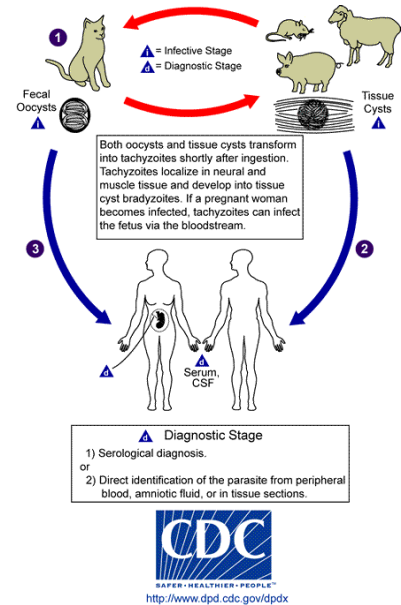


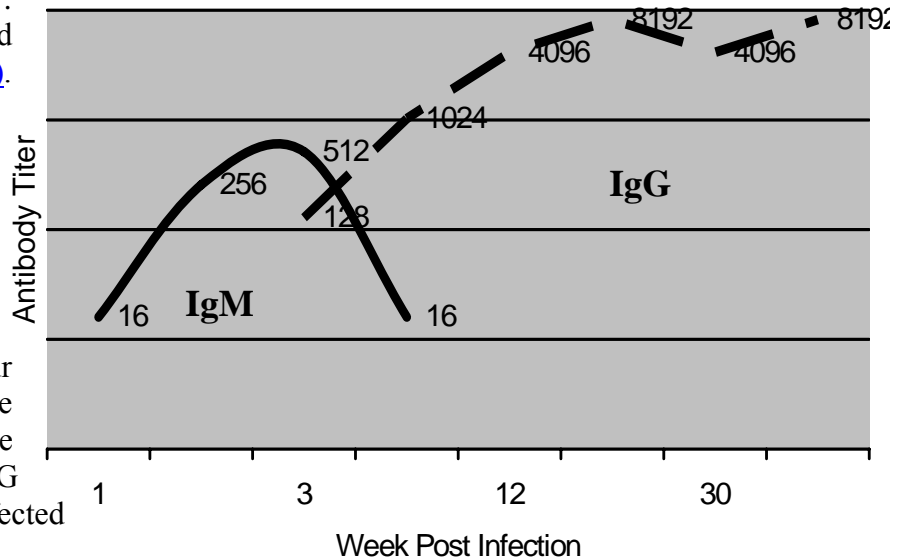
## Serologic Testing for *Toxoplasma gondii* at the University of Tennessee College of Veterinary Medicine

*Toxoplasma gondii* is an obligate intercellular parasite that infects a wide variety of warm blooded mammals and birds. Cats are the only known host where sexual multiplication occurs and results in the dissemination of large quantities of oocysts (egg-like structures), often in the millions, into the environment where they are capable of infecting all types of warm-blooded animals (wildlife, companion animals, domestic livestock), including people. Once infected, these “intermediate hosts” unwittingly contribute to the maintenance and perpetuation of *T. gondii* infection in host populations when the parasite multiplies asexually in the tissues of animals and people. The life cycle is completed when a cat consumes the infected tissues of an intermediate host and initiates a cycle of sexual multiplication. People become infected with *T. gondii* by ingesting oocysts shed by cats into the environment, or by consumption of the infected meat products of domestic food animals like poultry, pigs, and cows, and wildlife like turkey, deer, elk, bear, cougar and other species. Animals that graze, browse, and forage like cattle, sheep goats, deer, birds, and poultry are infected by ingestion of oocysts from fecally contaminated environmental sources like pastures, feed lots, and grain stores. Animals with carnivorous and omnivorous diets like dogs, cats, bear, boar, and other wildlife can be infected either by ingestion of oocysts from the environment, or by consumption of infected meat and edible tissues of their prey animals and scavenged carrion.



The spectrum of disease produced by *T. gondii* ranges from clinically inapparent to severe with neurologic impairment and death. Immunocompetent animals and people may experience transitory flu-like symptoms that include fever, lethargy muscle soreness and enlarged lymph nodes shortly after infection with the parasite. Following these symptoms in the early stage of the infection, the parasite becomes quiescent in the tissues and the host develops antibodies against the parasite that persist for the life of the host. The presence of a measurable antibody titer against *T. gondii* indicates that the host is infected with the parasite and that it is living in their tissues. If the host’s immunity remains intact there may not be any other occurrence of symptoms or disease. However, if the host becomes immunocompromised following chemotherapy or another disease it is possible that the parasite will become active and replicate in the host. Disease ensues from the destruction of host tissues caused by the asexual replication of the parasite, and its invasion of new host cells. When this occurs in the brain and associated tissues of the central nervous system, neurologic disease and perhaps death are eventual outcomes. All females (animal and people alike) are at risk for congenitally infecting their offspring when they acquire the parasite for the first time during pregnancy. Infections acquired during the 1<sup>st</sup> trimester usually result in spontaneous abortion or miscarriage. Offspring infected during the 2<sup>nd</sup> trimester may survive through birth, but with serious congenital deficits including neurologic impairment and possible death in the perinatal period. Infections acquired during the 3<sup>rd</sup> trimester often result in a less severe form of disease with complications like mild retardation and visual impairment that become evident later in life.

