Reviewer's report

Title: Performances of two different panfungal PCRs to detect mould DNA in formalin-fixed paraffin-embedded tissue: what are the limiting factors?

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Reviewer: Volker Rickerts

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Major revisions
1. The use of extraction negative controls in this study is important. However, it highlights a problem of broadrange fungal PCR assays applied to samples with low levels of target DNA.

DNA, that the authors rate as contamination is present in 5 clinical samples and “erratically identified in negative extraction controls... They were detected in single PCR reactions .... and could not be confirmed in a repeated testing ”. However, if a single positive PCR assay was counted as positive in other cases (sample no.5, line 551), it is unclear to me how the authors identify contamination versus true detection of causative fungi.

The same criteria need to be applied. For example, amplification of Cladosporium DNA, that is widespread in the environment, in the absence of hyphae suggesting a cladosporium infection may be a hot candidate for contamination instead of true infection. Given this, the claim of 100% specificity is dubious. Therefore, a report on the number of all extraction controls, negatives and positives and the sequencing results are needed together with clear criteria what makes a DNA amplification a positive result.

2. The sensitivity of the ITS-2 assay is within the range of previously published results, although the authors used more tissue than in other studies (see minor point 5). It has been previously documented that longer amplicons a less often amplified from FFPE samples. It would be interesting to compare the positivity of fungal PCR’s with the amplicon length to different length human amplicons done by the authors in a multiplex PCR (line 201). Unfortunately data on the different length amplicons are not reported in table 3.

Discretionary Revisions
1. The authors use terms like “broadrange panfungal” (line 111) and “potentially all fungal organisms” (116). I would feel more comfortable with the to use broadrange instead of panfungal, which may not exist.

2. The authors mention an “additional heating step” during the extraction procedure (173). It would be great to provide a rational for that.

3. The authors use the term “DNA-degrading”. As DNA extraction differs with different extraction procedures it may not be degradation of DNA that is the problem. I think crosslinking with proteins may be more accurate to describe this.
4. Discrepant PCR results and culture (line 355 ff): Mixed infections should be mentioned in the discussion of possible reasons for discrepant results. Case reports of mixed infections are clearly documented. In addition, culture positive sample no.2 in this series (line 509) may also be a mixed infection and the potential of preferential amplification by the ITS-assay should be addressed.

5. I miss a discussion on the number of inhibited samples in this study. How is this number compared to existing literature of fungal PCR from FFPE samples.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I have no competing interests