

# 1 Classification accuracy and compatibility across devices of a new 2 Rapid-E+ flow cytometer

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11 Matt Smith #1 (Citation: <https://doi.org/10.5194/egusphere-2024-187-CC1>)

12 The authors present a very interesting and robust study examining the classification accuracy and compatibility across devices  
13 of a new Rapid-E+ flow cytometer for examining airborne pollen. The paper is generally well written, although it could do  
14 with thorough editing with specific focus on the use of articles. I have listed some minor comments below that I hope will  
15 help.

16 **Reply:** The authors would like to thank Matt Smith for interest in the study and for his helpful comments, which we have used  
17 to improve our manuscript. Below we answer the questions and indicate the changes we have made to the revised manuscript.

18  
19 My one comment about the methods relates to the use of the Hirst type trap (Lines 161 to 165). When calibrating such sensitive  
20 instruments as the Rapid-E and Rapid-E+, it is important to remove as much uncertainty as possible. The authors might  
21 therefore consider counting whole slides from the Hirst type trap to reduce error. Obviously, this is not always feasible when  
22 examining whole seasons, but even examining a small subset of slides in this way might provide some interesting insights.  
23 Although I note that correlations were only conducted for or days when average pollen concentrations measured by the manual  
24 method exceeded 10 pollen m<sup>-3</sup> in order to reduce uncertainty.

25 **Reply:** The reviewer is correct that the standard method (EN16868) has large uncertainty that originates from different critical  
26 points (i.e. flow measurements, pollen identification, subsampling during analysing collected samples). Recent study by Mimic  
27 and Sikoparija (2021) confirmed that analysing 100% of samples coming from Hirst type traps is expected to improve  
28 comparison of time series obtained from different devices especially for low concentrations. However, as the reviewer correctly  
29 pointed out, analysing an entire sample under microscope for the entire season is nor realistic, the effect is quite small, and all  
30 measurement critical points exist in an automatic approach as well (Tummon et al. 2022), but still are not precisely quantified.  
31 This is why we followed the recommendations of the EN16868 norm: to limit the influence of measurement uncertainty when  
32 comparing results from different methods. We focused on daily values and only considered cases where a sufficient amount  
33 of pollen was detected. To clearly address this aspect, we have added the following sentence to section 3.3:

34 “Limited improvement in correlations could be expected if the measurement uncertainty of the standard Hirst volumetric  
35 method (EN16868), inherited from the subsampling during analysing collected samples, is eliminated by counting 100% of  
36 microscopic slides (Mimic and Sikoparija 2021). However, such analysis for the entire season is extremely difficult and even  
37 if done so, the effect is presumed to be small.”

38  
39 Minor comments

40 Line 47 - “which is a new model stemming from the PA-300 (Crouzy et al., 2016) and Rapid-E (Sauliene et al., 2019”).  
41 **Reply:** Corrected as suggested.  
42  
43 Line 49 – “In particular, Rapid-E+ samples at a flow rate of 5 l min<sup>-1</sup>”  
44 **Reply:** Corrected as suggested.  
45  
46 Line 53 – “Like its predecessor”  
47 **Reply:** Corrected as suggested.  
48  
49 Line 73 – “was trained in the Novi Sad laboratory”  
50 **Reply:** Corrected as suggested.  
51  
52 Line 74 – “owned by the City of Osijek in Croatia”  
53 **Reply:** Corrected as suggested.  
54  
55 Line 74 – “and the Finnish Meteorological Institute”  
56 **Reply:** Corrected as suggested.  
57  
58 Lines 79/80 – “for the Pannonian Plain” Lines 85/86 – “or capturing the main features”  
59 **Reply:** Corrected as suggested.  
60  
61 Line 91 – “Scientific names should be italics” (review throughout including figures and tables).  
62 **Reply:** The scientific names of the plant species from which pollen was used in the model training were written in italics  
63 (Table A2). For classes of pollen identified from aerobiological samples (automatic and manual) we did not use the taxonomic  
64 nomenclature because the pollen classes do not fully represent taxonomic categories. For example, in real time detections class  
65 *Artemisia* is trained on pollen from *Artemisia absintium* L., *Artemisia vulgaris* L., thus it cannot be fully representative for  
66 genus *Artemisia*. Similarly, in manual analysis the class *Artemisia* recorded in the given day could consist of pollen coming  
67 either from one or several species thus never being representative for the entire genus *Artemisia*. To address this, we have  
68 added the following info in the Table A2:  
69 “\* does not fully represent taxonomic rank (i.e. pollen in reference data coming only from one or several species of the  
70 respective taxonomic category) thus not written in italics”  
71  
72 Lines 98/99 – “To ensure identification”  
73 **Reply:** Corrected as suggested  
74  
75 Line 102 - by using a Swisens Atomizer  
76 **Reply:** Corrected as suggested  
77  
78 Line 193 – “It is interesting to note that after the start of rainfall the coarse particles”  
79 **Reply:** Corrected as suggested.  
80  
81 Line 196 – The following lacks clarity and should be rewritten "However, quite low flow rate"  
82 **Reply:** The sentence is rewritten and now reads:  
83 “However, following the equations given in Tummon et al. (2022), the flow rate of the Rapid-E+ (5 l min<sup>-1</sup>) is not sufficient  
84 to measure all relevant concentrations at sub hour temporal resolution with reasonably low uncertainty.”  
85  
86 Line 208 – “despite these pollen grains being morphologically similar” (note that the plural of pollen is pollen)  
87 **Reply:** Corrected as suggested.  
88  
89 Line 245 – “There was a clear tendency towards confusion of different pollen occurring”

