplasmosis diagnosed during life. *Clin Infect Dis*. 1997 Apr;24(4):676-683.
39. Chandenier J, Jarry G, Nassif D, et al. Congestive heart failure and myocarditis after seroconversion for toxoplasmosis in two immunocompetent patients. *Eur J Clin*.
40. Vogel N, Kirisits M, Michael E, et al. Congenital toxoplasmosis transmitted from an immunologically competent mother infected before conception. *Clin Infect Dis* 1996; 23: 1055–60.
41. Dunn D, Wallon M, Peyron F, et al, Mother-to-child transmission of toxoplasmosis: risk estimates for clinical counselling. *Lancet* 1999; 353: 1829–33.
42. Gavinet MF, Robert F, Firtion G, et al. Congenital toxoplasmosis due to maternal reinfection during pregnancy. *J Clin Microbiol*. 1997; 35:1276-1277
43. Frenkel, J.K., *Toxoplasma* in and around us. *BioScience* 1973; 23, 343–352.
44. McAuley J, Boyer K.M., Patel D, et al. Early and longitudinal evaluations of treated infants and children and untreated historical patients with congenital toxoplasmosis: the Chicago collaborative treatment trial. *Clin. Infect. Dis*. 18 (1), 38–72 (Erratum in: *Clin Infect Dis* 1994; 19(4): 820).
45. Guerina N.G., Hsu H.W, Meissner H.C, et al. Neonatal serologic screening and early treatment for congenital *Toxoplasma gondii* infection. *N. Engl. J. Med* 1994. 330, 1858–1918.

46. Montoya and Remington, Toxoplasmic Chorioretinitis in the Setting of Acute Acquired Toxoplasmosis, *Clin Infect Dis.* 1996;23(2):277-282
47. Glasner P D, Silveira C, Kruszon-Moran D, et al. An unusually high prevalence of ocular toxoplasmosis in southern Brazil. *Am J Ophthalmol.* 1992; 114:136–144.
48. Schwarzman, Maguire, *Systemic coccidia*,97;1141- 1151.
49. Mariuz P, Bosler EM, Luft BJ. Toxoplasma pneumonia, *Semin Respir Infect.* 1997;12(1):40-3.
50. Hofman P, Drici MD, Gibelin P, et al. Prevalence of toxoplasma myocarditis in patients with the acquired immunodeficiency syndrome. *Br Heart J.* 1993; 70:376–381.
51. Mary, A. S., and M. Hamilton.. Ventricular tachycardia in a patient with toxoplasmosis. *Br. Heart J.* 1973; 35:349-352
52. Reiter-Owana et al, Bulletin of the World Health Organisation, 1999, 77(11)
53. Skinner L J, Chatterton J M, Joss A W et al □ The use of an IgM immunosorbent agglutination assay to diagnose congenital toxoplasmosis. *Journal of medical microbiology* 1989; 28 (2) p. 125-128
54. Verhofstede C, Van Renterghem L, Plum J. Comparison of six commercial enzyme linked immunosorbent assays for detecting IgM antibodies against *Toxoplasma gondii*. *J Clin Pathol.* 1989; 42(12):1285-1290.
55. Naot Y, Desmonts G, and Remington J S. IgM enzyme-linked immunosorbent assay test for the diagnosis of congenital *Toxoplasma* infection. *J. Pediatr* 1981; 98:32-36.
56. Hedman K, Lappalainen M, Seppaia E, et al. Recent primary toxoplasma infection indicated by a low avidity of specific IgG. *J Infect Dis* 1989; 159:736-40.
57. Joynson D H M, Payne R A, Rawal B K. Potential role of IgG avidity for diagnosing Toxoplasmosis. *J Clin Pathol* 1990; 43:1032-1033
58. Policy guidelines on blood transfusion in Kenya, Nov 2011; 4,11.

59. Kenya National Bureau of Statistics, population and housing census 2009.
60. WHO Blood Safety and Availability. Fact Sheet No. 279, updated June 2013.
61. Al Omari A M, Prevalence of antibodies to *Toxoplasma gondii* among blood donors in Abha, Asir Region, south-western Saudi Arabia, *J Egypt Public Health Assoc.* 1994;69(1-2):77-88
62. Sundar P, Mahadevan A, Jayshree R S et al, *Toxoplasma* seroprevalence in healthy voluntary blood donors from urban Karnataka. *Indian J Med Res.* 2007; 126(1):50-5
63. Alvarado-Esquivel C, Mercado-Suarez M F, Rodríguez-Briones A, et al, Seroepidemiology of infection with *Toxoplasma gondii* in healthy blood donors of Durango, Mexico. *BMC Infectious Diseases* 2007, 7:75 doi:10.1186/1471-2334-7-75

Appendix 1:



Dr. Wambua D. Njale,
P. O. Box 14160-00100,
Nairobi,
Kenya.
27/08/2012.

The Director,
UNITID,
P.O. Box 19676-00202
Kenya National Hospital University Campus.

Dear Sir,

RE: REQUEST FOR THE USE OF THE UNITID LABORATORY SERVICES.

