Bibliography References

LDBIO DIAGNOSTICS

IMMUNOBLOTS & RAPID TESTS

for Parasitology and Mycology

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New products

Aspergillus ICT IgG IgM CE *Chronic Pulmonary Aspergillosis*

Evaluation of LDBio Aspergillus ICT Lateral Flow Assay for IgG and IgM Antibody Detection in Chronic Pulmonary Aspergillosis.

Elizabeth Stucky Hunter, Malcolm D Richardson, David W Denning. *J Clin Microbiol.* **2019** Aug 26;57(9):e00538-19.

Background: Detecting *Aspergillus*-specific IgG is critical to diagnosing chronic pulmonary aspergillosis (CPA). Existing assays are often cost- and resource-intensive and not compatible with resource-constrained laboratory settings. LDBio Diagnostics has recently commercialized a lateral flow assay based on immunochromatographic technology (ICT) that detects *Aspergillus* antibodies (IgG and IgM) in less than 30 min, requiring minimal laboratory equipment.

Methods: A total of 154 CPA patient sera collected at the National Aspergillosis Centre (Manchester, United Kingdom) and control patient sera from the Peninsula Research Bank (Exeter, United Kingdom) were evaluated. Samples were applied to the LDBio *Aspergillus* ICT lateral flow assay, and results were read both visually and digitally. Results were compared with *Aspergillus* IgG titers in CPA patients, measured by ImmunoCAP-specific IgG assays.

Results: For proven CPA patients versus controls, sensitivity and specificity for the LDBio *Aspergillus* ICT were 91.6% and 98.0%, respectively. In contrast, the routinely used ImmunoCAP assay exhibited 80.5% sensitivity for the same cohort (cutoff value, 40 mg of antigen-specific antibodies [mgA]/liter). The assay is easy to perform but challenging to read when only a very faint band is present (5/154 samples tested). The ImmunoCAP *Aspergillus* IgG titer was also compared with the *Aspergillus* ICT test line intensity or rate of development, with weak to moderate correlations.

Conclusion: The *Aspergillus* ICT lateral flow assay exhibits excellent sensitivity for serological diagnosis of CPA. Quantifying IgG from test line intensity measurements is not reliable. Given the short run time, simplicity, and limited resources needed, the LDBio *Aspergillus* ICT is a suitable diagnostic tool for CPA in resource-constrained settings.

Multicenter Evaluation of a Novel Immunochromatographic Test for Anti-aspergillus IgG Detection.

Raphaël P Piarroux, Thomas Romain, Aurélie Martin, Damien Vainqueur, Joana Vitte, Laurence Lachaud, Jean-Pierre Gangneux, Frédéric Gabriel, Judith Fillaux, Stéphane Ranque. *Front Cell Infect Microbiol*. **2019** Jan 31;9:12

Background: *Aspergillus* sp. fungi cause various diseases in both immunocompetent and immunocompromised patients. The most frequent *Aspergillus* disorders include chronic pulmonary aspergillosis (CPA), a life-threatening disease that affects at least 3 million people worldwide, and allergic bronchopulmonary aspergillosis (ABPA), which affects approximately 4.8 million severe asthmatic patients globally. Diagnosis of such diseases involves IgG serological testing; however, the

currently available anti-*Aspergillus* IgG detection assays are inappropriate for resource-poor laboratory settings, as they are expensive, rely on automated procedures, and require stable electrical power. Therefore, accurate CPA or ABPA diagnosis facilities are lacking in most low- and middle-income countries.

Methods: We evaluated a novel anti-*Aspergillus* antibody immunochromatographic test (ICT) that requires minimal laboratory equipment. Two evaluations were performed: a single-center 4-month prospective study in a French reference laboratory (44 cases/257 patients) and a retrospective study in five French reference laboratories (262 cases and 188 controls). We estimated the ICT indices for the diagnosis of chronic aspergillosis, and the test results were compared to those of anti-*Aspergillus* IgG immunoblot (IB) assay.

Results: Of the 713 patients included in the study, 306 had chronic aspergillosis. Test sensitivity and specificity were 88.9% (95%CI[85-92]) and 96.3% (95%CI[94-98]) for the ICT and 93.1% (95%CI[90-96]) and 94.3% (95%CI[92-96]) for the IB, respectively. Agreement between the two assays was almost perfect (kappa = 0.86).

