**Supplementary Appendix: Recent Advances in the Diagnosis of Talaromycosis**

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**(1) Recent change in *Talaromyces marneffei* nomenclature**

*T. marneffei* was previously classified under the *Penicillium* subgenus *Biverticillium* based on morphological features. Further phylogenetic research led to the transfer of *Penicillium* subgenus *Biverticillium* to *Talaromyces* and renaming of *Penicillium marneffei* to *T. marneffei* in 2011*1.* Other *Talaromyces* species have been reported to cause infections in humans, albeit rarely, including *T. amestolkiae*, *T. purpurogenus* and *T. piceu*s. *T. marneffei* can be readily differentiated from other *Talaromyces* as the only *Talaromyces* spp. that exhibits thermal dimorphism, switching from an environmental mold to yeast form at body temperature (≥ 35°C), and is among the few *Talaromyces* spp. that produce a bright red pigmentation in the mold form2,3.

**(2) Safety Considerations for the Laboratory Diagnosis of Talaromycosis**

T. marneffei is classified as a risk group 2 human pathogen; hence all open fungal cultures (even when working with the non-infectious yeast form) should be handled inside a class II Biological Safety Cabinet (BSCII) both in endemic and non-endemic settings, including procedures to extract fungal proteins for MALDI-TOF MS identification.

**(3) Antifungal Susceptibility Testing**

A major advantage of culture is the ability to perform antifungal susceptibility testing using either broth dilution methods or E-test. Like all dimorphic fungi, antifungal susceptibility of *T. marneffei* varies between the mold and yeast form4. The reported minimum inhibitory concentrations (MICs) among various studies and among various antifungal susceptibility testing methods are generally consistent. *T.* *marneffei* is highly susceptible to the azole drugs itraconazole, voriconazole, posaconazole, isavuconazole (0.001 µg/ml to 0.07 µg/ml)4-7, and 5-fluorocytosine (5-FC) (0.015 µg/ml to 1.0 µg/ml)7-9. MICs are intermediate for amphotericin B (0.12 µg/ml to 4.0 µg/ml)7 but clinically amphotericin B is very potent against talaromycosis10. MICs are higher for fluconazole (0.25 µg/ml to 16.0 µg/ml)8 and the echinocandins (2 to 8 µg/ml), suggesting that they are unlikely to be effective5,6,8,9. Studies of new antifungal drugs are limited but olorofim fosmanogepix, and oteseconazole have shown low MICs9. The MIC cut-off to define clinical activity of antifungal drugs against *T. marneffei* have not been established. However, in a recent study examining 101 cases of talaromycosis, cases of slow fungal clearance, defined as persistence of blood culture positivity after 2 weeks of antifungal therapy with amphotericin B, exhibited higher MICs for both voriconazole and fluconazole (*χ2* = 12.623, *P* < 0.001 and *χ2* = 9.356, *P* = 0.002, respectively), suggesting the possibility of drug resistance11.

**(4) Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry Commercial Databases**

*T. marneffei* identification has recently been added to the Bruker Biotyper system but still requires database expansion with local strains in endemic regions12. *T. marneffei* is not yet included in the Vitek MS IVD Database (Biomerieux, Marcy l’Etoile, France)13. In a recent evaluation of a new MALDI-TOF MS system, the Zybio EXS3000 (Zybio Inc., Chongqing, China) correctly identified 100% of the 135 *T. marneffei* strains from four distinct clusters8.

**(5) The role of antigen testing for screening, prognostication, and monitoring of treatment response**

Mp1p antigenemia detected by EIA has been shown to precede blood culture positivity by up to 16 weeks14,15, and therefore has the potential to be used for targeted screening of high-risk patients with advanced HIV disease before the onset of clinical symptoms16. This may facilitate disease prevention through a screen-and-treat strategy with the potential to reduce mortality17. Quantification of antigen levels is possible with the Mp1p EIA using a standard curve of known concentrations of the recombinant Mp1p, or of the Mp1p LFA using a lateral flow strip reader18-20. The ability to quantify antigen levels presents an opportunity to improve clinical management, in predicting disease severity, monitoring treatment response, and evaluating treatment effect of novel antifungal strategies.

**(6) Approaches to Increase the Diagnostic Sensitivity of *T. marneffei* PCR**

Whole blood as a specimen is shown to outperform plasma in sensitivity as it allows detection of both intracellular and extracellular fungal DNA. *T. marneffei* replicates inside macrophages, which may be lost during serum or plasma specimen processing21. As demonstrated for other fungal pathogens like *Aspergillus* and *Mucorales*, DNA extraction of high volumes of whole blood (≥ 1 – 3 mL), elution of DNA in smaller volumes (< 50 μl) and reducing the ratio of DNA extract to the final volume of PCR reaction may improve diagnostic yield, particularly in patients with negative blood culture22-26. The use of bead-beating prior to DNA extraction, to mechanically facilitate the release of fungal DNA from yeast cells, improves the recovery of fungal DNA, and requires only 1-2 minutes additional time with minimal equipment. For the extraction of *Histoplasma* spp. DNA, bead-beating has been shown to increase the yield 100-fold26. In a recent evaluation of a 5.8S qPCR assay, DNA extraction using the MasterPure Yeast DNA Purification kit with bead beating on whole blood demonstrated the highest analytical sensitivity to date of 1 yeast cell ml-1 and high clinical sensitivity of 88%21. The addition of bead beating prior to DNA extraction by the MasterPure Yeast DNA Purification kit resulted in improved assay performance compared to sonication or to no bead beating (*P* = 0.0064).”

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