My name is Dr. Wambua D. Njale. I am a post graduate student pursuing my M.Vet degree in Internal medicine. I am currently in my 2nd year, working on my thesis, which is assessing the prevalence of *Toxoplasma gondii* infection in donated blood at the National Blood Transfusion Service. I will be assessing for the presence of IgG and IgM markers of exposure and recent infection respectively, as well as assessing IgG avidity in the past via IgG's to ascertain how recent the infections truly are.

I humbly request your assistance in carrying out this study by allowing me to use your staff and your personnel (Cell technician). I will provide all the test kits and reagents necessary.

The study protocol has already been presented to the Department of Medicine and has been approved. I've attached the correct paper of the study.

Kindly consider my request.

Sincerely,

Dr. Wambua D. Njale.

Dr. Ogugi / *Approved*

Please assist the student

Approved: [Signature] 28/8/12

[Signature]
23/8/12

Telephone: NBTS (HQs)
020-2012867



KENYA NATIONAL BLOOD
TRANSFUSION SERVICE (K.N.B.T.S)
P.O. BOX 29804-00202
NAIROBI

MINISTRY OF MEDICAL SERVICES

Ref. No:.....ST/7/1/7 Vol. I

Date....., 20.....
3rd September, 2012

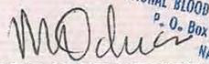
The Director
University of Nairobi
Ethical Research Committee
NAIROBI
Kenya.

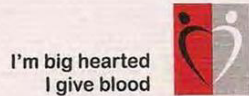
RE: STUDY ON PREVALENCE OF TOXOPLASMOSIS IN BLOOD DONORS

Dr. Wambua Ndele has expressed interest to carry the above study at National Blood Transfusion Service (NBTS) for her Mmed thesis.

NBTS has granted her permission to use samples from VNRBD to carry out the study.

We wish her well.


HEAD
NATIONAL BLOOD TRANSFUSION SERVICES
P. O. Box 29804 - 00202
NAIROBI.
DR. MARGARET ODUOR
Head-National Blood Transfusion Services



IT'S SAFE AND IT SAVES

Study proforma

RBTC code.....

Venue site.....

District

Date.....

Donor Ref Number	D.O.B/ Age	Gender	Level of education	Occupation	No of previous donations

Results

Donor Ref Number	Blood group	HIV	Hep B	Hep C	VDRL	Toxo IgG	Toxo IgM	Toxo IgG avidity.

Appendix 4:

TOXO IgM

ELISA Test for the Detection of IgM Antibodies to *Toxoplasma Gondii* in Human Serum

Package Size

[REF] ⁶	51109	96 Tests	Complete Test Kit
[IVD]			

Intended Use

The TOXO IgM ELISA is intended for the detection of Immunoglobulin M (IgM) class antibodies to *Toxoplasma gondii* in human serum.

Toxoplasma infects nearly all mammals and birds. It is the most widely distributed of all intracellular parasites. Humans become infected through contamination with feces or uncooked meat, or through direct inoculation via blood transfusions or congenital transmission.

Pregnant women who acquire toxoplasmosis during the first trimester have a 25% risk of fetal transmission resulting in spontaneous abortions, stillborns, or severe disease. Sixty five percent of infants born to women infected during the third trimester have subclinical infection with ultimately 85% developing chorioretinitis or neurological sequelae.

Principle - Classic EIA -

The HUMAN TOXO IgM ELISA is based on the classical ELISA technique. The microtiter strip wells as a solid phase are coated with purified *Toxoplasma gondii* membrane antigens (TOXO-Ag). In the first incubation step corresponding specific antibodies (TOXO-IgM-Ab) present in patient specimens or controls bind to the antigens at the solid phase. The sample dilution buffer contains anti-human IgG to prevent rheumatoid factor (RF) interference and competition from specific IgG present in the specimen.

At the end of the incubation unbound components are washed out. For the second incubation step anti-IgM conjugate (anti-human IgM antibodies, peroxidase conjugated) is added which binds specifically to IgM class antibodies resulting in the formation of typical immunocomplexes. After a second washing step to remove excess conjugate, TMB/Substrate is added (Step 3). A blue colour develops changing to yellow after stopping the reaction. The intensity of the colours is directly proportional to the TOXO-IgM-Ab concentration in the specimen.

The absorbance of calibrators and specimen is determined by using an ELISA microplate reader (HUMAREADER). Results for patient samples are obtained by comparison with a cut-off value.

Reagents and Contents

[MIC]	12	Microtiter Strips (in 1 strip holder) (Code TOX M) 8-well snap-off strips coated with <i>Toxoplasma gondii</i> membrane antigen	
[NC]	2.5 ml	TOXO IgM Negative Control (green cap) ready for use, human	
[PC]	2.5 ml	TOXO IgM Positive Control (red cap) ready for use, human	
[DIL-M] 5111	100 ml	Dilution Buffer IgM (blue cap) ready for use, coloured green Phosphate buffer NaCl Albumin Anti-human-IgG (goat)	pH 6.5 ± 0.2 10 mmol/l 8 g/l 10 g/l
[CON]	12 ml	Anti-IgM Conjugate (white cap) ready for use, coloured red Anti-human IgM (rabbit), peroxidase-conjugated	
[WS] 5102	50 ml	Washing Solution (white cap) Concentrate for about 1000 ml Tris buffer NaCl	pH 7.2 ± 0.2 10 mmol/l 8 g/l
[SUB] 5103	15 ml	Substrate Reagent (black cap) ready for use, colourless to bluish 3,3',5,5'-tetramethylbenzidine (TMB) Hydrogen peroxide	pH 3.7 ± 0.2 1.2 mmol/l 3 mmol/l
[STOP] 5104	15 ml	Stop Solution (red cap) Sulphuric acid, ready for use	0.5 mol/l
	2	Adhesive Strips	

Preservatives: Total concentration < 0.1%

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and controls should be handled as potentially infectious. The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or controls should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

[STOP] irritates eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

After opening reagents have to be used within 60 days.

