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An inter-laboratory multi-centre evaluation of the performance of Mucorales PCR assays when testing serum specimens: A study by the ISHAM Fungal PCR Initiative and the ModiMucor study group -FPCRI Mucor Laboratory Working group

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culture (Layios et al., 2014). In that case the isolate was identified by ITS sequencing. Therefore the present case is the first describing the preliminary identification of *Mortierella* spp. with a conventional method. It is important to note that the conventional method could identify *Mortierella* spp. seven days earlier than ITS sequencing. It is still not clear if the present case with *Mortierella* spp. is colonization or an infection. The patient is currently being followed up clinically and further lower respiratory samples will be taken up. In the laboratory it feels comfortable to have access to both conventional and modern diagnostic methods. The present study describes the importance of hands on experience in diagnosis of rare fungal pathogens.

P164

An inter-laboratory multi-centre evaluation of the performance of Mucorales PCR assays when testing serum specimens: A study by the ISHAM Fungal PCR Initiative and the ModiMucor study group - FPCRI Mucor Laboratory Working group (A. Alanio, M. Cogliati, S. Fuchs, F. Hagen, C. Halliday, R. Hare, C. Klaassen, M. Lackner, M. Lengerova, W. Posch, B. Sendid, J. Springer, B. Willinger) - ModiMucor study group (F. Botterel, M. E. Bougnoux, S. Bretagne, C. Damiani, F. Dalle, J. Denis, M. Gitts-Muselli, X. Iriart, F. Morio, P. Poirier, E. Scherer)

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Objectives: Real-time qPCR detection of Mucorales DNA in serum, plasma and BAL fluid has been shown to be sensitive and early tool for diagnosing mucormycosis. Several qPCR assays have been developed, but little comparison and no standardization is available. In 2016, the Mucorales Laboratory Working Group of the ISHAM Fungal PCR Initiative (FPCRI) organised inter-laboratory evaluations of Mucorales PCR assays currently in use, with the objectives of: 1) determining the uniformity of qualitative detection (positive/negative) and 2) assessing qPCR performance. Participants were members of the FPCRI, laboratories involved in the ModiMucor* study and three other French laboratories which have already implemented this tool to diagnose mucormycosis.

Methods: For the 1st panel (A), four sera (2ml/sample) were inoculated with genomic DNA (between 27 to 116 pg of DNA/mL of serum) from four different mucorales species (*Rhizomucor pusillus*, *Lichtheimia corymbifera*, *Cunninghamella bertholletiae*, *Rhizopus oryzae*). For the 2nd panel (B), six sera (2 ml/sample) were inoculated with three concentrations of *R. pusillus* and *L. corymbifera* equivalent to one, 10 and 100 genomes/mL of serum. For each panel, a negative control serum was also sent. Twenty laboratories analysed the panel A (10 FPCRI, two Modimucor/FPCRI, seven Modimucor, three others French laboratories) and 22 laboratories analysed the panel B (two more FPCRI laboratories). All participants were requested to use their own DNA extraction methodology. The 12 French participants used the same qPCR technique (combination of three qPCR assays targeting the four most frequent genera: *Lichtheimia*, *Mucor*, *Rhizopus*, *Rhizomucor*) (1). For panel B, only quantitative PCR results were analysed (two techniques that used conventional PCR were excluded). Twenty-two laboratories returned multiple datasets, leading to 26 different datasets for analysis: - 16 datasets using a single technique (1) - 10 datasets using other techniques (Four Pathonostics MucorGenius®, Six "in-house" assays, (2-5), three of which were not previously described).

Results: For panel A, all datasets were negative when testing the negative control serum. Conversely, 85-90% of the sera inoculated with "common" Mucorales (*R. pusillus*, *R. oryzae*, *L. corymbifera*) were positive. Detection of sera containing *C. bertholletiae* DNA was lower (12%). For panel B, the probability of qPCR positivity increased predictably with DNA quantity, generating a mean PCR efficiency of 0.85. Detection of *Lichtheimia* DNA was optimal, irrespective of the technique. Regarding the qPCR systems, Millon et al. and MucorGenius® techniques generated greater positivity rates, with earlier Cq values.

