

Towards Universal Screening for Toxoplasmosis: Rapid, Cost-Effective, and Simultaneous Detection of Anti-*Toxoplasma* IgG, IgM, and IgA Antibodies by Use of Very Small Serum Volumes

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Rapid, cost-effective, and early determination of the serological status of potentially infected individuals, particularly pregnant women, can be critical in preventing life-threatening infections and subsequent fetal congenital abnormalities. An article in this issue of the *Journal of Clinical Microbiology* (X. Li, C. Pomares, G. Gonfrier, B. Koh, S. Zhu, M. Gong, J. G. Montoya, and H. Dai, *J Clin Microbiol* 54:1726–1733, 2016, <http://dx.doi.org/10.1128/JCM.03371-15>) describes an innovative multiplexed immunoassay that offers a path toward universal screening.

Toxoplasma gondii is a protozoan parasite that is infectious to all warm-blooded terrestrial and marine animals (1). Infection can be acquired congenitally (2, 3), by ingesting viable cysts in undercooked meat (4, 5), or by inhalation or ingestion of oocyst-contaminated soil or water (6, 7). Although *T. gondii* infection is largely asymptomatic in healthy humans, congenital toxoplasmosis can be devastating, leading to encephalomyelitis, convulsive seizures, respiratory problems, and fetal or infant death (2).

Because of the grave effects of congenital toxoplasmosis, it is very important to accurately and expeditiously identify pregnancy-related *Toxoplasma* infections. However, the idea of universal screening for toxoplasmosis is very controversial for a variety of reasons, including the low prevalence of infection in certain countries and the reliability of diagnostic tests. Universal screening is mandatory in certain countries, such as Austria and France, while Canada and Brazil conduct routine maternal screening to identify and assist seronegative women with preventive measures, as well as to institute early treatment of infections acquired during pregnancy (3, 8–11). Conversely, universal screening is not routinely performed and, in fact, has been discouraged in countries where the prevalence of the disease is very low, such as the United Kingdom, Norway, and the United States (3, 7, 12).

Definitive diagnostic tests for *T. gondii* infection include the gold-standard Sabin-Feldman dye test, detection of serum antibodies by enzyme-linked immunosorbent assay (ELISA), modified agglutination test, PCR, and a more recently commercially available multiplex immunoassay, the BioPlex 2200 system (13–17). Despite the availability of these tests, universal screening remains elusive. There is some speculation that the Sabin-Feldman dye test may be cost prohibitive and too labor-intensive to set up, while many of the commercial tests that compare their results to the Sabin-Feldman dye test have been reported to have difficulty reaching 100% correlation, as well as the potential for slower detection of infection. In particular, there is currently no reference diagnostic test available for assessing IgM and IgA anti-*Toxoplasma* antibodies. Given these limitations, Xiaoyang Li and colleagues have developed and described, in this edition of the *Journal of Clinical Microbiology*, a plasmonic gold chip multiplex immunoassay capable of simultaneously measuring IgG, IgM, and IgA antibodies against *Toxoplasma* with high sensitivity, specific-

ity, positive predictive value, and negative predictive value in as little as 5 μ l of patient serum (18).

The simultaneous detection of all three immunoglobulin isotypes, IgG, IgM, and IgA, should offer a more complete picture of the infection status of the patient. Together, these three antibody isotypes could provide information regarding seroconversion and whether the infection is acute, chronic, or reactivated. With the exception of the BioPlex 2200 (which does not measure IgA), all currently available tests would have to be run individually, thus requiring significantly larger serum sample volumes (5 to 50 μ l for plasmonic gold chip- and bead-based assays versus ≥ 100 μ l for ELISA and other assays) and an increase in reagents and labor. One solution to this problem would be to perform these assays in a multiplex format where the three antibody isotypes are measured simultaneously by using a much smaller sample volume than traditional methods. The utility and benefits of multiplex immunoassays have been widely published. There are multiple reports of serological multiplex immunoassays for the measurement of antibodies to a wide range of pathogens, including *T. gondii*, *Escherichia coli* O157:H7, *Helicobacter pylori*, HIV, and noroviruses (19–21). However, multiplex immunoassays also present challenges and limitations that include cross-reactivity and assay sensitivity and specificity that must be addressed before they can be used as definitive diagnostic tools (22).

Major hurdles confronting the development of antibody screening tools include the use of invasive procedures, the need for trained personnel, and the costs involved in blood collection. To

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overcome these hurdles, research laboratories have been studying the efficacy of replacing serum with saliva as the diagnostic fluid of choice for measuring immune responses to pathogen exposure. Saliva collection is noninvasive and easy to perform and requires minimal training. Moreover, saliva has been shown to be an ideal matrix for exposure and infection studies, as demonstrated by the numerous reports of very good correlation of antibody responses in paired serum and saliva samples (23–26). The combination of speed and the ability of multiplex immunoassays to measure multiple analytes simultaneously in very small sample volumes with the use of an easily collected noninvasive matrix like saliva may potentially have a significant positive impact on the ability to provide universal screening for not only *T. gondii* infection but also for a host of other infections.

In summary, the application of multiplex immunoassay technologies such as the plasmonic gold chip assay along with less invasive sample collection procedures for the measurement of antibodies against *T. gondii* and other infectious microorganisms may provide more sensitive and specific tools to better implement routine screening for various infections and autoimmune disorders.

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