Updates in Immunoassays: Parasitology

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LEARNING OBJECTIVES:

1. Review the five parasitic infections targeted by the CDC that require public attention.
2. Discuss the immunoassays available to detect *Toxocara* and *Toxoplasma*.
3. List FDA-cleared immunoassays used to identify parasitic infections.
4. Describe issues related to ANA testing.
5. Examine the role of CRP and procalcitonin as biomarkers in bacterial sepsis.

ABBREVIATIONS: ANA - anti-nuclear antibody; CDC - Centers for Disease Control and Prevention; CLIA - chemiluminescent immunoassay; CRP - C-reactive protein; CT - computerized tomography; DFA - direct fluorescent antibody; EIA - enzyme immunoassay; ELFA - enzyme-linked fluorescent assay; ELISA - enzyme-linked immunosorbent assay; FDA - Food and Drug Administration; IF - immunofluorescent; IFA - indirect fluorescent antibody; IgE - immunoglobulin E; IgG - immunoglobulin G; IgM - immunoglobulin M; IIF - indirect immunofluorescence; IUIS - International Union of Immunological Societies; MRI - magnetic resonance imaging; O&P - ova and parasite; PCT - procalcitonin; RNP - ribonucleoprotein; Sm - Smith; SS - Sjögren's syndrome; TES-Ag - *Toxocara* excretory-secretory antigen; WHO = World Health Organization.

INDEX TERMS: Toxoplasmosis; enzyme immunoassay; enzyme-linked fluorescent assay; anti-nuclear antibodies; C-reactive protein; procalcitonin.

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Parasitic disease, although once considered a disease acquired abroad or in developing countries, is very much a problem in the US. According to the Centers for Disease Control and Prevention (CDC), there are approximately 7.4 million cases of trichomoniasis; 2 million cases of giardiasis; 300,000 cases of cryptosporidium; and 400–4,000 cases of congenital toxoplasmosis in the US per year.[1] The CDC has targeted five parasitic infections that are often neglected but require public health attention. They include Chagas disease, cysticercosis, toxocariasis, toxoplasmosis, and trichomoniasis.[2]

Laboratory diagnosis for Chagas disease, cysticercosis, and trichomoniasis relies on either microscopic examination (Chagas disease and trichomoniasis), through magnetic resonance imaging (MRI) or computerized tomography (CT, cysticercosis).[3,4] Toxocariasis and toxoplasmosis are diagnosed through serological methods.

Toxocariasis

Although the enzyme-linked immunosorbent assay (ELISA) for *Toxocara* excretory-secretory antigen (TES-Ag), confirmed by western blot, is the serological assay used to determine a positive result, caution should be taken in interpretation since present-day tests cannot differentiate between a past or recent infection. [5,6,7] Blood eosinophil counts and total serum IgE levels should also be used in conjunction with exposure history and serological and clinical findings.[5,6,7,8] Most clinical laboratories do not routinely test for toxocariasis and refer specimens to reference laboratories for evaluation.

Toxoplasmosis

According to the CDC, toxoplasmosis is one of the primary causes of death in the US due to foodborne illness.[9] Although it is estimated that more than 60

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million individuals harbor the parasite with no significant consequences, caution is taken when a pregnant female or someone in an immunocompromised state acquires the infection.[9] *Toxoplasma gondii*, the parasite responsible for causing toxoplasmosis, is acquired through eating contaminated and undercooked meat, by accidently ingesting the oocysts after eating unwashed vegetables and fruits, or after cleaning a litter box and not following proper hand washing procedures.[10,11] Consequences are severe to the developing fetus if a pregnant female acquires a primary infection; therefore, an accurate and rapid diagnosis is essential to prevent symptoms such as blindness, mental disability and brain damage in the newborn.[8,12]

The most common way to diagnosis *Toxoplasma gondii* is through serological techniques. Enzyme immunoassays (EIA), chemiluminescent immunoassays (CLIA), enzyme-linked fluorescent assays (ELFA) and indirect fluorescent antibody (IFA) assays are available that can detect both IgM and IgG levels. There are several companies that market these methodologies. Although many tests have been cleared by the Food and Drug Administration (FDA) and have been available for use since 1977, Table 1 lists only those FDA-cleared assays for toxoplasmosis testing in the last 10 years.[13] The complete list of cleared assays can be found at reference 13 in this manuscript.