Conclusion: Despite the diversity of techniques, Mucorales DNA detection in sera was very reproducible with little inter-laboratory variability providing support for including Mucorales PCR in the EORTC/MSG definitions of invasive fungal disease and for its use in the clinical diagnosis of mucormycosis *ModiMucor**: French prospective multicenter study for evaluation of Mucorales PCR (PHRC (Projet Hospitalier de Recherche Clinique) national-Modimucor 2014-A00580-47.) **References** 1- Millon et al. CID 2013 2- Springer et al. J. Med. Microbiol. 2016 3- Lengerova et al. J Clin Microbiol 2014 4- Machouart et al. J Clin Microbiol 2006 5- Hrnčirova K., J Clin Microbiol 2010

P165

Comparison of a point-of-care (PreventID®) with the DermaGenius® Nail real-time PCR kit for the detection of dermatophytes species in nail samples

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Objectives: Compare the performance of a point-of-care test for the detection of dermatophyte fungi (PreventID®) with the DermaGenius® 2.0 Nail real-time multiplex PCR assay on a set of clinical nails. Both methods are compared to microscopy as the gold standard method.

Methods: Left over nail samples were split into different parts. The culture and microscopy of all nail samples was performed by an external clinical microbiology laboratory. One part of the nail sample was used for DNA-extraction with the PathoNostics Extraction kit and identification of the pathogen was enabled by using the DermaGenius® 2.0 Nail real-time multiplex PCR. Finally, an immunochromatographic point-of-care test (PreventID® Dermatophyte) was applied to determine the presence of dermatophyte-derived antigens in the nails.

Results: Microscopy of the nails was considered as the gold standard. The DermaGenius® 2.0 Nail real-time multiplex PCR was able to detect all positive samples, resulting in a sensitivity of 100%. Specificity was however lower (83%) due to detection of dermatophyte DNA in microscopy-negative samples. Interestingly, these samples were culture-positive. Culture resulted both in a reduced specificity (67%) and sensitivity (57%). Both the sensitivity (79%) and specificity (83%) for the PreventID® Dermatophyte test were promising. Although the PCR assay is sensitive, the clinical relevance is still a matter of debate. High Ct-values indicate limited amounts of DNA and do not always represent a clear infection. Interestingly, the Ct-values for samples which were negative with the PreventID® test were between 26-33 with the DermaGenius® real-time PCR. In addition, readability of the test strips was limited in some samples while Ct-values of 22-28 with the DermaGenius® PCR was obtained, indicating a high fungal load. These samples were also positive with microscopy.

Conclusion: Microscopy and the DermaGenius® 2.0 Nail real-time PCR showed a good correlation and are useful for rapid identification of dermatophyte infection. For specific pathogen identification, the DermaGenius® PCR can be used. Culture can result in false negatives and in combination with a long turnaround time, it has limited value in routine diagnostics. The use of an immunochromatographic point-of-care test is uncommon in dermatophyte diagnostics but was included in this study to determine its suitability. Although the PreventID® Dermatophyte test results in a sensitivity of 79%, some dermatophyte infections with low Ct-values were missed. The difference in sensitivity could be explained by the fact that the PreventID® test is using a protein target while the DermaGenius® PCR detects DNA, which is normally not influenced by treatment. But more important is that the interpretation of the PreventID® test is based on visual inspection which makes the test less reliable. The PreventID® should be compared in a larger sample set to determine suitability for routine dermatophyte diagnostics. Currently, microscopy and the DermaGenius® 2.0 Nail real-time PCR seem to be the most reliable and suitable methods for diagnosis of dermatophyte infections in nails.

P166

Polymerase chain reaction on respiratory tract specimens of immunocompromised patients to diagnose Pneumocystosis - A systematic review