[MIC] (Code: TOX M)

– sealed in an aluminium bag with a desiccant.

– must be at room temperature before opening.

– unused: return with the desiccant to the zip-lock bag and store in this way at 2...8°C.

Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to **room temperature** (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Notes

The **general purpose reagents** **[DIL-M]** 5111, **[WS]** 5102, **[SUB]** 5103, **[STOP]** 5104 are interchangeable between different lots and kits. For **IgM tests** use only IgM dilution buffer **[DIL-M]** 5111.

All other reagents are **specific for the individual package lot** and must not be interchanged with other lots. No reagents of other manufacturers should be used along with reagents of this kit.

Working Wash Solution [WASH]

– dilute **[WS]** 5102 1+20 with fresh deionised water, e.g. 50 ml **[WS]** 5102 + 1000 ml = 1050 ml.

– Stability: **1 week at 2...8°C.**

Specimen

Serum

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 7 days at 2...8°C or longer at -20°C. **Freeze and thaw once only.** Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record specimens and controls carefully on the spread sheet supplied with the kit.

P4: **[MIC]** - select the required number of Microtiter Strips.

P5: **Run duplicates** for controls. Pipette controls and specimen on the **bottom** in the microwells.

P6: **Always add reagents in the same order and timing to minimise reaction time differences between wells.** This is important for reproducible results. Pipetting of specimens should not exceed 5 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: **Avoid/remove air bubbles** prior to incubations and reading of absorbance.

P8: **[SUB]** – incubate in the dark. **[SUB]** initiates a kinetic reaction, which is terminated by **[STOP]**.

P9: **[DIL-M]** – turbidity after addition of the sample has no influence on the results.

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove Adhesive Strips, aspirate off the contents into 5% sodium hypochlorite solution and add [WASH] to each well, aspirate off after 30 sec. soak time and repeat washing.

W2: In case of automatic washers prime with [WASH] and wash strips 4 resp. 5 times. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.

Sample Preparation:

Dilute the patient's sera 1+100 with [DIL-M] 5111, e.g. 10 µl serum + 1 ml [DIL-M] 5111, mix thoroughly (see P9).

Incubate diluted samples at least 5 min. prior to further processing.

Diluted samples can be stored up to 24 h at 2...8°C before testing. Controls are ready for use.

Step 1	Well [µl]			
	A1 Blank	B1/C1 [NC]	D1/E1 [PC]	F1... Sample
[NC] in duplicate	--	100	--	--
[PC] in duplicate	--	--	100	--
Diluted samples	--	--	--	100
[MIC] cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash 4 times as described (see W1 - W3)				
[WASH]	350	350	350	350
Step 2				
[CON]	--	100	100	100
[MIC] cover with Adhesive Strips				
Incubate 30 min. at 17...25°C				
Wash 5 times as described (see W1 - W3)				
[WASH]	350	350	350	350
Step 3				
[SUB] 5103	100	100	100	100
Incubate 15 min. at 17...25°C (see P8)				
[STOP] 5104	100	100	100	100
Mix carefully				
Zero the ELISA microtiter plate reader (HUMAREADER) using the substrate blank in well A1.				
Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of the reaction, using a reference wavelength of 630-690 nm (if available).				

Calculation of Control Values and Cut-off

Mean absorbance values of [NC] in wells B1 and C1 (MNC) and [PC] in wells D1 and E1 (MPC) are calculated according to:

$$MNC = \frac{A_{450}(B1) + A_{450}(C1)}{2}; \quad MPC = \frac{A_{450}(D1) + A_{450}(E1)}{2}$$

$$\text{Cut-off value COV} = MNC + 0.2 \times MPC$$

The test run may be considered valid provided that the following criteria are met:

1. Substrate blank in well A1 < 0.150
2. MNC ≤ 0.250
3. MPC ≥ 0.400
4. MPC : MNC ≥ 3

Interpretation of Results

$A_{450}(\text{patient}) \geq \text{COV} + 20\%$: anti-TOXO-IgM-Ab-positive
 $A_{450}(\text{patient}) < \text{COV} - 20\%$: anti-TOXO-IgM-Ab-negative

Due to physiological and analytical variations patient results lying 20% above or below the calculated cut-off are equivocal. It is recommended to measure these samples in parallel with a fresh sample taken 7 to 14 days later, each in duplicate. The trend between the specific antibody levels should be used for interpretation, also taking into consideration the specific IgG concentration (HUMAN ELISA IgG), the patient history and additional investigations. Repeatedly reactive or equivocal samples may be subjected to a confirmatory test.