Conclusion: As this ICT displays good diagnostic performance and complies with the ASSURED (Affordable, Sensitive, Specific, User-friendly, Equipment-free, and Delivered) criteria, we concluded that this anti-*Aspergillus* antibody ICT can be used to diagnose *Aspergillus* diseases in resource-poor settings.

Toxoplasma WB IgA CE *Ocular toxoplasmosis*

Comparison of immunoblotting (IgA and IgG) and the Goldmann-Witmer coefficient for diagnosis of ocular toxoplasmosis in immunocompetent patients.

Thibaud Mathis, Sylvain Beccat, Pascal Sève, François Peyron, Martine Wallon, Laurent Kodjikian. *Br J Ophthalmol*. **2018** Oct;102(10):1454-1458

Background: Ocular toxoplasmosis (OT) is a common cause of posterior uveitis worldwide. The diagnosis of OT is based on clinical findings, but in most cases, laboratory tests are required to confirm the aetiology, especially when other diseases are suspected. The aim of this study was to evaluate which methods, between the Goldmann-Witmer coefficient (GWC) and immunoblotting (IB) with both IgG and IgA, in aqueous humour (AH) samples, can be the most sensitive to diagnose OT, in current practice, especially in the first three weeks.

Methods: Retrospectively reviewed records of 87 consecutive patients who had underwent AH and serum sample, 42 patients with suspected OT and 45 patients with suspected other ocular inflammatory diseases. All samples were analysed by both GWC and IB.

Results: The GWC was significant in 47.6% of patients presenting with suspected OT. The intraocular production of specific antibody anti-Toxoplasma gondii IgG and IgA was revealed by IB in 71.4% of samples. The combination of these two methods increased the sensitivity to 76.2%. Based on the interval between symptom onset and paracentesis, IB had a greater sensitivity than GWC when sample of AH was taken in the first three weeks (64.7% vs 23.5%, P=0.039), while the difference between the sensitivity of IB and GWC was less important in cases with an interval >3 weeks (76% vs 64% P=0.625).

Conclusion: IB seems to be more useful than the GWC if only one of these methods can be performed, especially during the first three weeks after symptom onset.

Next products

Aspergillus WB IgE *Allergic Broncho Pulmonary Aspergillosis*

Interest of Aspergillus fumigatus Western Blot assay for differential diagnostic between IgE sensitization and Allergic Broncho Pulmonary Aspergillosis.

Raphaël P Piarroux, Jean-Christophe Dubus, Martine Reynaud-Gaubert, Marion Gouitaa, Stéphane Ranque, Joana Vitte. 9th Trends in Medical Mycology. **2019**. P126

Objectives: Diagnosis of Allergic Broncho Pulmonary Aspergillosis (ABPA) is complex and based on a multi-criteria definition. The detection and quantitation of immunoglobin E (IgE) responses to Aspergillus fumigatus (Af) is one of the major criteria for its definition, but also reflect sensitization to Af. In the past few year a new approach assaying IgE responses to molecular antigens has emerged, enhancing the discrimination between Af sensitization and ABPA,but does not allow a clear-cut distinction between these two diseases. We recently reported that a Western Blot detecting IgE antibodies specific for Aspergillus fumigatus could be helpful in separating those two entities. This work aimed to complete the evaluation of Af IgE WB assay as a differentiating tool between Af-sensitization and ABPA.

Methods: 221 sera with known sIgE reactivity (21 ABPA, 200 Af-sensitized) were assayed with the LDBIO Asp II WB. Sera were collected 2014-2018 and previously assayed with ImmunoCap® to Af extract and molecular antigens. We evaluated the ability of WB LDBIO to detect Af sensitization and to discriminate between ABPA and Af-sensitized patients, relying on ImmunoCap® and clinical chart conclusions as a reference.

Results: Samples displayed 0 to 10 bands in the 10-37 kDa range. Among those bands, 4 were most frequent and therefore considered as major bands (16, 18-20, 22 and 30 kDa) while the others were considered as minor bands. WB positivity was defined by the presence of 2 major bands.

21/21 ABPA and 115/200 Af sensitizations sera were detected by WB (61% sensitivity). WB positivity was strongly correlated to specific IgE level and was positive 95% (75/79) of the time if IgE to Af were >2kUa/l.

Band patterns formed various profiles for discrimination between ABPA and Af-sensitization. Of those profiles, one (5 detectable bands, regardless of minor/major classification) had 100% sensitivity (21/21) and 91% specificity (201/221) for ABPA diagnostic. However, we preferred to use a more specific profile with 2 major bands and 2 minor bands outside of the 16-30 kDa range. This profile had 90% sensitivity (19/21) and 95.5% specificity (211/221).