In May 2011, the FDA cleared the first test that is able to determine whether a pregnant woman acquired a toxoplasma infection within the past four months.[14] According to the press release, "The VIDAS TOXO IgG Avidity assay can be used to rule out recent *Toxoplasma gondii* infection. The test works by detecting how strongly IgG avidity antibodies bind to the *Toxoplasma gondii* antigens in the assay. IgG avidity antibodies from infections older than four months bind tightly with the antigens, while IgG avidity antibodies

Table 1. FDA Cleared Assays in Toxoplasma Testing[13]

Trademark	Methodology	Manufacturer	Date Cleared
VIDAS® TOXO IgG Avidity Assay	ELFA	bioMerieux, Inc	5/18/11
ADVIA Centaur® Toxoplasma lgG (Toxo G) Assay	ELISA	Siemens Healthcare Diagnostics Inc.	5/4/11
Platelia™ Toxo IgM	ELISA	Bio-Rad Laboratories	7/1/09
Elecsys Toxo IgG Immunoassay and Elecsys PreciControl Toxo IgG	CLIA	Roche Diag. Corp	6/9/08
Access Toxo IgG Assay, Access Toxo IgG Calibrators, Access Toxo IgG QC	ELISA	Beckman Coulter, Inc	5/23/08
DiaSorin LIAISON® TOXO IgM	CLIA	DiaSorin S.P.A.	2/8/06
Access® Toxo IgG Assay on Access® Immunoassay Systems	CLIA	Beckman Coulter, Inc.	8/8/03
Access® Immunoassay System Toxo IgM II Assay	CLIA	Beckman Coulter, Inc.	6/2/03
Bio-Rad Platelia® Toxo IgM TMB	ELISA	Bio-Rad Laboratories	9/30/02
Bio-Rad Platelia® Toxo IgG TMB	ELISA	Bio-Rad Laboratories	9/30/02
IMMULITE® and IMMULITE® 2000 Toxoplasma IgM	CLIA	Diagnostic Products Corp.	1/10/02

ABBREVIATIONS: CLIA: chemiluminescent immunoassay, ELFA: enzyme-linked fluorescent assay, ELISA: enzyme-linked immunosorbent assay, IgG: immunoglobulin G, IgM: immunoglobulin M, QC: quality control, TMB: tetramethylbenzidine

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from infections acquired in the past four months form weaker bonds."[14] The test is marketed by bioMerieux, Inc., and utilizes ELFA technology in the identification process.[15,16]

Giardia and Cryptosporidium Testing

In the US, one of the most common intestinal parasitic infections affecting humans is *Giardia*.[17] *Giardia* infections are acquired by swallowing cysts from contaminated food or water[18] and can be identified by microscopic examination following routine ova and parasite (O&P) protocols or by antigen detection testing. *Cryptosporidium*, also an intestinal parasite, is similar to *Giardia* in that it is acquired after accidently ingesting the parasite from contaminated water, soil, or food sources and causes diarrheal disease. According to the CDC, several public outbreaks have been attributed to ingesting municipal water or recreational water contaminated with *Cryptosporidium*.[19] Approximately 748,000 new cases of *Cryptosporidium* occur in the U.S. per year.[20]

There are several assays available that identify both *Giardia* and *Cryptosporidium* infections either alone or in combination by applying EIA, direct fluorescent antibody assays (DFA), or by lateral flow assays. Table 2 lists those FDA cleared assays for *Giardia* and/or *Cryptosporidium* testing in the last 10 years.[21] The complete list of cleared assays can be found at reference 21 in this manuscript.