Samples from patients with infectious mononucleosis may give an equivocal or low positive result. This may be due to a reactivation of Toxo IgM antibody production. The possibility of an infectious mononucleosis infection should therefore be investigated before interpretation of results.

If an ELISA reader is not available a visual interpretation of results is possible:

- * The substrate blank in well A1 should appear colourless.
- * A specimen can be considered positive if the colour of the sample well is definitely stronger than the colour of the [NC] wells B1/C1.

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via

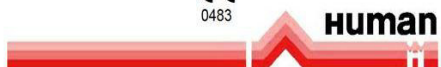
www.human.de/data/gb/vr/el-toxom.pdf or
www.human-de.com/data/gb/vr/el-toxom.pdf

Literature

1. Engvall, E., Perlmann, P., Enzyme linked immunosorbent assay (ELISA), Quantitative assay for immunoglobulin G. *Immunochemistry* 8, 871-874 (1971)
2. Engvall, E., Perlmann, P., Enzyme linked immunosorbent assay (ELISA), III. Quantitation of specific antibodies by enzyme-labelled anti-immunoglobulin in antigen coated tubes, *J. Immunol.* 109, 129-135 (1972)
3. Remington, J.S., Klein, J.O., Infectious diseases of the fetus and newborn infant. Sanders, Philadelphia, London, Toronto (1976)
4. Bidwell, D.E. et al., Enzyme-immunoassays for viral diseases. *J. Infect. Dis.* 136, Supplement 274-278 (1977)
5. Volk, W.A. *Essentials of Medical Microbiology*, Second ed., J.B. Lippincott Company, Philadelphia, New York, San Jose, Toronto, 728-279 (1982)
6. ISO 15223 Medical devices – Symbols to be used with medical device labels, labelling and information to be supplied.

EL-TOXOM
INF 5110901 GB
01-2004-13

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0483



Human Gesellschaft für Biochemica und Diagnostica mbH
Max-Planck-Ring 21 · D-65205 Wiesbaden · Germany
Telefon: +49 6122 9988 0 · Telefax: +49 6122 9988 100 · eMail: human@human.de

Appendix 5:

TOXO IgG

ELISA Test for the Detection of IgG Antibodies to *Toxoplasma Gondii* in Human Serum

Package Size

[REF]	51209	96 Tests	Complete Test Kit
[IVD]			

Intended Use

The TOXO IgG ELISA is intended for the detection of Immunoglobulin G (IgG) class antibodies to *Toxoplasma gondii* in human serum.

Toxoplasma infects nearly all mammals and birds. It is the most widely distributed of all intracellular parasites. Humans become infected through contamination with feces or uncooked meat, or through direct inoculation via blood transfusions or congenital transmission.

Pregnant women who acquire toxoplasmosis during the first trimester have a 25% risk of fetal transmission resulting in spontaneous abortions, stillbirths, or severe disease. Sixty five percent of infants born to women infected during the third trimester have subclinical infection with ultimately 85% developing chorioretinitis or neurological sequelae.

Principle - Classic EIA -

The HUMAN TOXO IgG ELISA is based on the classical ELISA technique. The microtiter strip wells as a solid are coated with *Toxoplasma* antigens (TOXO-Ag) prepared from sonicated whole *Toxoplasma gondii* parasites (Tachyzoites). In the first incubation step corresponding specific antibodies (TOXO-IgG-Ab) present in patient specimens or controls bind to the antigens at the solid phase. At the end of the incubation unbound components are washed out. For the second incubation step anti-IgG conjugate (anti-human IgG antibodies, peroxidase conjugated) is added which binds specifically to IgG class antibodies resulting in the formation of typical immunocomplexes. After a second washing step to remove excess conjugate, TMB/Substrate is added (Step 3). A blue colour develops changing to yellow after stopping the reaction. The intensity of the colour is directly proportional to the TOXO-IgG-Ab concentration in the specimen.

The absorbance of calibrators and specimen is determined by using an ELISA microplate reader (HUMAREADER). Results for patient samples are obtained either by comparison with a cut-off control or in IU/ml by quantitative estimation using a calibration curve constructed with the help of the cut-off and 3 positive controls.

Reagents and Contents

[MIC]	12	Microtiter Strips (in 1 strip holder) (Code TOX G) 8-well snap-off strips coated with sonicated <i>Toxoplasma gondii</i> antigen	
[NC]	2.5 ml	TOXO IgG Negative Control (green cap) ready for use, human	
[CC]	2.5 ml	TOXO IgG Cut-Cff Control (white cap) ready for use, human	5.0 IU/ml
[PCL]	2.5 ml	TOXO IgG Positive Control Low (red cap) ready for use, human	30 IU/ml
[PCM]	2.5 ml	TOXO IgG Positive Control Medium (red cap) ready for use, human	100 IU/ml
[PCH]	2.5 ml	TOXO IgG Positive Control High (red cap) ready for use, human [CC], [PCL], [PCM], [PCH]-Calibrated against the 2 nd International Standard Preparation (WHO anti- <i>Toxoplasma</i> serum).	200 IU/ml
[DIL-G]	100 ml	Dilution Buffer IgG (white cap) Ready for use, coloured green	pH 6.5 ± 0.2
[5121]		Phosphate buffer	10 mmol/l
		NaCl	8 g/l
		Albumin	10 g/l
[CON]	12 ml	Anti-IgG Conjugate (white cap) Ready for use, coloured red Anti-human IgG (rabbit), peroxidase-conjugated	
[WS]	50 ml	Washing Solution (white cap) Concentrate for about 1000 ml	pH 7.2 ± 0.2
[5102]		Tris buffer	10 mmol/l
		NaCl	8 g/l
[SUB]	15 ml	Substrate Reagent (black cap) ready for use, colourless to bluish	pH 3.7 ± 0.2
[5103]		3,3', 5,5'-tetramethylbenzidine (TMB)	1.2 mmol/l
		Hydrogen peroxide	3 mmol/l

