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SUBJECT REVIEW

SUSPICION IN THE FORM OF INFECTION IS THE BASIS FOR SELECTING THE APPROPRIATE METHOD FOR EXAMINING THE TOXOPLASMOSIS DISEASE OF BENDS THAT HAVE NO SYMPTOMS FROM PATIENTS.

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Abstract

Toxoplasmosis is a contagious serious disease that spreads all over the world in human and animals, its caused by *Toxoplasma gondii* parasite, it has the ability to infect various tissues in body patients, the importance of human infection appear in the occurrence of some severe cases in patients, the symptoms appear as a fever and tremors, as well as lymphatic dysfunction hepatitis, and the encephalitis which caused in birth defects. The diagnosis of disease does not depend on the clinical symptoms in patents, but we can diagnosis of the disease by the examination of body fluids using one of the diagnostic tests such as biological tests, serological tests, tissue examination, and molecular methods according of site and form of infection.

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Introduction:-

The diagnosis of *Toxoplasma gondii* parasitic infection does not depend on the presence of clinical signs and symptoms, whether animal or human, because the incidence of this parasite without symptoms of Kindle is similar to many of the symptoms of common diseases such as influenza. Therefore, many serological, other in both animal and human (Edvinsson, 2006), and the absence of symptoms of the disease on the injured makes us wonder what are the methods used to diagnose the incidence of toxoplasmosis? What are their foundations? When will we choose the appropriate method of examination? Therefore, we will discuss the most important diagnostic methods for parasites according to research and references as follows:

Parasite Isolation

It is one of the biological methods for the diagnosis of *T. gondii* parasites, which is the injection of the patient's tissue from the lymph nodes, muscle tissue, and various body fluids such as embryonic fluid, blood, and spinal cord fluid (Murray *et al.*, 2003). Newborns through parasitic isolation of blood and body fluids (Jalalou, 2010), this is a strong evidence of infection. However, such isolation takes time to allow for early diagnosis.

Direct microscopic Examination

This is the simplest and fastest test. However, this technique is very sensitive because it depends on the investigation of the presence of the rapid phase of breeding Tachyzoite in the centrifugal deposits of body fluids such as blood, spinal cord devices after being installed on glass slides using methyl alcohol, dye and taste under the light microscope under the major force or oil lens (Paniker, 2002).

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Skin sensitivity Test

This test is known as the delayed skin hypersensitivity test where cellular immunity, which is positive for several months, is used in this test. Toxoplasmin is used as an antigen, injected as a pure solution, and a redness of 5 cm within 48 hours is a positive result (Abd-AL- Hameed, 2007).

Diagnosis by Computerized Tomography Scan (CT.Scan) and Magnetic Resonance Imaging (MRI)

This method is one of the modern methods used to diagnose parasitic infection in the brain, depending on the imaging of the brain tissue, where the diagnosis of any case of inflammation of the brain resulting from the curve of the universe, and found that the use of magnetic imaging MRI is the most sensitive method of CT class. Scan in patients with cerebral palsy (Berroya *et al.*, 2001).

(DT) Sabin Feldman dye test

This type of test is called Methylene blue dye test. This test uses live parasites in its rapid reproductive phase and is the first serological test used to detect the antibodies specific to toxoplasmosis by researchers Feldman and Sabin in 1948 (Tenter *et al.*, 2000).). The test is easily tested and applied under laboratory conditions. It is used to diagnose toxoplasmosis 1 to 2 weeks after infection and reaches the highest level of antibody after 16-5 weeks. However, it is a disadvantage of this test because it is highly expensive and dangerous to employees because it is used by animals, (Dubey,2007).

Direct heamagglutinatio test

The first to describe and use the DAT is Fulton and Turk in 1959, which is a fast-paced, non-hazardous test using the formalin-infected antigen. Antibodies to the parasite surface are identified as in the most sensitive and specific test of dye (DT) in the discovery of the underlying infection of macular degeneration (Dubey, 2008).

