

## ***Interactive comment on “Annual distribution of allergenic fungal spores in atmospheric particulate matter in the eastern mediterranean; a comparative study between ergosterol and quantitative PCR analysis” by N. Lang-Yona et al.***

**Anonymous Referee #2**

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General Comment: The paper is exploiting molecular analytical methods to quantify fungal (general and species specific) prevalence in atmospheric environment. In the analysis, the results of the molecular approach is benchmarking with the 'ergosterol' marker resulted from analytical approach (GC-MS) published by the same group. While the intention to exploit more 'precise' methodologies in quantifying the fungal prevalence is appreciated, the current method comparison seems inadequate.

Specific comment: 1. Quality control in interpreting qPCR is very important. Yamamoto et al. (2011) study provided a rather solid and concrete quality control (MDL, COV, etc)

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studies on house dust. What will be the corresponding MDL, COV for the present study?

2. The conversion of the qPCR results to spore concentration depends on validated calibration curves. While the standard curves of specific allergenic groups can be prepared by using the known species/or member species, how is the total spores using the universal fungal primer was referencing to? What are the precision and accuracy will this standard curve be?

3. It seems that the authors had used two different thermal cycles in running the PCRs. Pg 28694, line 2: initial 20s denaturation and enzyme activation at 95oC, followed by 45 cycles of 3s denaturation at 95oC, and 30s annealing and extension at 60oC; while in line 25 of the same page: 50oC for 2 min, 95oC for 15 min of initial denaturation and 45 cycles at 95oC for 15s dissociation and 60oC for 1 min of annealing and extension. We know that the PCR products depends much on conditions of the thermal cycle. Why there are two different operations and how will this affect the results and their interpretations?

Overall, quality assurance is needed to build confidence on the results reported.

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