[STOP]	15 ml	Stop Solution (red cap)	
[5104]		Sulphuric acid, ready for use	0.5 mol/l
	2	Adhesive Strips	

Preservatives: Total concentration < 0.1%

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and controls should be handled as potentially infectious. The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or controls should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

[STOP] irritates eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.
After opening reagents have to be used within 60 days.

[MIC] (Code: TOX G)

- sealed in an aluminium bag with a desiccant.
- must be at room temperature before opening.
- unused: return with the desiccant to the zip-lock bag and store in this way at 2...8°C.

Do not touch the upper rim or the bottom of the wells with fingers.

Reagent Preparation

Bring all reagents to room temperature (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Notes

The general purpose reagents [DIL-G] 5121, [WS] 5102, [SUB] 5103, [STOP] 5104 are interchangeable between different lots and kits. For IgG tests use only IgG dilution buffer [DIL-G] 5121.

All other reagents are specific for the individual package lot and must not be interchanged with other lots. No reagents of other manufacturers should be used along with reagents of this kit.

Working Wash Solution [WASH]

- dilute [WS] 5102 1+20 with fresh deionised water, e.g. 50 ml [WS] 5102 + 1000 ml = 1050 ml.
- Stability: 1 week at 2...8°C.

Specimen

Serum

Do not use highly lipemic or hemolysed specimens.

Specimens may be stored for 7 days at 2...8°C or longer at -20°C.

Freeze and thaw once only. Thawed specimen must be homogenised. Eliminate particulate matter by centrifugation or filtration.

Procedure

Follow the procedure exactly as described.

Procedural Notes

P1: Do not mix caps of vials (risk of contamination). Do not use reagents after their expiration date.

P2: Do not use reagents that could be contaminated or look or smell different than usual.

P3: Record specimens and controls carefully on the spread sheet supplied with the kit.

P4: [MIC] - select the required number of Microtiter Strips.

P5: Run duplicates for controls. Pipette controls and specimen on the bottom in the microwells.

P6: Always add reagents in the same order and timing to minimise reaction time differences between wells. This is important for reproducible results. Pipetting of specimens should not exceed 5 minutes. Otherwise pipette the calibration curve in the indicated positions at half way time of the series. If more than 1 plate is used, repeat the dose response curve for each plate.

P7: Avoid/remove air bubbles prior to incubations and reading of absorbance.

P8: [SUB] - incubate in the dark. [SUB] initiates a kinetic reaction, which is terminated by [STOP].

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high absorbance.

W1: Remove Adhesive Strips, aspirate off the contents into 5% sodium hypochlorite solution and add [WASH] to each well, aspirate off after 30 sec. soak time and repeat washing.

W2: In case of automatic washers prime with [WASH] and wash strips 4 resp. 5 times. Ensure the washer fills all wells completely and aspirates off efficiently after 30 sec. (remaining liquid: < 15 µl).

W3: After washing, remove remaining liquid by tapping the plate upside down on tissue paper.

Pipetting Scheme

Reagents and specimens should be at room temperature before use.

Sample Preparation:

Dilute the patient's sera 1+100 with [DIL-G] 5121, e.g. 10 µl serum + 1 ml [DIL-G] 5121, mix thoroughly.

Diluted samples can be stored up to 48 h at 2...8°C before testing.

Controls are ready for use.

Step 1	Well [µl]			
	A1 Blank	B1/C1 [NC]	D1/C2 [PC]	D2... Sample
[NC] in duplicate	--	100	--	--
[CC] in duplicate, D1/E1	--	--	100	--
[PCL] in duplicate, F1/G1	--	--	100	--
[PCM] in duplicate, H1/A2	--	--	100	--
[PCH] in duplicate, B2/C2	--	--	100	--
Diluted samples	--	--	--	100

[MIC] cover with Adhesive Strips

Incubate 30 min. at 17...25°C

Wash 4 times as described (see W1 - W3)

[WASH]	350	350	350	350
--------	-----	-----	-----	-----

Step 2

[CON]	--	100	100	100
-------	----	-----	-----	-----

[MIC] cover with Adhesive Strips

Incubate 30 min. at 17...25°C

Wash 5 times as described (see W1 - W3)

[WASH]	350	350	350	350
--------	-----	-----	-----	-----

Step 3

[SUB] 5103	100	100	100	100
------------	-----	-----	-----	-----

Incubate 15 min. at 17...25°C (see P8)

[STOP] 5104	100	100	100	100
-------------	-----	-----	-----	-----

Mix carefully

Zero the ELISA microtiter plate reader (HUMAREADER) using the substrate blank in well A1.