(Modified Agglutination Test (MAT)

Is a test developed and modified by Remington and Desmants in 1980. It was also developed in 1987 by Dubey and Desmant. It is used to detect IgG antibody antibodies by using antigen allergens by destroying IgM antibodies in the patient's serum by using a solution Buffer solution dilute and compound (2-mercapto ethanol)

(IHAT) Indirect Haemagglutination Test

This test was first used (Jacobas and Lunde) in 1957 and is used to kill the infected antigen associated with red blood cells of sheep treated with Pyruvic Aldehyde, as the antibodies bind to sensitive red blood cells.

Latex agglutination test (LAT)

This test is one of the tests to detect antibody antibodies of the conical curved parasite because of its placidity and sensitivity to the host (Salibay *et al.*, 2008). The test kit contains a substance that is attached to the monophyte, soluble molecules as well as parasite antigens. This is because it is used to determine the presence of IgG antibodies as evidence of chronic infection and IgM for latex Antibody Antigen Complex, as well as its ease, sensitivity and privacy, it has the right cost, time and effort to do it (Duck, 2009).

(Complement Fixation Test (CFT)

This test has been widely used in Europe to diagnose toxoplasmosis. Dissolved antigens have been prepared in peritoneal fluid from injected, infected or other animals. However, the defect in this test is the lack of good sensitivity which makes it rarely used today (Rafil , 2008).

Indirect Fluoresnt Antibody Test (IFAT)

This test was used for the first time by Goldman in 1957 to detect conical parasitic antibodies and is widely used but requires a fluorescent microscopy. This test is based on the principle of antigen binding with the specific antibodies found in the patient's serum where it can detect antibodies of the type (IgM), which indicates the presence of acute infection. This test is highly sensitive and easy to administer, allowing positive results at very early stages of the disease, as well as being safer, less expensive and more widely used (Cole *et al.*, 2010).

(Enzyme Linked Immune Sorbent Assay (ELISA)

The first was used in 1971 in the Netherlands by Van-Waeman and Schurs. Nato and Remington have developed the general technique for detecting ELSA antibodies in the blood of the umbilical cord (ELISA, 2004). ELISA includes several types: double ELISA , Micro ELISA, Macro ELISA, and Dot ELISA, which use IgM, IgG, IgG antibody

detection is useful for detecting previous infections with elevated IgG antibody in patients with chronic infection. IgM parasite antibodies are the most commonly used for detecting infection. When the IgM antibody appears shortly after infection (3-5 days), the ELISA method of IgM does not produce false positive results with a Rheumatoid Factor (RF). Several studies have indicated that the IgM antibody continues for 12 years after infection (Montoya, 2002).

IgE antibodies can be detected in serum ELISA in adults and newborns. Blood IgE concentrations in the blood are lower than those of IgM, IgG. This is useful in the diagnosis of newly acquired infections, and IgA antibody can be detected by ELISA. Remain in the blood for 2-3 months, which is useful in the diagnosis of infection in adults (Wilson *et al.*, 1997).

The antigens are used to treat the antibodies specific to the IgG and IgM antibodies. The IgM antibody is raised shortly after stimulation; decreases more rapidly than the IgG, while the antibody IgG antibody (Yazar *et al.*, 2006). This test was found to be 100% effective, 83.3% (Yazar *et al.*, 2006).

Molecular Tests:

Examples of these tests are Polymerase Chain Reaction (PCR), which has a high ability to diagnose the pathogenic organisms, these tests are highly sensitive as they can diagnose the presence of parasites even if there is one parasite in the sample is also highly susceptible to the dissection of unsatisfactory species of pathogen (Al-abodi *et al.*, 2015). One of the main barriers to using this test is the high cost of these tests, as well as the high level of expertise needed (Sebastiaan *et al.*, 2007). The PCR-based methods have been successful in detecting the three species of lamp and have been selected for clinical studies (Calderaro *et al.*, 2006 ; Al-Mayali and Al-abodi, 2017). This technique has evolved into a number of methods that are derived and the most recent and accurate is the polymerase reaction technique in real time. The following is a definition of this technique:

Real time polymerase chain reaction (RT_ PCR)