Conclusion: This study shows the potential of WB in the work-up of IgE responses to Af in asthmatics and cystic fibrosis patients: WB was able to discriminate between ABPA and Af-sensitized patients, with a sensitivity of 90% and a specificity of 95.5%. In the same population, the criteria defined by ISHAM (tIgE>1000 kUI/l and Af IgE>0.35 kUa/l) had 48% sensitivity (10/21) and 98.2% specificity (217/221).

Of note, among the WB negatives, 4 were positive to another mold (Alternaria alternata), thus WB could avoid unnecessary diagnostic difficulties due to such cross-reactivity.

A multicenter evaluation is now needed to confirm those results, but WB shows a great potential for second-line diagnostic tool when ABPA is suspected.

TOXO II WB IgM *Toxoplasma seroconversion*

Diagnostic accuracy of toxoplasma western blot test in suspected seroconversion in pregnancy: a multicentric study.

Valeria Meroni, Alfonso Corcione, Luigia Scudeller, Marie-Pierre Brenier-Pinchart, Hélène Fricker-Hidalgo, Coralie L'Ollivier, Hervé Pelloux, Luc Paris. *6tn Internal Congress Of Congnital Toxoplasmosis*. **2019**. Poster.

Introduction: Toxoplasmosis is a parasitic infection usually asymptomatic that could be life threatening for immunosupressed patients and can cause severe illness if transmitted from the mother to the fetus. Due to the high sensitivity mandatory for screening tests, the automated tests, widely employed for Toxoplasma gondii serology can yield false positive results due to cross and/or non-specific reaction Therapy could reduce the transmission and the severity of congenital toxoplasmosis only if maternal infection is treated in the first weeks after infection. On the other hand, therapy with spiramycin, given most of the time, when IgM are found to be positive, could delay IgG production and mask seroconversion. There is the need of second level test to early detect real toxoplasmic seroconversions that could combine high sensitivity for IgM detection and high specificity. We planned a European multicentre study with 4 reference center for the diagnosis of toxoplasmosis: Pitié-Salpétrière Paris, CHU Grenoble-Alpes Grenoble, La Timone Marseille, IRCCS Policlinico San Matteo Pavia to evaluate the diagnostic accuracy of the new LDBIO-TOXO II IgG/ IgM Immunoblot (IgG- IgM WB) by LdBio Lyon - France. We also looked at the immunodominant antigen in the IgM Wb.

Materials and Methods: The leftover of laboratory samples stored after clinical diagnosis of 343 sera 234 corresponding to 96 toxoplasmic seroconversions (2 to 3 sera/patient) and 169 sera corresponding to 69 patients with cross reactions and/or non-specific IgM (1 to 3 sera/patient) were retrospectively analysed. All the patients had a documented seroconversion with the last IgG /IgM negative to the first IgG /IgM positive or a false positive result without seroconversion as previously analysed in the different laboratories with panel of different serological techniques. All fully anonymised samples processing had been performed in blind by LDBio. To validate WB we performed two different analyses: concordance (Cohen's kappa) with final diagnosis (seroconversion vs false positive) and diagnostic validity (sens, spec etc).

Results: For IgM and IgG type II WB the concordance with the final diagnosis was really very good K= 0,83 and K=85, respectively. Kappa is even higher in analyses where equivocal results are considered positive: K= 0,89 and K= 0,89. Among the discordant results, there are 4 cases in which the appearance of IgM and 46 cases in which the appearance of IgG was recorded by Wb before the traditional tests. Sensitivity of IgM WB was 100%, of IgG WB 93,8% , Specificity was 87%, 95.7%, respectively. Looking at the most antigenic bands, P30 was recorded in all but one positives samples and P40 in all but 5.

Conclusion: LDBio Type II IgG /IgM western blot could be a valuable help in quick and precise diagnosis of early seroconversion also in spiramycin treated pregnant women. The IgM Wb detected all the seroconversion (earlier in 4 cases than traditional test) and discriminated very well the false positive test. The definition of immunodominant band will be very helpful in the interpretation of the test.

Evaluations

Echinococcus IgG serology

Evaluation of Nine Commercial Serological Tests for the Diagnosis of Human Hepatic Cyst Echinococcosis and the Differential Diagnosis with Other Focal Liver Lesions: A Diagnostic Accuracy Study.