Measure the absorbance at 450 nm as soon as possible or within 30 min. after terminating of the reaction, using a reference wavelength of 630-690 nm (if available).

Calculation of Control Values and Cut-off

Mean absorbance values of [NC] (MNC), of [CC] (MCC) and of [PCL], [PCM], [PCH] (MPCL, MPCM, MPCH) are calculated according following example:

$$MCC = \frac{A_{450}(D1) + A_{450}(E1)}{2}$$

The test run may be considered valid provided that the following criteria are met:

1. Substrate blank in well A1 < 0.150
2. MNC ≤ MCC
3. MPCM ≥ 0.750
4. MPCM : MNC ≥ 5

Interpretation of Results

$A_{450}(\text{patient}) \geq MCC + 15\%$: anti-TOXO-IgG-Ab-positive
 $A_{450}(\text{patient}) < MCC - 15\%$: anti-TOXO-IgG-Ab-negative

Due to physiological and analytical variations patient results lying 15% above or below the calculated cut-off are equivocal. It is recommended to measure these samples in parallel with a fresh sample taken 7 to 14 days later, each in duplicate. The trend between the specific antibody levels should be used for interpretation, also taking into consideration the patient history and additional investigations. Repeatedly reactive or equivocal samples may be subjected to a confirmatory test.

Quantitative Estimation of Toxoplasma IgG in Patient Samples

For a quantitative estimate of Toxoplasma IgG antibody levels of positive specimens in IU/ml, the MCC and the 3 MPCs (low, medium, high) (ordinate) are plotted in a graph versus their corresponding *T. gondii* IgG concentrations of 5, 30, 100, and 200 IU/ml (abscissa).

The estimate of levels in patient sera are read off the graph using their individual A_{450} .

Patient sera with A_{450} greater than the [PCH] (200 IU/ml) should be further diluted with dilution buffer IgG, and reassayed before estimating antibody levels.

The clinical significance of changes in *Toxoplasma gondii* specific IgG levels must be interpreted with care.

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/el-toxog.pdf or
www.human-de.com/data/gb/vr/el-toxog.pdf

Literature

1. Engvall, E., Perlmann, P., Enzyme linked immunosorbent assay (ELISA), Quantitative assay for immunoglobulin G. *Immunochemistry* 8, 871-874 (1971)
2. Engvall, E., Perlmann, P., Enzyme linked immunosorbent assay (ELISA). III. Quantitation of specific antibodies by enzyme-labelled anti-immunoglobulin in antigen coated tubes, *J. Immunol.* 109, 129-135 (1972)
3. Remington, J.S., Klein, J.O., Infectious diseases of the fetus and newborn infant. Sanders, Philadelphia, London, Toronto (1976)
4. Bidwell, D.E. *et al.*, Enzyme-immunoassays for viral diseases. *J. Infect. Dis.* 136, Supplement 274-278 (1977)
5. Volk, W.A. *Essentials of Medical Microbiology*. Second ed., J.B. Lippincott Company, Philadelphia, New York, San Jose, Toronto, 728-279 (1982)
6. ISO 15223 Medical devices – Symbols to be used with medical device labels, labelling and information to be supplied.

EL-TOXOG
 INF 5120901 GB
 01-2004-13



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human



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ndix 6:

NovaLisa TM

Toxoplasma gondii IgG Avidity Test

0483

Enzyme immunoassay for the avidity determination of IgG-class antibodies to Toxoplasma gondii in human serum

Enzymimmunoassay zur Bestimmung der Avidität von IgG-Antikörpern gegen Toxoplasma gondii in Humanserum

Only for in-vitro diagnostic use

English: Page 2 to 4

Deutsch: Seite bis

Francais: Page à

Italiano: da Pagina a

Espanol: Página a

For further languages please contact our authorized distributors.

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ENGLISH 0483

1. INTRODUCTION

The avidity determination is a diagnostic method which is used to differentiate a recent (acute) and a more distant (past)

infection with *Toxoplasma gondii* in patient sera. Avidity is the binding force of the antibody (serum specimen) with the

corresponding antigen.

Low avid IgG antibodies in the early stage of infection can be differentiated from high avid antibodies associated with a past

infection.

The determination of IgG antibody avidity is an additional analysis to the classic serology in regard to the status of a

Toxoplasma gondii infection.

2. INTENDED USE

The NovaTec *Toxoplasma gondii* IgG avidity test is intended to differentiate between past and acute infection.

3. PRINCIPLE OF THE ASSAY

Microtiter strip wells coated with *Toxoplasma* antigen are incubated with diluted serum specimen (dual pipetting). After washing

one well is incubated with avidity reagent and the corresponding well with washing buffer. In this step the low avidity antibodies

are removed from the antigens whereas the high avidity ones are still bound to the specific antigens.

Anti human IgG labelled with peroxidase is added. The immuncomplex is visualized with TMB to give a blue reaction product.

Stop solution is added to stop the reaction and changing the colour of the reaction product into yellow. Absorbance at 450 nm is

read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

Avidity Reagent* : 1 bottle containing 15 ml of an Urea solution, coloured blue, ready to use, white cap.