A new scientific technique combining the amplification of the DNA sample with the detection of the amount and quality of the amplification output in a single reaction tube, which protects the samples from contamination by opening and closing the reaction tubes ((Abdul-Ghani, 2011). This technique is based on double dinucleotide reagents, which are a radioactive dye associated with each detector that has a sequence complementary to one of the resulting tapes. A fluorescent dye, called a Reporter, is connected to the 5-pin reagent the third part of the reagent is connected to another dye called the Quencher dye. When the laser is emitted by laser or halogen, the dye absorbs the energy of the photons and then transfers it to the absorbent dye via the Fluoresce resonance energy transfer (FRET). (Shibli *et al.*, 2008)

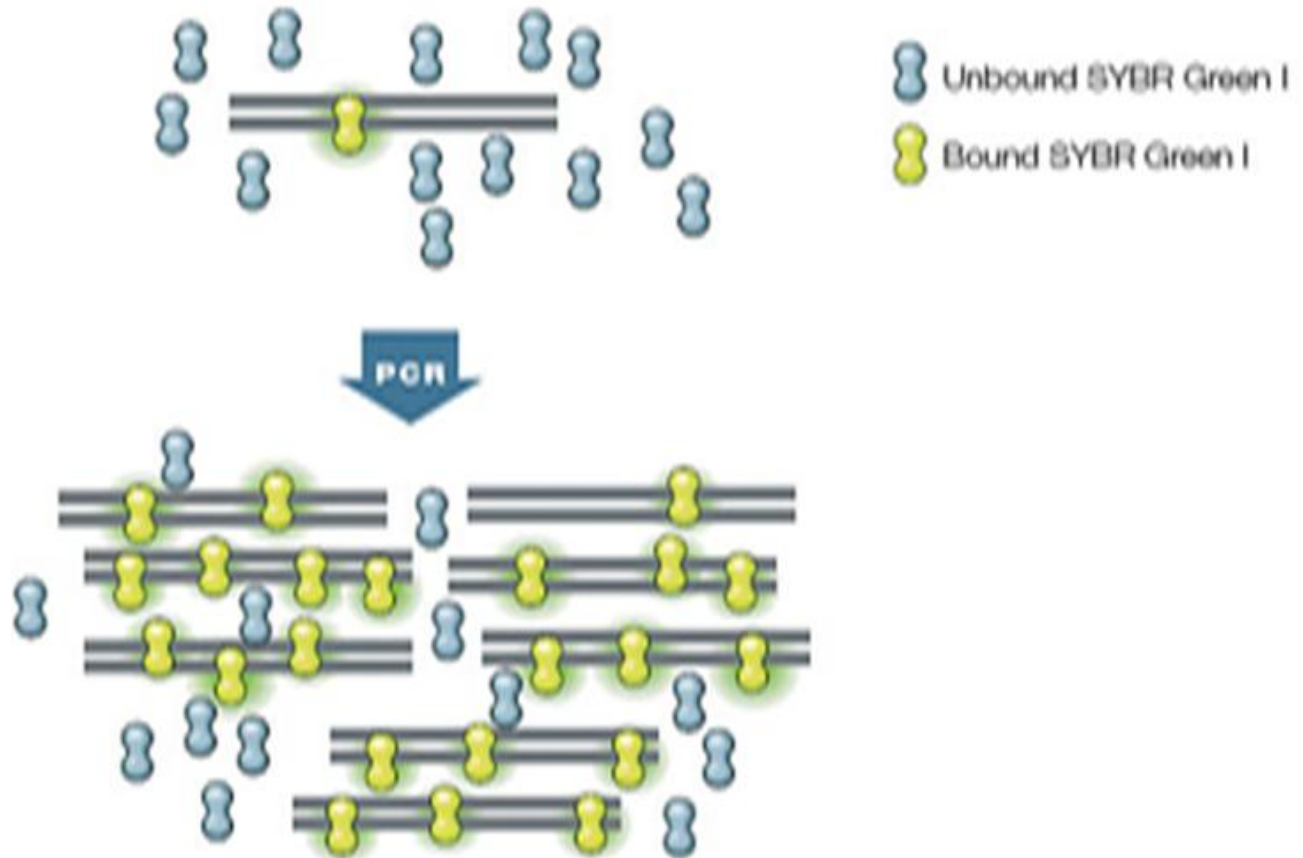
Basics of polymerase chain reaction technology in Real-Time PCR:

The RT-PCR method was first used by Higuchi and his group (1993). The RNA is usually the basis of the real time reverse transcriptase technique and cannot be used as a template in RT-PCR. Therefore, the first step is to transform the template RNA to cDNA in the reverse transcription process through which the gene expression can be measured by RT-PCR technology that can control real-time DNA amplification. This process is carried out by means of special chemicals and tools consisting of a special radioactive dye and sensors that include:

Green Cyberspace Syber green I dye:

It is the most common pigment in the RT-PCR device and is associated with a non-specific type of DNA tape (Abdul-Ghani, 2011; Menotti *et al.*, 2010), which is non-radioactive when placed in a normal solution but lighted and increased 1000 fold when connected with the dsDNA. The green cypress dye is also associated with the polymerase chain reaction products that lead to increased radioactive dye in sequence and can then be determined.

Figure 1:-Cyber green dye is increasingly radioactive when its parts are connected with the dsDNA tape (Dorak, 2006).



Hydrolysis probe dyes:

The TaqMan probe (e.g. the Beacons molecules) is used in this method, which depends on the decomposition of the probe associated with the Quencher dye to produce the Reporter flouochrome dye in the Taq polymerase sequencing phase, which results in the separation of the Reporter dye from the Quencher dye, It is clear that during successive sessions, the sparkle is further enhanced by the expansionary asble and by the increasing color of the Free Reporter (Gibson *et al.*, 1996 ; Wallon *et al.*, 2010).