Francesca Tamarozzi , Silvia Stefania Longoni , Ambra Vola, Monica Degani, Stefano Tais, Eleonora Rizzi, Marco Prato, Salvatore Scarso, Ronaldo Silva, Enrico Brunetti, Zeno Bisoffi, Francesca Perandin. *Diagnostics* **2021**, *11*(2), 167.

Background: The differential diagnosis of hepatic cystic echinococcosis (CE) may be challenging. When imaging is insufficient, serology can be applied, but no consensus diagnostic algorithm exists.

Methods: We evaluated the performances of nine serological tests commercialized in Europe for the diagnosis of "echinococcosis". We performed a diagnostic accuracy study using a panel of sera from patients with hepatic CE (*n* = 45 "liquid" content stages, *n* = 25 "solid" content stages) and non-CE focal liver lesions (*n* = 54 with "liquid" content, *n* = 11 with "solid" content). The diagnosis and staging of CE were based on ultrasound (gold standard). Nine commercial seroassays (5 ELISA, 2 WB, 1 Chemiluminescence Immunoassay [CLIA] and 1 Immunochromatographic test [ICT]) were the index tests.
instructions by investigators blinded to PAMF-TSL results. Sensitivity and specificity were calculated.

Results: Sensitivity (Se) ranged from 43 to 94% and from 31 to 87%, and specificity (Sp) from 68 to 100% and from 94 to 100%, when borderline results were considered positive or negative, respectively. Three seroassays (2 ELISA, 1 WB) were excluded from further analyses due to poor performances. When tests were combined, Sp was 98-100%. The best results were obtained using the WB-LDBIO alone (Se 83%) or as a third test after two non-WB tests (Se 67-86%).

Conclusion: A validated WB or two non-WB tests, read with stringent criteria (borderline = negative and considered positive only if concordant positive), possibly confirmed by the WB, appear sensible approaches.

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Diagnostic Performances of Commercial ELISA, Indirect Hemagglutination, and Western Blot in Differentiation of Hepatic Echinococcal and Non-Echinococcal Lesions: A Retrospective Analysis of Data from a Single Referral Centre.

Ambra Vola, Tommaso Manciulli, Annalisa De Silvestri, Raffaella Lissandrin, Mara Mariconti, Mar Siles-Lucas, Enrico Brunetti, and Francesca Tamarozzi. *Am. J. Trop. Med. Hyg.*, 101(6). **2019**. pp. 1345–1349.

Background: The diagnosis of cystic echinococcosis (CE) is based on imaging. Serology supports imaging in suspected cases, but no consensus exists on the algorithm to apply when imaging is inconclusive.

Methods: We performed a retrospective analysis of serology results of patients with untreated hepatic CE and non-CE lesions, seen from 2005 to 2017, to evaluate their accuracy in the differential diagnosis of hepatic CE. Serology results of three seroassays for echinococcosis (ELISA RIDASCREEN, indirect hemagglutination (IHA) Cellognost, and Western blot LDBIO) and clinical characteristics of eligible patients were retrieved. Patients were grouped as having active or inactive CE and liquid or solid non-

CE lesions. Sensitivity, specificity, and diagnostic accuracy were compared between scenarios encompassing different test combinations. Eligible patients included 104 patients with CE and 257 with non-CE lesions.

Results: Sensitivity and diagnostic accuracy of Western blot (WB) were significantly higher than those of the following: 1) IHA or ELISA alone, 2) IHA+ELISA interpreted as positive if both or either tests positive, and 3) IHA+ELISA confirmed by WB if discordant. The best performances were obtained when WB was applied on discordant or concordant negative IHA+ELISA. Analyses performed within "active CE (n = 52) versus liquid non-CE (n = 245)" and "inactive CE (n = 52) versus solid non-CE (n = 12)" groups showed similar results. Specificity was high for all tests (0.99–1.00) and did not differ between test combination scenarios.

Conclusion: WB may be the best test to apply in a one-test approach. Two first-level tests confirmed by WB seem to provide the best diagnostic accuracy. Further studies should be performed in different settings, especially where lower test specificity is likely.

Leishmania IgG serology

Evaluation of six commercial kits for the serological diagnosis of Mediterranean visceral leishmaniasis.