Performance control ** : 1 bottle containing 3 ml of a high avidity diluted serum coloured yellow, ready to use, green cap

* contains 0.1 % Bronidox L

** contains 0.02 % Kathon and 0.02% Bronidox

4.2. Additional materials required but not provided

NovaTec CE-labelled *Toxoplasma gondii* IgG ELISA, [REF] TOXG0460

5. STABILITY AND STORAGE

The reagents are stable up to the expiry date stated on the label when stored at +2...+8 °C.

After first opening the reagents are stable up to the expiry date.

6. REAGENT PREPARATION

The avidity reagent and performance control are ready to use.

Crystallisation of the avidity reagent may occur at low temperature.

Therefore it is very important to bring the avidity reagent and control to room temperature (+20Ń +25°C) before

starting the test run!

7. ASSAY PROCEDURE

Use procedure and test preparation as mentioned in the NovaTec Toxoplasma gondii IgG package insert [REF] TOXG0460

The performance of the avidity test is different as follows:

Dual pipetting of the serum samples and control:

A clean, disposable tip should be used for dispensing the control and serum samples.

1. Wells A1/A2 are used for the substrate blank.
2. Dispense 100 μ l performance control in wells B1/B2.
3. Dispense 100 μ l diluted serum sample (1+100) in wells C1/C2.

Dispense 100 μ l diluted serum sample (1+100) in wells D1/D2 etc. Cover the wells with foil.

4. Incubate for 1 hour \pm 5 min at 37°C \pm 1°C
5. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 μ l of washing solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next

step! (See step 4, package insert Toxoplasma gondii IgG , [REF] TOXG0460)

Note: Washing is critical! Insufficient washing results in poor precision.

6. Dispense 100 μ l of Avidity reagent in wells B1, C1, D1, E1 etc.
Dispense 100 μ l of diluted (1+19) Washing solution in wells B2, C2, D2, E2 etc.
7. Incubate for exactly 5 min at room temperature (+20 to +25°C).
8. Repeat step 5.

9. Dispense 100 µl Toxoplasma anti-IgG Conjugate into all wells except in the blank wells (A1/A2). Cover with foil.

10. Following the instructions 6. - 11. mentioned in the package insert of Toxoplasma gondii IgG ELISA, [REF]

TOXG0460.

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8. RESULTS

8.1. Assay validation criteria

In order for an assay to be considered valid, the following criteria must be met:

- □ Substrate blank in A1/A2: Absorbance value lower than 0.100.
- □ Performance control in B1/B2: Avidity (%): exact value and range are indicated on the label

If these criteria are not met, the test is not valid and must be repeated.

8.2. Calculation of Results

With patient samples having an absorbance value lower than the cut-off determined by the regular NovaTec Toxoplasma gondii

IgG ELISA you may not proceed. These samples contain no antibodies to Toxoplasma at all or a concentration of Toxoplasma

IgG antibody that is low to evaluate IgG avidity. For each patient sample or control calculate the ratio between the absorbance

of the well dispensed with Avidity reagent and the absorbance of the well dispensed with Washing buffer multiplied by 100:

Absorbance (sample or control) Avidity reagent x100 = Avidity(%)

Absorbance (sample or control) Washing buffer (diluted 1 +19)

Samples or controls with an absorbance greater than the measuring range of the ELISA reader (over f / error) at 450 nm the

absorbance of these samples must be read at 405 nm. The calculation of the Avidity (%) is the same as with 450 nm.

8.3. Interpretation of Results

Avidity (%) > 40 Toxoplasmosis antibody with high avidity -- □ □ Past infection

Avidity (%) ~~140~~ Toxoplasmosis antibody with low avidity - - □ □ Acute or recent infection

9. SPECIFIC PERFORMANCE CHARACTERISTICS

9.1. Precision

Intraassay

Sample n Mean avidity(%) CV (%)

High avidity serum 24 89.0 4.1

High avidity serum 22 77.1 4.0

Low avidity serum 20 24.7 4.6

Interassay

Sample n Mean avidity(%) CV (%)

High avidity serum 6 88.8 2.4

High avidity serum 6 78.8 3.2

Low avidity serum 6 25.8 3.9

9.2. Performance Characteristics

The NovaTec Toxoplasma gondii IgG Avidity Test has been evaluated for use in Toxoplasmosis with acute and past infection

sera. A total number of 69 patient samples were tested. These sera were supplied by the Institute of Parasitology, University

Bonn.

Total: 69 patient sera

Acute Infection

Past Infection

Total

Inst. of Parasitology 25 44 69

NovaTec Avidity Test 23 41 64

Discrepancy sera:

NovaTec Avidity Test 3 f Acute 2 f Past 5

Agreement 92% 93.2% 92.7%

These five discrepant sera were retested in an Avidity ELISA of another manufacturer. Four sera were in accordance to

NovaTec Avidity ELISA and 1 serum was different to the NovaTec Test.

9.3. Interferences / Cross reactivity

Regarding the interferences and cross reactivity please refer to the package insert of *Toxoplasma gondii* IgG [REF] TOXG0460.