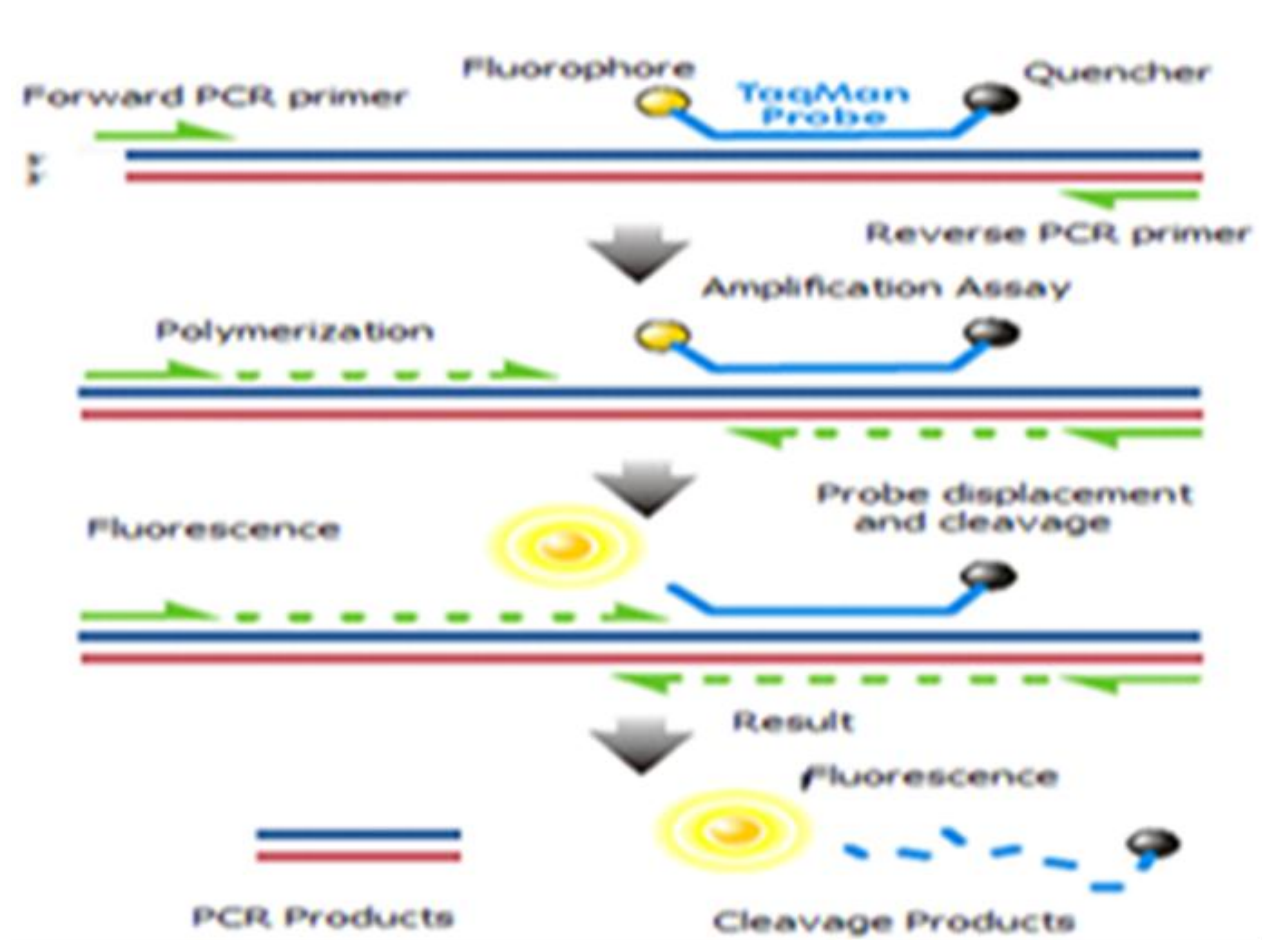


Figure 2:-The chemical mechanism of the TaqMan probe (Dorak, 2006).

Hybridization Technique:

The probe senses the Fluorochrome dye at the third end and the other senses a second dye when the two colors are in a similar space (approximately 0-1 nucleotides) and that the light emitted from the dyed dye will evoke the receiving dye and result in the emission of the fluorescent dye can be determined during the annealing phase. In the first part of the fermentation phase of polymerase chain reaction and after each successive cycle of PCR, the number of hybrid and polycarbonate probes will increase and result in more brilliance. Joint processes between reverse transcription Real-time polymerase chain reaction is very effective when gene expression is measured (Dorak and Tervli, 2009). to analyze the results using RT-PCR, two methods are used:

First: absolute quantity.

Second: Relative Quantitative (Livak and Schmittgen, 2001).

The absolute quantity depends on the comparison between the Threshold cycle (Ct) of the test samples with the standard curve and figure (3):