Maude F Lévêque, Emilie Battery, Pascal Delaunay, Badre Eddine Lmimouni, Karim Aoun, Coralie L'Ollivier, Patrick Bastien, Charles Mary, Christelle Pomares, Judith Fillaux, Laurence Lachaud. *PLoS Negl Trop Dis.* **2020** Mar 25;14(3):e0008139.

Background: Zoonotic visceral leishmaniasis (VL) is endemic in the Mediterranean basin. However, large-scale comparative analyses of the commercial kits for the serological diagnosis of this neglected disease are lacking. This study compared the performances of four enzyme-linked immunosorbent assays (ELISA) and two immunochromatographic tests (ICT) as screening tests for the serodiagnosis of human VL in the Mediterranean region.

Methodology/principal findings: Serum samples from 319 patients living in France, Tunisia or Morocco were tested using two ICT (IT LEISH and TruQuick LEISH IgG/IgM Meridian) and four ELISA reagents (NovaLisa Leishmania infantum IgG, Bordier Leishmania infantum, Ridascreen Leishmania IgG, and Vircell Leishmania). The population with proven VL (n = 181) included 65 immunocompromised patients. Significantly higher percentages of false-negative results were obtained with all assays in immunocompromised patients, compared with the immunocompetent population. In the whole population, sensitivity and specificity ranged from 80.7% to 93.9% and from 95.7% to 100%, respectively. The maximum accuracy was observed with the Bordier and Vircell ELISA kits (96.2%), and the lowest accuracy with Ridascreen reagent (88.7%). New thresholds of positivity are proposed for the Bordier, Vircell and NovaLisa ELISA kits to achieve 95% sensitivity with the highest possible specificity. Western blot (WB), used as a confirmation method, showed 100% sensitivity and identified 10.1% of asymptomatic carriers among the control population from the South of France.

Conclusions/significance: This is the first study that compared commercially available kits for VL serodiagnosis in the endemic region of the Mediterranean basin. It provides specific information about the tests' performance to help clinicians and biologists to select the right assay for VL screening.

Point-of-Care Toxoplasma serology

High performance of a novel point-of-care blood test for *Toxoplasma* infection in women from diverse regions of Morocco.

Bouchra El Mansouri, Fatima Amarir, François Peyron, El Bachir Adlaoui, Raphaël Piarroux, Joseph Lykins, Majda El Abbassi, Nesma Nekkal, Nadia Bouhlal, Kamar Makkaoui, Amina Barkat, Aziza Lyaghfouri, Ying Zhou, Samira Rais, Mounia Oudghiri, Ismail Elkoraichi, Mustapha Zekri, Nezha Belkadi, Hajar Mellouk, Mohamed Rhajaoui, Allal Boutajangout, Abderrahim Sadak, Denis Limonne, Rima McLeod & Kamal El Bissati. *Emerging Microbes & Infections*. **2021**. 10:1, 1675-1682.

Background: Point-of-care (POC) testing for Toxoplasma infection has the potential to revolutionize diagnosis and management of toxoplasmosis, especially in high-risk populations in areas with significant environmental contamination and poor health infrastructure precluding appropriate follow-up and preventing access to medical care. Toxoplasmosis is a significant public health challenge in Morocco, with a relatively heavy burden of infection and, to this point, minimal investment nationally to address this infection.

Methods: Herein, we analyse the performance of a novel, low-cost rapid test using fingerstick-derived whole blood from 632 women (82 of whom were pregnant) from slums, educational centres, and from nomad groups across different geographical regions (i.e. oceanic, mountainous) of Morocco.

Results: The POC test was highly sensitive and specific from all settings. In the first group of 283 women, sera were tested by Platelia ELISA IgG and IgM along with fingerstick whole blood test. Then a matrix study with 349 women was performed in which fingerstick – POC test results and serum obtained by venipuncture contemporaneously were compared. These results show high POC test performance (Sensitivity: 96.4% [IC95 90.6–98.9%]; Specificity: 99.6% [IC95 97.3–99.9%]) and high prevalence of Toxoplasma infection among women living in rural and mountainous areas, and in urban areas with lower educational levels.

Conclusions: The high performance of POC test confirms that it can reduce the need for venipuncture and clinical infrastructure in a low-resource setting. It can be used to efficiently perform seroprevalence determinations in large group settings across a range of demographics, and potentially expands healthcare access, thereby preventing human suffering.

Evaluation of Three Point-of-Care Tests for Detection of Toxoplasma Immunoglobulin IgG and IgM in the United States: Proof of Concept and Challenges.