Miscellaneous Testing

Although anti-nuclear antibody (ANA) tests do not identify infectious agents but screen for autoimmune disorders, this test is performed on a routine basis in most clinical laboratories. In the November 2011 issue of Clinical Laboratory News an article was published entitled *Antinuclear Antibody Testing Dilemmas* where the author discussed some of the issues encountered with ANA testing.[22] To summarize the article, the use of indirect immunofluorescent testing (IIF), which is considered the gold standard for ANA testing, has several limitations when compared to some of the newer EIA and multiplex methodologies that are available. For example, manual tests are not standardized; interpretation of results is subjective; the pattern identified is not always reflective of the disease state; is time consuming to set up; and roughly 15% of positive lupus patients are missed.[22] In addition, false positives can occur since approximately 13% of healthy individuals test positive with a titer of 1:80 leaving it difficult for the clinician to interpret whether the result is clinically significant.[22] The advantages are that most ANA tests can detect more than 100 autoantibodies; IIF

Table 2. FDA-Cleared Assays in Giardia/Cryptosporidium Testing[21]

Trademark	Methodology	Manufacturer	Cleared
GIARDIA/CRYPTOSPORIDIUM QUIK CHEK	Rapid membrane EIA	TECHLAB, Inc.	8/18/11
Giardia Fecal Antigen Detection Lateral Flow Kit	Lateral flow	IVD Research, Inc.	1/14/09
GIARDIA/CRYPTOSPORIDIUM QUIK CHEK	ELISA	TECHLAB, Inc.	11/7/05
Xpect™ Giardia Lateral Flow Assay	Lateral flow	Remel Inc	11/18/03
Xpect™ Giardia/Cryptosporidium Lateral Flow Assay	Lateral flow	Remel Inc.	11/10/03
GIARDIA II	ELISA	TECHLAB, Inc.	11/4/03
Cryptosporidium/Giardia Direct Fluorescence Antigen Detection Kit	DFA	IVD Research, Inc.	3/5/03
Giardia Antigen Detection Microwell ELISA Assay	ELISA	IVD Research, Inc.	9/17/02

Abbreviations: DFA: direct fluorescent antibody, ELISA: enzyme-linked immunosorbent assay, EIA: enzyme immunoassay

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assays yield both pattern and titer results; and offer good sensitivity for autoimmune disorders such as lupus, mixed connective tissue disease, and drug-induced lupus with 85%, 100%, and 100% sensitivity, respectively.[22]

Although some of the newer EIAs are automated, easy to use, and can run on instruments already utilized in the clinical laboratory for other assays, these assays do not test for as many antigens as the IIF tests and do not provide a pattern and titer which most clinicians rely on when assessing their patients.[22]

Since controversy exists, the American College of Rheumatology issued a position paper in 2009 specifying recommendations for ANA testing.[23] These recommendations were approved by the board of trustees the same year and are as follows: "the immunofluorescent (IF) ANA test should remain the gold standard for ANA testing; hospital and commercial laboratories using bead-based multiplex platforms or other solid phase assays for detecting ANAs must provide data to ordering physicians on request that their assay has the same or improved sensitivity and specificity compared to the IF ANA; in-house assays for detecting ANA as well as anti-DNA, anti-Sm, anti-RNP, anti-Ro/SS-A, anti-La/SS-B, etc. should be standardized according to national (CDC) and/or international (WHO, IUIS) standards; and laboratories should specify the methods utilized for detecting ANAs when reporting their results."[22,23] The article ended with a statement made by John L. Carey, MD, vice-chair of pathology and laboratory medicine at Henry Ford Health System in Detroit. He noted that several labs perform an EIA initially if the preferred method is not specified on the laboratory order. If the EIA is positive, confirmation is done with an IIF where the pattern type and titer are both reported out. He stated by using this algorithm, clinicians can determine the significance and relevance of a positive ANA test.[22]