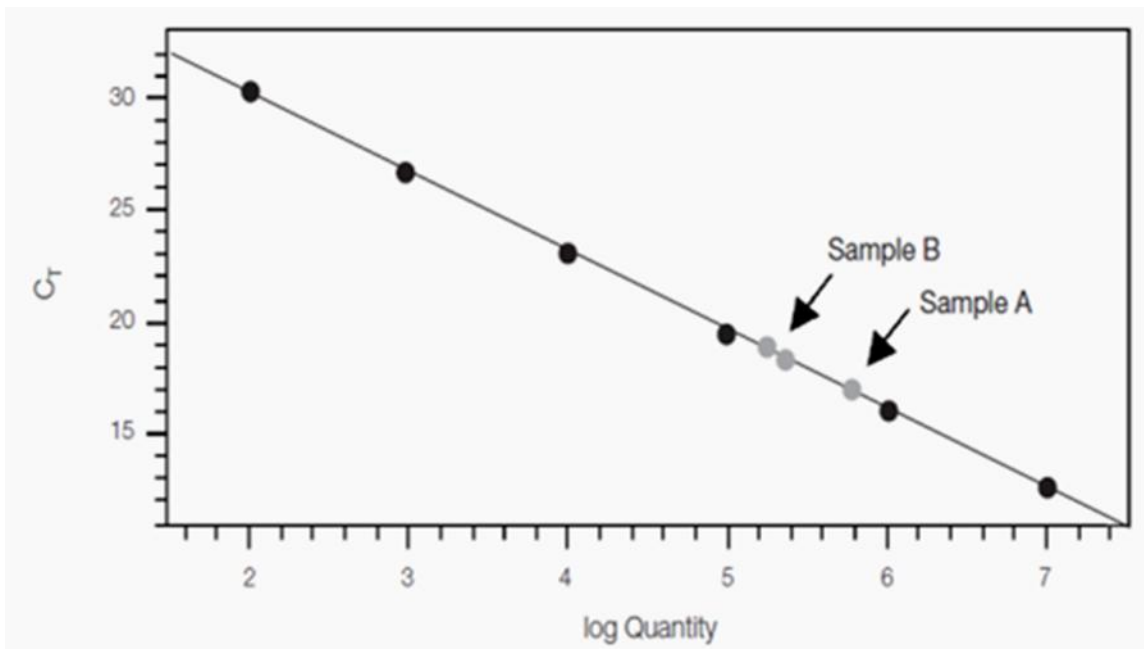


Figure 3:-An example of absolute quantitative measurement using the standard curve to determine the number of copies of β -actin in A and B samples unknown and diluted 10 times from a known concentration of the template (102-107 β -actin) and analyzed with two samples Unknown.

Threshold Cycle (C_t) tells the first signal or radiation of the dye when it binds to nucleic acids when DNA is doubled. This cycle is measured at the exponential phase, for the amplification zone the PCR output is multiplied by each cycle according to Figure (4).

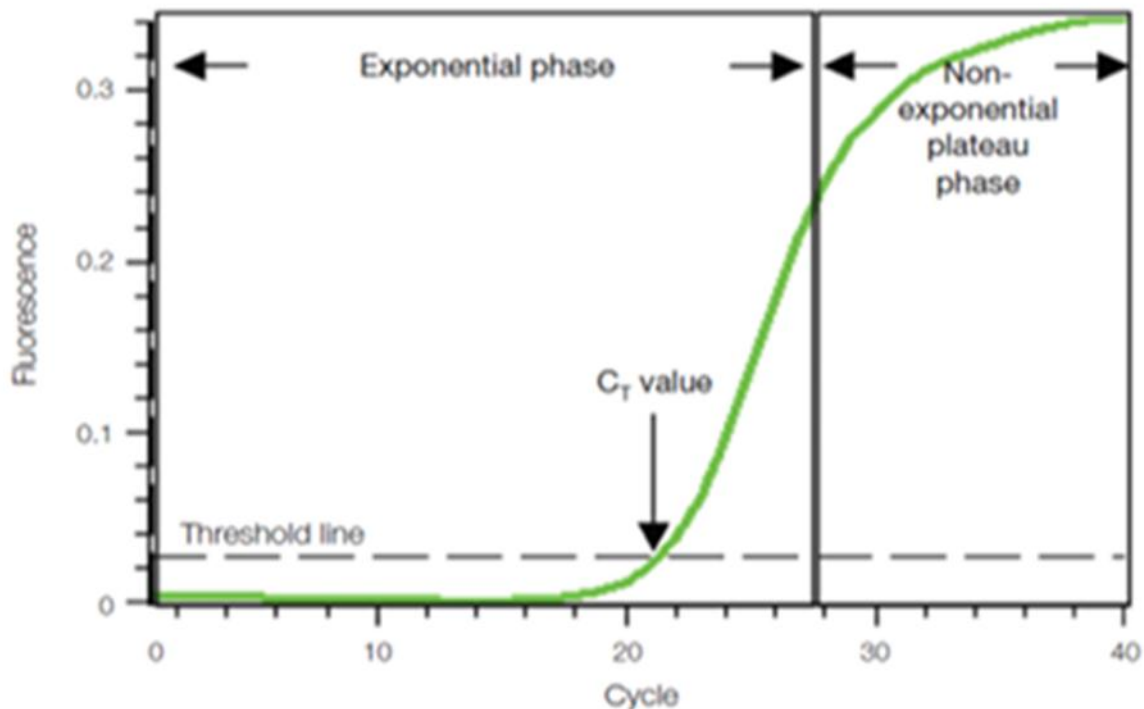


Figure 4:-A phase of amplification of the exponential and non-exponential DNA Plateau phase

The cDNA is the determinant of absolute quantity, and the relative quantity describes the change in the target gene expression, to determine the change in gene expression, three methods are used:

Method 1: (Livac method) $\Delta\Delta C_t$.

Method 2: (Ct method).

Method 3: Pfuffl method (Livak and Schmittgen, 2001).

There are many applications for the use of RT-PCR in medical fields, for example to diagnose HIV (Human Immunodeficiency Virus) (Menotti *et al.*, 2010) and in laboratories for the diagnosis of incurable diseases such as avian and swine flu.

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