Carlos A Gomez, Laura N Budvytyte, Cindy Press, Lily Zhou, Rima McLeod, Yvonne Maldonado, Jose G Montoya, Despina G Contopoulos-Ioannidis. *Open Forum Infect Dis*. **2018** Oct 29;5(10):ofy215.

Background: The cost of conventional serological testing for toxoplasmosis discourages universal adoption of prenatal monthly screening programs to prevent congenital toxoplasmosis. Point-of-care (POC) technology may constitute a cost-effective approach.

Methods: We evaluated the diagnostic accuracy of 3 Toxoplasma POC tests against gold-standard testing performed at Palo Alto Medical Foundation Toxoplasma Serology Laboratory (PAMF-TSL). The

POC tests included the following: Toxo IgG/IgM Rapid Test (Biopanda) and the OnSite Toxo IgG/IgM Combo-Rapid-test that detect IgG and IgM separately, and the Toxoplasma ICT-IgG-IgM-bk (LDBIO) that detects either or both immunoglobulin IgG/IgM in combination. Samples were selected from PAMF-TSL biobank (n = 210) and Centers for Disease Control and Prevention Toxoplasma 1998 Human Serum Panel (n = 100). Based on PAMF-TSL testing, Toxoplasma-infection status was classified in 4 categories: acute infections (n = 85), chronic infections (n = 85), false-positive Toxoplasma IgM (n = 60), and seronegative (n = 80). The POC testing was performed in duplicate following manufacturer's instructions by investigators blinded to PAMF-TSL results. Sensitivity and specificity were calculated.

Results: A total of 1860 POC tests were performed. For detection of Toxoplasma IgG, sensitivity was 100% (170 of 170; 95% confidence interval [CI], 97.8%-100%) for all 3 POC kits; specificity was also comparable at 96.3% (77 of 80; 95% CI, 89.5%-98.9%), 97.5% (78 of 80; 95% CI, 91.3%-99.6%), and 98.8% (79 of 80; 95% CI, 93.2%-99.9%). However, sensitivity for detection of Toxoplasma IgM varied significantly across POC tests: Biopanda, 62.2% (51 of 82; 95% CI, 51.4%-71.9%); OnSite, 28% (23 of 82; 95% CI, 19.5%-38.6%); and LDBIO combined IgG/IgM, 100% (82 of 82; 95% CI, 95.5%-100%). Diagnostic accuracy was significantly higher for the LDBIO POC kit. The POC kits did not exhibit cross-reactivity for false-positive Toxoplasma-IgM sera.

Conclusions: The 3 evaluated POC kits revealed optimal sensitivity for Toxoplasma-IgG antibodies. The LDBIO-POC test exhibited 100% sensitivity for the combined detection of IgG/IgM in acute and chronic Toxoplasma infection. Biopanda and Onsite POC tests exhibited poor sensitivity for Toxoplasma-IgM detection.

Toxoplasma IgG serology

Performance of seven commercial automated assays for the detection of low levels of anti-Toxoplasma IgG in French immunocompromised patients.

Tiphaine Douet, Catherine Armengol, Elena Charpentier, Pamela Chauvin, Sophie Cassaing, Xavier Iriart, Antoine Berry, Judith Fillaux. *Parasite*. **2019**;26:51.

Background: Immunocompromised patients are at high risk for the development of severe toxoplasmosis from tissue cyst reactivation, the most frequently, or from recently acquired acute infections. Knowledge of serologic status is therefore crucial. Screening for toxoplasmosis is sometimes performed while patients are already immunocompromised and have a low or even undetectable IgG titer by routine automated enzyme immunoassays. The aim of this study was to assess the sensitivity and specificity of seven reagents for the detection of low levels of IgG. Sera from 354 patients were collected and analysed.

Results: Elecsys® offered the best analytic performances, superior to those of Architect® and Platelia®, which were superior to those of Access II® and TGS TA®. Vidas II® and Liaison II® reagents exhibited poor analytical performances in this cohort. For Elecsys®, Platelia® and Architect®, new thresholds for the grey zone and positive zone have been defined to improve the sensitivity of these reagents while maintaining excellent specificity.

Conclusions: Commercialized assays for toxoplasmosis screening are not suitable for IgG low-level detection in patients without adapting the supplier thresholds to avoid false negative results and risk generalized toxoplasmosis.

