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 grass 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 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 grains. Well-defined, sharp images were obtained by fine focus and zoom combinations to enhance certainty and recognition speed. Secondly, using a custom application, each pollen grain was identified and counted on-screen by "point and click" or "screen touch", simultaneously counting and permanently anchoring opaque 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 need for manual counting devices, commonly associated with mechanical and human errors, was eliminated. Error free counts can be obtained with increased speed, therefore, improving the overall efficiency of the process and the EEU system as a whole.
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 exposure 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 rotorods 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 paced lab environment. This software did not, however, provide the EEU with the ability to perform rapid counting with visual assurance at a modest cost nor provide the need for flexibility of technique. 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 requirement 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 visual clarity 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.

Methods

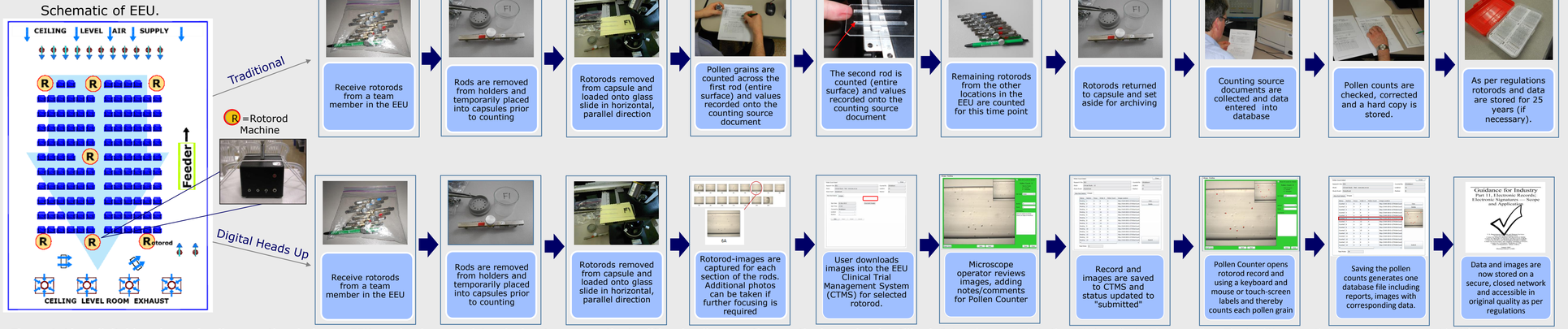

Figure 1: Live, digital imaging and pollen counting during an EEU session has a variety of advantages over the traditional pollen counting method previously utilized. Rotorods are used in the EEU to collect airborne pollen. The traditional method uses microscopy to identify and count the pollen for each time point and a source document is completed that is later inputted into a data management system. Capturing the full rod as a compilation of digital images and on-screen counting results in the generation of one database file including reports, images with corresponding data. Imaging and counting can be performed separately.

Results

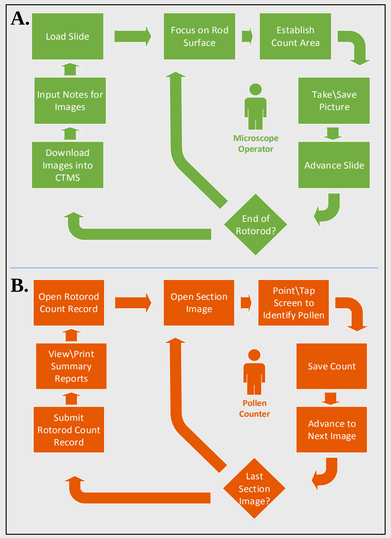

Figure 2: Employing assembly line tactics decreases process related errors. One person can focus on capturing the rods as images (A.), while a second person performs pollen counts on a screen.

This sub-study was self-funded.

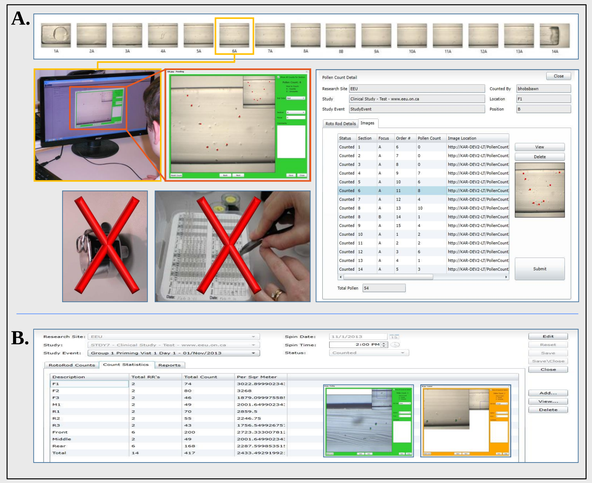

Figure 3: Visually guided, permanent pictures of the rotorod sections verify that a pollen sample was taken during an EEU study as well as validate the count data. A. Retaining the assemblage of rotorod pictures per given sampling session allows a repeatable representation of the results. Quality assurance (i.e. additional counting) of retained graphics can be completed easily without errors associated with re-handling of the rotorod. Archiving the rotorod itself can lead to marred and contaminated rods and hence misinterpretation. B. Double-counting or miscounting is eliminated by limiting stage manipulation during the identification and counting process. Manual tally devices are no longer a source of additional error. Double-data entry rules can be incorporated in the CTMS. Furthermore, real-time data analysis of counts can be performed easily without double data entry or human performed calculations. All data is summarized and viewable through a validated CTMS.

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*.,Aerobiologia, 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 the site 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 of 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. Retaining 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 Pollen Counter can now 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, GCP, and the FDA's 21 CFR PT11 were being met with the previous method, the new method incorporates the requirements in a validated central 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 proper archiving of all data and rods (as per regulations). Only one site member requires microscope training, the remaining team members only require pollen recognition training. Head's-up digital counting will be implemented in future EEU trials to support our internal mandate for continuous process improvement.