Short Communication: an abbreviated method for the Quality Control of pollen counters

MATT SMITH[1], JOSE OTEROS[2], CARSTEN SCHMIDT-WEBER[2, 3], JEROEN T M BUTERS[2]

[1]Institute of Science and the Environment, University of Worcester, UK

[2]ZAUM – Center of Allergy & Environment, Technical University Munich/ Helmholtz Center Munich, Germany

[3]Member of the German Center for Lung Research (DZL), Munich, Germany

Correspondence: Dr Matt Smith, School of Science and the Environment, University of Worcester, Henwick Grove, Worcester, WR2 6AJ, United Kingdom. E-mail: aeromattsmith@gmail.com

Abstract

We present an abbreviated method for conducting large scale Quality Control (QC) exercises over limited time periods, which was used for examining the proficiency of technicians involved in the Bavarian ePIN network. The goal was for technicians to have their analysis skills evaluated at least twice: (1) by having at least one of their slides successfully checked by other counters in the ePIN network and (2) by successfully examining at least one additional slide from other sites. Success was judged as a Relative Difference (RDif %) $\leq$ 30% between the two daily average pollen concentrations. A total of 21 sites participated in the ePIN QC exercise. All of the results for Total Pollen had RDif % <30%. Only 5 results had RDif > 30%, 3 for *Betula* and 2 for Poaceae pollen. Of these, 3 were slides containing < 40 pollen/m^3 daily average and 2 were for sites that had microscopes with small fields of view and examined <10% of the slide surface. More than 80% of the participants had at least two slides successfully checked by someone else in the network, and all of the participants had one slide successfully examined. The latter is comparable to a traditional ring test where only one slide is sent to participating sites. The method described here enabled a large number of technicians to be examined in a short period of time and represents a viable alternative to other approaches that can take many months to complete.

Key words: Aerobiology; *Betula* pollen; Poaceae pollen; Quality Assurance; Quality Control

We present the results of a novel method for conducting large scale QC exercises over limited time periods, which was adopted by the electronic Pollen Information Network for Bavaria, Germany (ePIN) for examining the proficiency of technicians examining *Betula* and Poaceae pollen. This phase of the ePIN study aimed to identify optimal sites (number and locations) for placing a network of automated pollen monitoring systems based on BAA500 methodology (the BAA500 is a fully automated, image recognition-based pollen monitoring system produced by Helmut Hund GmbH) (Oteros et al. 2015). This was achieved by building an intensive network of 27 volumetric Hirst (1952) type samplers in Bavaria. Due to the large volume of samples generated in a such short period of time, which was too much for one institute to deal with, we employed experts from across Europe to simultaneously count the slides.

All pollen counters (henceforth referred to as technicians) involved in the ePIN study were required to participate in a Quality Control (QC) exercise. Unfortunately, performing an inter-laboratory ring test using the same sample slide, as recommended by the European Aerobiology Society's Working Group on Quality Control (Galán et al. 2014), was not practicable on this occasion because the ePIN study was constrained by time and results were required before the project finished (in the same calendar year as the study started). In comparison, the QC exercise for *Ambrosia* pollen took a total of 531 days from when the exercise commenced until all 69 analysts reported their results (Sikoparija et al. 2017).

Preparations began on 15.01.15 and the network was disassembled before the end of 2015. The full network was operational for approximately 7 months (15.03.15 until 15.09.15) (Table 1). The ePIN project took a reasonable amount of care to ensure that the data were reproducible. Where possible, atmospheric concentrations of pollen were collected and analysed following the European Aerobiology Society (EAS) minimum recommendations (Galán et al. 2014). The atmospheric

samplers used in the study were all of the Hirst (1952) design, which ensured that data were comparable between sites. The Hirst type traps sample at a continuous volume of 10 l/min, drawing in 14.4 m^3 of air every day. All traps were calibrated for the correct flow using the same rotameter eliminating flow error (Oteros et al. 2017). All slides were prepared in a central laboratory by the same technicians using the same protocol, and marked so the transects could be identified. Slides were examined by light microscopy, and pollen grains were identified at x 400 magnification. Pollen were counted along 4 horizontal transects, in 12hr intervals, according to the standardized method of the German Pollenfluginformationsdienst (PID) (Winkler et al. 2001). All data were entered into a custom-made Excel spreadsheet supplied to technicians at the beginning of ePIN. Raw counts were converted into concentrations and expressed as pollen/m^3 daily average. Experienced technicians from existing pollen-monitoring networks were recruited for analysing the slides.