Ocular toxoplasmosis

Biological Diagnosis of Ocular Toxoplasmosis: a Nine-Year Retrospective Observational Study.

Valentin Greigert, Alexander W Pfaff, Arnaud Sauer, Denis Filisetti, Ermanno Candolfi, Odile Villard. *mSphere*. **2019** Sep 25;4(5):e00636-19.

Background: Ocular toxoplasmosis (OT), i.e., the ocular manifestation of Toxoplasma gondii infection, is one of the leading causes of posterior uveitis. While ocular lesions are often typical, atypical forms often require biological confirmation of the diagnosis. Our study sought to review the biological OT diagnoses made in our laboratory to further assess the role of each test in the diagnostic procedure.

Results: All ocular samples sent to our laboratory over the last 9 years for OT diagnosis were included. These samples were analyzed using T. gondii PCR and antibody detection by means of immunoblotting and Candolfi coefficient (CC) determinations, either alone or in combination. Since serum analysis is required to interpret both the CC and immunoblotting, blood serology for T. gondii was also performed in most cases. Of the 249 samples analyzed, 80 (32.1%; 95% confidence interval [95%CI], 26.3 to 37.9) were positive for OT. Of these 80 cases, 52/80 (65.0%; 54.6 to 74.5) displayed a positive PCR, 15/80 (18.8%; 10.2 to 27.3) a positive CC, and 33/80 (41.3%; 95%CI, 30.5 to 52.0) a positive immunoblot result. Overall, 63 of the 80 OT diagnoses (78.8%; 95%CI, 69.8 to 87.7) were made on the basis of a single positive test result.

Conclusions: Our study results remind us that current biological diagnostic tools for OT must be employed in combination to obtain an optimal diagnosis based on the precious ocular fluids sampled by ophthalmologists. Clinicobiological studies that are focused on correlating the performances of the different tests with clinical features are critically needed to improve our understanding of the pathophysiology and diagnosis of OT.

Importance: Ocular toxoplasmosis (OT), a parasitic infection of the eye, is considered to be the most important infectious cause of posterior uveitis worldwide. Its prevalence is particularly high in South America, where aggressive Toxoplasma gondii strains are responsible for more-severe presentations. The particular pathophysiology of this infection leads, from recurrence to recurrence, to potentially severe vision impairment. The diagnosis of this infection is usually exclusively based on the clinical examination. However, the symptoms may be misleading and are not always sufficient to confirm a diagnosis of OT. In such cases, biological tests performed by means of several techniques on blood and ocular samples may facilitate the diagnosis. In this study, we analyzed the tests that were performed in our laboratory over a 9-year period every time OT was suspected. Our report highlights that the quality of ocular sampling by ophthalmologists and combinations of several techniques are critical for a reliable biological OT diagnosis.

Schistosoma serology

Accuracy of parasitological and immunological tests for the screening of human schistosomiasis in immigrants and refugees from African countries: An approach with Latent Class Analysis.

Beltrame A, Guerriero M, Angheben A, Gobbi F, Requena-Mendez A, Zammarchi L, et al. *PLoS Negl Trop Dis*. **2017.** 11(6): e0005593.

Background: Schistosomiasis is a neglected infection affecting millions of people, mostly living in sub-Saharan Africa. Morbidity and mortality due to chronic infection are relevant, although schistosomiasis is often clinically silent. Different diagnostic tests have been implemented in order to improve screening and diagnosis, that traditionally rely on parasitological tests with low sensitivity. Aim of this study was to evaluate the accuracy of different tests for the screening of schistosomiasis in African migrants, in a non-endemic setting.

Methodology/Principal findings: A retrospective study was conducted on 373 patients screened at the Centre for Tropical Diseases (CTD) in Negrar, Verona, Italy. Biological samples were tested with: stool/urine microscopy, Circulating Cathodic Antigen (CCA) dipstick test, ELISA, Western blot, immune-chromatographic test (ICT). Test accuracy and predictive values of the immunological tests were assessed primarily on the basis of the results of microscopy (primary reference standard): ICT and WB resulted the test with highest sensitivity (94% and 92%, respectively), with a high NPV (98%). CCA showed the highest specificity (93%), but low sensitivity (48%). The analysis was conducted also using a composite reference standard, CRS (patients classified as infected in case of positive microscopy and/or at least 2 concordant positive immunological tests) and Latent Class Analysis (LCA). The latter two models demonstrated excellent agreement (Cohen's kappa: 0.92) for the classification of the results. In fact, they both confirmed ICT as the test with the highest sensitivity (96%) and NPV (97%), moreover PPV was reasonably good (78% and 72% according to CRS and LCA, respectively). ELISA resulted the most specific immunological test (over 99%). The ICT appears to be a suitable screening test, even when used alone.

