A post-hoc qualitative analysis of real time heads-up pollen counting versus traditional microscopy counting in the Environmental Exposure Unit (EEU)

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Abstract

RATIONALE: A custom digital imagery method for real time identification and counting of pollen was qualitatively evaluated in the Environmental Exposure Unit (EEU).

METHODS: Airborne pollen was collected in the EEU via a Rotorod® impact sampler. The pollen grains on each sampling rod were counted using both traditional and Heads-up counting microscopy. The heads-up technique incorporated a microscope camera to create an on-screen image of the sampling rod. Firstly, unique images were created by manually advancing the stage, without duplicating previously captured pollen. Well-defined, sharp images were obtained by fine focus and custom settings for both illumination and objective combinations to enhance contrast and recognition speed. Secondly, using a custom application, pollen grains were identified and counted on-screen with “point and click” or “screen touch”, simultaneously counting and permanently anchoring spuge dots to the pollen grain locations. Counts were stored in real time on a central database.

RESULTS: Increased clarity of the pollen grains resulted in higher counting accuracy. Duplicate counting of pollen grains was eliminated by digitally labelling counted grains. Additional advantages of Heads-up counting: automatic counting, no human errors, 100% capture efficiency without human intervention. Improved image quality and reduced counting errors due to more controlled, human-free, automated counting. The digital images are also retained for off-line analysis.

CONCLUSIONS: This validated heads-up counting technique will allow for an increased response time to changes in the EEU pollen levels. This advancement could also enhance pollen counting processes followed by others using direct microscopy pollen counting techniques.

Background

The Environmental Exposure Unit (EEU) is a unique, internationally recognized research facility that allows for the evaluation of concentration of groups of 5 to 150 volunteers to ambient levels of airborne allergens such as ragweed and grass pollen.

Traditional pollen recognition, for the purpose of quantification during clinical research in the EEU, is being challenged. The EEU has previously utilized greased rods and simple microscopy techniques to identify the pollen of study. New recognition software is available for purchase using specialized automated microscopes to facilitate this process in a regular 8-hour lab environment. This software did not, however, provide the EEU with the ability to perform rapid counting with visual confirmation at study sites.

The EEU needs to maintain compliance with all regulatory requirements including the FDA’s 21 CFR PT 11. This need for conformity presents a challenging but necessary task for the development of a system that will meet the requirements for customization of existing data capture methods and the integration of the digital formats into the existing research infrastructure.

Objective

This study examined the ability to maintain and/or enhance the visualization of airborne pollen in its digital format to provide flexibility for future EEU studies to quantify pollen exposure and generate a system to archive the images, data, and resulting reports for future data verification if required.

Results

The Rotorod Count System is a new research tool used in the EEU to collect airborne pollen. The traditional method was microscopy in slides and count live pollen for each time point and a dataset is complete that is later input to a data management system. Capturing the full rods as a compilation of digital images and screen counting results in the generation of one database file including reports, images with corresponding data. Imaging and counting can be performed in parallel.

Summary & Discussion

Historically, the EEU has utilized rotorod impact sampling combined with traditional microscopy to identify and quantify pollen grains. This is a well-recognized method that “provides consistent and reliable measurements of ragweed pollen concentrations” (Heffer M et al. Aerobiology, 2005; 21: 233-239). To meet regulatory requirements, data is entered in a customizable data management system, quality checked, and the sampling rods are physically archived for 25 years.

Modifying the traditional process we intended to enhance the ability to recognize specific particles, therefore, reducing possible recognition errors, lessening stage manipulation and mechanical counting errors, and bundle the counting, data entry, error checking, reporting, and archiving as integrated processes into our Clinical Trial Management System. The process of recognition and counting is split into two separate processes; capturing images and counting pollen allowing a single member to focus on one task only. Errors during the handling of the sampling rods is often unavoidable, however, by creating digital images of the entire rod retaining the physical rods for recounting or archival is no longer required.

No assumptions are made that the microscope operator correctly identified all the pollen grains, proof of the count is still visual but immediately reviewable. The traditional method required remounting sampling rods on slides and incurring further error due to marring or misplacement of the rod. Reducing the number of pollen counting staff required is also a possibility. Redating qualified and experienced microscope operators is still a necessity, however only one microscope fitted with a camera needs to be used to create the sampling rod images. The counting process does not require the use of a microscope which allows for flexibility of location for the counting process. The Rotorod Counter can focus on identification of the pollen of interest, rather than operating the microscope. With access to the CTMS via our closed network and a qualified laptop or computer, the counting can be done anywhere. Although, the regulatory requirements relating to Health Canada, GCPS and the FDA’s 21 CFR PT11 were being met with the previous method, a new method incorporates the requirements in a validated system using current technologies and allowing the process to grow and improve with advancing technology.

Conclusions

Heads-up digital pollen counting is slower at times than the traditional method, however it does allow for processing thousands of pollen grains and rods (as per regulations). Only one also member requires microscope training, the remaining team members only require pollen recognition training. Heads-up digital counting will be implemented in future EEU trials to support our internal mandate for continuous process improvement.