A total of 20 sites were available to participate in the ePIN QC exercise for *Betula* pollen and 21 sites in the QC exercise for Poaceae pollen. Six additional sites belonging to ePIN were independent and conducted their own QC. The goal of the ePIN QC survey was for technicians in charge of each site to have their analysis skills evaluated at least twice; a minimum of one slide from their site successfully checked by other counters in the ePIN network and by successfully examining at least one additional slides from another site. Note that "success" was judged as a Relative Difference (RDif %) $\leq$ 30% between the two daily average pollen concentrations following Comtois et al. (1999). This was considered to be the "recommended standard" that technicians should attain. The QC exercise was carried out in two parts: (1) QC for airborne *Betula* pollen; (2) QC for airborne Poaceae pollen:

Part 1: At the end of the airborne *Betula* pollen season, technicians were contacted and asked to send 4 slides they had been analysing to the Project Manager of ePIN at the Centre of Allergy & Environment (ZAUM) in Munich, Germany. It was requested that each of these slides should contain a minimum of 40 *Betula* pollen/m^3 and maximum of 300 *Betula* pollen/m^3 daily average. Low values (< 40 pollen/m^3) were excluded due to the fact that they can cause problems in the QC process as variations of even a few pollen grains can cause the RDif % to exceed 30%. The upper limit was selected because it represents the sort of levels often encountered on daily slides, but these levels are not excessively high and should not unduly increase the work for the participants.

Part 2: The same method for conducting the QC exercise for *Betula* pollen was used for Poaceae. The main difference being that technicians were contacted at the end of the Poaceae pollen season and asked to send 3 slides they had analysed to the Project Manager of ePIN. It was requested that each of these slides should contain a minimum of 40 Poaceae pollen/m^3 and maximum of 300 Poaceae pollen/m^3 daily average.

The Project Manager of ePIN collated the slides, re-labelled them, and then sent a selection back out to participating sites. All technicians were expected to re-analyse pollen slides from other participants in ePIN (not the slides they had already analysed). The slides were re-labelled so that the technicians remained anonymous. Not all requested slides were examined in the QC survey. In order to aid analysis, and to determine possible causes of error, technicians were requested to record all pollen types listed in the ePIN protocol present on the slides and not just *Betula* or Poaceae. Daily average airborne *Betula* or Poaceae pollen concentrations that varied by > $\pm$ 30% were deemed outside the limits of the QC survey and required further investigation.

Twenty sites were included in the ePIN QC survey for *Betula.* A total of 46 results were submitted for Total Pollen and 48 results for *Betula* pollen (i.e. daily airborne pollen concentrations submitted by two technicians for the same sample). It was encouraging to see that all the results for "Total Pollen" had a Relative Difference of 30% or less (Fig. 1A). Furthermore, out of a total of 48 daily average *Betula* pollen concentrations included in the ePIN QC, only 3 had RDif > 30% (Fig. 1B). Seventeen technicians (85%) managed to have their analysis skills evaluated at least twice during the ePIN survey for *Betula* pollen before it ended. Nonetheless, the technicians responsible for the remaining 3 sites did succeed in either submitting at least one slide to the QC exercise for *Betula* that was successfully checked by other technicians, or they successfully examined at least one slide from another site. To put this into context, this is comparable to the results of a traditional inter laboratory ring-test where only one slide is sent round for participants to analyse.