Conclusions: The rapid test ICT was the most sensitive test, with the potential of being used as a single screening test for African migrants.

Recommendations/review

Toxoplasma serology

Serological diagnosis of Toxoplasma gondii infection: Recommendations from the French National Reference Center for Toxoplasmosis.

Odile Villard, Bernard Cimon, Coralie L'Ollivier, Hélène Fricker-Hidalgo, Nadine Godineau, Sandrine Houze, Luc Paris, Herve Pelloux, Isabelle Vilena, Ermano Candolfi. *Diagn Microbiol Infect Dis*. **2016** Jan;84(1):22-33.

Toxoplasmosis manifests no clinical signs in 80% of cases in immunocompetent patient, causing immunization characterized by the persistence of cysts, particularly in brain, muscles, and retina. Assessing the serological status, based on testing for serum toxoplasma IgG and IgM antibodies, is essential in cases that are increasingly at risk for the more severe disease forms, such as congenital or ocular toxoplasmosis. This disease also exposes immunosuppressed patients to reactivation, which can lead to more widespread forms and increased mortality. By interpreting the serological results, we can estimate the risk of contamination or reactivation and define appropriate prophylactic and preventive measures, such as hygienic and dietetic, therapeutic, biological, and clinical follow-up, according to the clinical context. We hereby propose practical approaches based on serological data, resulting from a consensus of a group of experts from the French National Reference Center Network for Toxoplasmosis, according to both routine and specific clinical situations.

Ocular toxoplasmosis

When biology supports clinical diagnosis: review of techniques to diagnose ocular toxoplasmosis.

Valentin Greigert, Elsa Di Foggia, Denis Filisetti, Odile Villard, Alexander W Pfaff, Arnaud Sauer, Ermanno Candolfi. *Br J Ophthalmol.* **2019**. **0**:1–5.

Toxoplasmosis is a common infection whose worldwide prevalence is estimated at 30%, with large disparities across the world. Among infected subjects, the prevalence of ocular toxoplasmosis (OT) is, however, limited to about 2% in Europe and 17% in South America.
In France, it is estimated that about 1 000 000 patients present either active OT or subsequent chorioretinal scars. Toxoplasma gondii is the first cause of posterior uveitis worldwide, responsible for retinochoroiditis, at times associated with anterior uveitis. To date, there is no consensus yet on how to diagnose OT, which is often based only on clinical presentation. Nevertheless, OT-associated symptoms are often atypical and misleading.

Over the last 20 years, tremendous progress has been made in biological tools, enabling parasitologists to confirm the diagnosis in most suspected cases of OT.
Using anterior chamber puncture, a safe and fast procedure, ophthalmologists sample aqueous humour for analysis using multiple techniques in order to reach high specificity and sensitivity in OT diagnosis.
In this article, we present the different techniques available for the biological diagnosis of OT, along with their characteristics, and propose a diagnostic algorithm designed to select the best of these techniques if clinical examination is not sufficient to ascertain the diagnosis.

Aspergillosis diagnosis

Diagnostic Aspects of Chronic Pulmonary Aspergillosis: Present and New Directions.

Bayu A. P. Wilopo, Malcolm D. Richardson & David W. Denning. *Current Fungal Infection Reports*. **2019**. 13:292–300.

Purpose of Review: Diagnosis of chronic pulmonary aspergillosis (CPA) is important since many diseases have a similar appearance, but require different treatment. This review presents the well-established diagnostic criteria and new laboratory diagnostic approaches that have been evaluated for the diagnosis of this condition.

Recent Findings: Respiratory fungal culture is insensitive for CPA diagnosis. There are many new tests available, especially new platforms to detect Aspergillus IgG. The most recent innovation is a lateral flow device, a point-of-care test that can be used in resource-constrained settings. Chest radiographs without cavitation or pleural thickening have a 100% negative predictive value for chronic cavitary pulmonary aspergillosis in the African setting.

Summary: Early diagnosis of CPA is important to avoid inappropriate treatment. It is our contention that these new diagnostics will transform the diagnosis of CPA and reduce the number of undiagnosed cases or cases with a late diagnosis.