90 **Reply:** Corrected as suggested.

91

92 Table 1 - It would be interesting to see the correlation coefficients for Taxaceae/Cupressaceae combined and for the Urticaceae  
93 family, as many pollen monitoring networks do not separate these into different genera due to the difficulty in identification.

94 **Reply:** We have calculated correlations for Taxaceae/Cupressaceae (sum of Taxus and Juniperus in Rapid-E+ data), Urticaceae  
95 (sum of Urtica and Parietaria in Rapid-E+ data) and Cannabaceae (sum of Cannabis and Humulus in Rapid-E+ data), and  
96 added coefficients into Table 1. Also, we added the following sentence to the results section:

97 “Merging Rapid-E+ measurements for classes that are difficult to identify by manual method (i.e. Taxus and Juniperus, Urtica  
98 and Parietaria, Cannabis and Humulus) did not improve the correlations (Table 1).”

99

100 Line 256 – “Repeating it for each device in a network is unfeasible”

101 **Reply:** Corrected as suggested.

102

103 Lines 261/262 – The following text lacks clarity and needs reworking, perhaps linked to another sentence "Demonstrated for  
104 Rapid-E, the problem also existed for Rapid-E+ (Fig. 4)".

105 **Reply:** The text is now rewritten and reads:

106 “As a result, classification performance falls when a model trained on a reference dataset from one device is tested on a  
107 reference dataset from another one, which was demonstrated for Rapid-E (Matavulj et al. 2021). The same problem exists in  
108 Rapid-E+ (Fig. 4).”

109

110 Line 263 - pollen not pollens

111 **Reply:** Corrected as suggested.

112

113 Line 274 - pollen not pollens

114 **Reply:** Corrected as suggested.

115

116 Line 277 – “Although this was not seen for all pollen types, there are pollen classes with comparable”

117 **Reply:** Corrected as suggested.

118

119 Line 317 – “datasets, the creation of which is a highly demanding process”.

120 **Reply:** Corrected as suggested.

121

122

## 123 **References**

124 Mimic, G., Sikoparija, B.: Analysis of airborne pollen time series originating from Hirst-type volumetric samplers—  
125 comparison between mobile sampling head oriented toward wind direction and fixed sampling head with two-layered  
126 inlet, *Aerobiologia* 37:321-331, <https://doi.org/10.1007/s10453-021-09695-7>, 2021.

127 Tummon, F., Bruffaerts, N., Celenk, S., Choël, M., Clot, B., Crouzy, B., Galán, C., Gilge, S., Hajkova, L., Mokin, V.,  
128 O’Connor, D., Rodinkova, V., Sauliene, I., Sikoparija, B., Sofiev, M., Sozinova, O., Tesendic, D. and Vasilatou, K.:  
129 Towards standardisation of automatic pollen and fungal spore monitoring: best practises and guidelines, *Aerobiologia*.  
130 <https://doi.org/10.1007/s10453-022-09755-6>, 2022.