C-Reactive Proteins and Procalcitonin in Bacterial Sepsis

C-reactive proteins (CRP) are acute-phase proteins that are elevated during the inflammatory response. Elevated levels are found as a consequence of tissue injury and are found in bacterial infections, post-surgery, in malignancies, and in approximately 70 different disease states and conditions.[24] Since levels can be markedly elevated in an inflammatory response, as much as a 100-fold increase in concentration, CRPs serve as excellent markers for inflammation and in bacterial sepsis.[24] Another biomarker used for bacterial sepsis is procalcitonin (PCT), a prohormone to calcitonin that is elevated in response to bacterial and toxin production.[25] The conversion of procalcitonin to calcitonin is prevented due to the release of various cytokines and endotoxins during bacterial invasion thereby causing PCT levels to rise.[25]

A large literature search was conducted by Pierrakos and Vincent in 2010 that yielded 3370 studies evaluating biomarkers in sepsis with a total of 178 biomarkers.[26] The overall conclusion of this extensive review was that while both PCT and CRP are the most widely used markers for bacterial sepsis, both have limitations such as less than 90% sensitivity and specificity (PCT) and the inability to differentiate between sepsis and another inflammatory response.[26] In addition, a 2011 article written by Woodworth entitled *Procalcitonin: The Answer to the Sepsis Dilemma* investigated the use of PCT as a biomarker for sepsis. The conclusions were: "to date, few studies have addressed the utility of PCT to predict sepsis in "real time." Until large real time studies with well-defined patient populations are completed, the utility of PCT to predict sepsis will remain controversial."[27]

Although controversy exists regarding the use of PCT as a predictor of sepsis in critically ill patients,[26,27,28,29,30] the FDA cleared the VIDAS® B·R·A·H·M·S PCT assay marketed by bioMérieux in 2007.[31] The assay runs on the VIDAS® and utilizes ELFA technology.[32] The assay is intended for use on the first day a severely ill patient is admitted to one of the critical care units to determine the threat of progression to severe sepsis and septic shock.[31] In a statement made by Stéphane Bancel, bioMérieux Chief Executive Officer, "PCT testing can be used as an aid to provide an early indication of need for aggressive interventions and can potentially improve patient outcomes and increase chances of survival."[31] Regardless of the controversy that exists, the assay provides a means to measure PCT levels when evaluating cases of bacterial sepsis.

Summary

Although most clinical laboratories use microscopy and routine O&P procedures when identifying parasitic

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infections, there are several parasites that are better detected through serological means. *Toxoplasma*, *Giardia*, and *Cryptosporidium* were discussed along with immunoassays used for their detection. Immunoassays provide quick results and are less labor intensive than specimen concentration and slide preparation for microscopic examination. These assays are easy to use and provide sensitive and specific results. Some clinical laboratories no longer perform O&Ps in house and refer specimens to reference laboratories for evaluation. By using immunoassays, some of the more common parasites can be identified in a timely manner reducing turn-around times.

Some controversy exists over the use of IIF and EIA tests used for ANA testing along with measuring CRPs and PCT as predictors of bacterial sepsis and septic shock. Regardless of the methodology discussed in this series of articles, there are pros and cons to the various immunoassays available. Determining the most appropriate assay based on patient population and volume is governed by the institution and its patients' needs.

In conclusion, immunoassays, whether manual or automated, are easy to use, cost effective and allow the medical laboratory professional to provide quick and accurate results to the clinician so the most appropriate treatment can be administered to the patient. The ultimate goal of healthcare professionals is to provide the highest quality of medical care in a timely manner. The use of immunoassays in the clinical laboratory allows the healthcare team to successfully achieve this goal.

The author endorses no company or product and has no financial gain or interest in the products presented.

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