Note: The results refer to the groups of samples investigated; these are not guaranteed specifications.

10. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values. Diagnosis of an

infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into

consideration clinical history, symptomatology as well as serological data.

In immunocompromized patients and newborns serological data only have restricted value.

A result of high avidity can not exclude the possibility of a recent infection.

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11. PRECAUTIONS AND WARNINGS

□ □In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is

intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure,

the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits

with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well

as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is

responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The

manufacturer is not liable for any results by visual analysis of the patient samples.

□ □Only for in-vitro diagnostic use.

□ □All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-

HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded

and handled as potentially infectious.

□ □Do not interchange reagents or strips of different production lots.

□ □No reagents of other manufacturers should be used along with reagents of this test kit.

- □ Do not use reagents after expiry date stated on the label.
- □ Use only clean pipette tips, dispensers, and lab ware.
- □ Do not interchange screw caps of reagent vials to avoid cross-contamination.
- □ Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- □ After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- □ To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- □ The NovaLisa[®] Avidity Test is only designed for qualified personnel who are familiar with good laboratory practice.

WARNING: Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

11.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

12. ORDERING INFORMATION

Prod. No.: TOXGA460 Toxoplasma gondii IgG Avidity Test (96 Determinations)

BIBLIOGRAPHY / LITERATUR / BIBLIOGRAPHIE / BIBLIOGRAFIA /
BIBLIOGRAFÍA

M.H. Beaman, R.E. McCabe, S-Y. Wong, J.S. Remington, *Toxoplasma gondii*, Inc: Principles and Practice of Infectious

Diseases, G.L. Mandell, J.E. Bennet, R. Dolin, eds., Churchill Livingstone Publ., Fourth edition, p. 2455-2475 (1995)

Katalin N. Horvath, Zsuzsanna Szenasi, Jozsef Danka and Istvan Kucsera: *Acta Parasitologica*, 50 (3), p.255 - 260 (2005)

K. Janitschke, *J. Lab Med*, 29(6), p. 439- 445 (2005)

Hedman K., Lappalainen M., Seppala I., Recent primary *Toxoplasmosa* infection indicated by a low avidity specific IgG,

J. Infect. Dis. 159, p. 736 - 740 (1989)

Joynson, DHM, Payne RA, Rawal BK, Potencial role of IgG avidity for diagnosing *Toxoplasmosis*, *J. Clin. Pathol.* 43, p: 1032-1033 (1990)

Symbols Key/ Symbolschlüssel/ Explication des symboles/ Legenda/ Símbolos

Manufactured by / Hergestellt von/ Fabriqué par/ Prodotto da/ Fabricado por

In Vitro Diagnostic Medical Device/ In Vitro Diagnosticum/ Dispositif médical de diagnostic *in*

vitro/ Diganostico *in vitro*/ Producto para diagnóstico In vitro

Lot Number/ Chargenbezeichnung/ Numéro de lot/ Lotto/ Número de lote

Expiration Date/ Verfallsdatum/ Date de péremption/ Scadenza/ Fecha de caducidad

Storage Temperature/ Lagertemperatur/ Température de conservation/ Temperatura di

conservazione / Temperatura de almacenamiento

CE Mark/ CE-Zeichen/ Marquage CE / Marchio CE/ Marca CE

[REF] Catalogue Number/ Katalog Nummer/ Référence du catalogue/ Numero di codice/
Número de

Catálogo

Consult Instructions for Use/ Gebrauchsanweisung beachten/ Consulter la notice d'utilisation/

Consultare le istruzioni/ Consulte las Instrucciones de Uso

Contains sufficient for `n_ tests/ Ausreichend für `n_ Tests/ Contenu suffisant pour `n_ tests/

Contenuto sufficiente per `n_ saggi/ Contenido suficiente para _n_ tests

Avidity reagent/ Avidity Reagenz

Performance control/ Funktionskontrolle

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SCHEME OF THE ASSAY

Toxoplasma gondii IgG Avidity Test

Assay Preparation

Prepare reagents and samples as described.

Establish the distribution and identification plan for all specimen and controls on the
form supplied in the kit.

Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

Sustrate

blank

(A1/A2)

Performance

control

B1

Performance

control

B2

Sample

diluted

1+100

e.g.C1

Sample

diluted

1+100

e.g.C2

Performance

control-

- 100 \neq 100 \neq - -

Dual pipetting

Sample

(1+100)

- - 100 \neq 100 \neq

Dual pipetting

Cover wells with foil

Incubate for 1 h at 37°C

Wash each well three times with 300 µl of washing solution

Avidity

reagent

- 100 µl 100 µl

Washing

solution - 100 µl 100 µl

Cover wells with foil

Incubate for 5 min at room temperature

Wash each well three times with 300 µl of washing solution

Conjugate - 100 µl 100 µl 100 µl 100 µl

Cover wells with foil

Incubate for 30 min at room temperature in the dark

Wash each well three times with 300 µl of washing solution

TMB 100 µl 100 µl 100 µl 100 µl 100 µl

Incubate for exactly 15min at room temperature in the dark

Stop

solution

100 µl 100 µl 100 µl 100 µl 100 µl

Photometric measurement at 450 nm (reference wavelength: 620 nm)

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