The Modified Agglutination Test (MAT) is used for detection of *T. gondii* antibodies in blood, serum, and other bodily fluids from client owned animals submitted to our laboratory at the University of Tennessee College of Veterinary Medicine. The MAT uses formalin fixed *Toxoplasma* tachyzoites to detect IgG antibodies produced by the host animal against the parasite as it invades and replicates in the infected animal's tissues. We believe that the MAT is an ideal test for this purpose in veterinary healthcare settings because it does not require *species specific conjugates* used in serologic assays based on the immunofluorescent antibody (IFA) and enzyme-linked immunosorbent assay (ELISA) methods. The MAT had a sensitivity and specificity of 82.9% and 90.29% when compared against other commercially available serologic assay methods for detection of *T. gondii* IgG antibodies in US raised domestic swine (Dubey et al 1995). The performance of the MAT for detection of IgM antibodies against *Toxoplasma gondii* in naturally infected animals is less clear and there have been few systematic evaluations of the assay for this purpose. In a limited study conducted in our laboratory, IgM antibodies were detected 1 to 2 weeks prior to the development of a measurable IgG antibody titer in experimentally infected opossums.



In our laboratory, we have successfully used the MAT to detect *T. gondii* antibodies in the serum and plasma of a wide variety of companion animal and wildlife species including but not limited to: cats, dogs, cattle, sheep, goats, llama, domestic and feral swine, deer, moose caribou, Australian kangaroos and wallabies, Malagasy lemur species, African and South American primates, marine mammals, various bird species, chickens and other poultry. We have also used the MAT to detect *T. gondii* antibodies in ocular fluid of domestic cats, cerebro-spinal fluid of cats and dogs, and domestic livestock, and quite interestingly the breast milk of a lactating Asian elephant at the Knoxville Zoo. We have found that the performance of the MAT is not unduly compromised with hemolyzed or lipemic serum samples, and that the assay performed consistently even with whole-blood samples from an infected subject that were dried on filter paper for several weeks.

An algorithm for interpreting *T. gondii* serologic test results produced by our laboratory at UTCVM is presented in the following table. The high specificity of the MAT for measuring IgG supports the interpretation that an individual with a positive antibody titer is infected with *T. gondii*. In our experience we have found that individuals with a positive IgG titer of 32 or greater will continue to test positive on repeated follow-up examinations. In most cases, the titer will actually increase exponentially over time until it reaches a plateau usually between 512 and 8192. The primary problem with testing for anti-*Toxoplasma* IgM is lack of specificity, or the inability to distinguish a "True-Negative" from a "False-Positive" test result. Two outcomes are commonly associated with samples submitted to our laboratory: (1) individuals with a positive

IgM result and negative IgG on repeated follow-up examinations, and (2) individuals with a positive IgM result that subsequently develop a positive IgG titer on repeated follow-up examinations, usually within a 3 to 4 week period. In the former case, we are fairly confident in ruling out *T. gondii* as a potential etiology associated with the symptoms of the clinical complaint because there has been no subsequent development of *T. gondii* specific IgG antibodies. At the present time, there do not appear to be any chemotherapeutic regimens that are effective for killing *T. gondii* parasites during the IgM phase of the host immune response that will prevent the subsequent development of IgG antibodies. In the second situation, the detection of *T. gondii* specific IgG antibodies on the follow-up examination conducted within 3 weeks of the initial IgM titer retrospectively confirms that the patient is infected with *T. gondii* parasites. We recommend starting treatment with appropriate anti-*Toxoplasma* therapy for patients with a high index of suspicion that test positive for IgM by the MAT. This treatment can be suspended, at the clinician's discretion, if the patient fails to develop the predicted *T. gondii* specific IgG response on the follow-up examination. Patients with confirmed *T. gondii* infections (IgG positive by MAT) should receive chemotherapy with an appropriate anti-*T. gondii* regimen until clinical signs resolve or improve to the attending clinician's satisfaction. There is no scientific basis for expecting that the patient will seroconvert to negative IgG status following treatment. *Toxoplasma gondii* IgG antibodies persist for the life of the infected host even while the parasite is quiescent. In clinically ill animals with previously detected *T. gondii* infections, attention should be directed at identification of additional underlying causes for the immunosuppressive disease while simultaneously treating with anti-*Toxoplasma* drugs.

**Table 1.** Interpretation of serologic results obtained by Modified Agglutination Test (MAT) for *Toxoplasma gondii* performed at University of Tennessee College of Veterinary Medicine Diagnostic Parasitology Laboratory.

<b>IgG Result (Titer)</b>	<b>IgM Result (Titer)</b>	<b>Interpretation</b>
Negative (<32)	Negative (<512)	No serological evidence of <i>Toxoplasma</i> infection
Negative (<32)	Positive (≥512)	Suspect early (acute) infection with <i>Toxoplasma</i> ; obtain new specimen and test 2-3 weeks later. IgG positive test confirms suspicion.
Equivocal (≤32)	Equivocal (≥512)	Subsequent re-testing fails to demonstrate measurable IgG antibody titer (≥32); investigate other causes for clinical symptoms
Positive (≥32)	Positive (≥512)	Possible acute infection with <i>Toxoplasma</i> ; obtain new specimen and test for rising IgG titer; 4-fold increase in IgG titer confirms suspicion.
Positive (≥32)	Negative or Equivocal (≤512)	Infection with <i>Toxoplasma</i> for probably 1 year or longer; obtain new specimen and test for rising IgG titer; 4-fold increase in IgG titer confirms suspicion. If no change in titer assume chronic infection