Forty-one slides were entered into the QC survey for Poaceae pollen. The goal of having 2 slides successfully examined (RDif % $\leq$ 30%) was achieved by 17 (81%) of the sites that were entered into the ePIN QC for Poaceae, and all technicians successfully examined one slide in the QC exercise. As with the *Betula* QC, all the results for Total Pollen on the Poaceae slides had Relative Difference $<^{\pm}$ 30% (Fig. 2A). There was more variation for the results of Poaceae pollen compared to Total Pollen, and 2 daily average Poaceae pollen concentrations included in the ePIN QC had RDif > 30% (Fig. 2B).

The area of the slide examined is likely to make a noticeable difference between counts (Comtois et al. 1999), and this sampled area is influenced by the microscope's field of view and the amount of magnification used. A general recommendation is that at least 10% of the slide should be examined (Mandrioli et al. 1998; Sikoparija et al. 2011; Galán et al. 2014). This project

did not insist on analysing a minimum of 10% of the sample by optical microscopy because the network, and all historical pollen time series, followed the standardized German method (VDI4252-4, 2016). As a result, 4 sites examined <10% of the slide because the technicians used microscopes that had a small field of view. However, future work of this kind should consider the field of view of the microscope. The usefulness of using a square eye-piece graticule should also be considered, as this is sometimes used to further reduce the area of the slide examined.

The method described here enabled a large number of technicians to be examined in a very short period of time (i.e. weeks) and represents a viable alternative to other approaches that can take many months to complete. However, the authors would like to stress that this method should not replace the Quality Control Exercises coordinated by the European Aerobiology Society's Working Group on Quality Control, which remains the benchmark in aerobiology (Galán et al. 2014; Sikoparija et al. 2017).

This study was extremely ambitious in the time allotted, and the main reason why some counters did not examine at least two slides can be attributed to logistics. The results also show that the biggest factors affecting reproducibility of the analysis were slides containing insufficient pollen for analysis (i.e. < 40 pollen/m^3) and microscopes with a small field of view reducing the area of slide examined (i.e. <10%) rather than the ability of technicians to successfully identify pollen.

Acknowledgments

The authors would like to thank all participants in ePIN for making this work possible, especially the pollen counters. This publication was funded by the Bavarian State Ministry of Health and Care

grant G47-G8180.1-2014/5-25 and the Bavarian State Ministry of the Environment and Consumer Protection grant TKP01KPB-69312.

References

Comtois P, Alcazar P, Neron D. 1999. Pollen counts statistics and its relevance to precision. Aerobiologia, 15, 19-28.

Galán C, Smith M, Thibaudon M, Frenguelli G, Oteros J, Gehrig R, Berger U, Clot B, Brandao R. 2014. Pollen monitoring: minimum requirements and reproducibility of analysis. Aerobiologia, 30, 385-395.

Hirst JM. 1952. An automatic volumetric spore trap. The Annals of Applied Biology, 39, 257-265.

Mandrioli P, Comtois P, Levizzani V. 1998. Methods in Aerobiology, Bologna, Pitagora Editrice.

Oteros J, Buters J, Laven G, Roseler S, Wachter R, Schmidt-Weber C, Hofmann F. 2017. Errors in determining the flow rate of Hirst-type pollen traps. Aerobiologia, 33, 201-210.

Oteros J, Pusch G, Weichenmeier I, Heimann U, Möller R, Röseler S, Traidl-Hoffmann C, Schmidt-Weber C, Buters JTM. 2015. Automatic and Online Pollen Monitoring. International Archives of Allergy and Immunology, 167, 158-166.

Sikoparija B, Galán C, Smith M, EAS_QC_Working_Group. 2017. Pollen-monitoring: between analyst proficiency testing. Aerobiologia, 33, 191.

Sikoparija B, Pejak-Sikoparija T, Radisic P, Smith M, Galan Soldevilla C. 2011. The effect of changes to the method of estimating the pollen count from aerobiological samples. Journal of Environmental Monitoring, 13, 384-390.

Winkler H, Ostrowski R, Wilhelm M, K-C. B. 2001. Polleninformationsdienst SD:

Pollenbestimmungsbuch der stiftung Deutscher Polleninformationsdienst. Berlin