This PC-104 computer control system is designed to be worn by workers on the back. The cascade impactor with the miniature motors are worn on the chest while the air sampling pump is attached to the belt. The second computer is to be controlled by the industrial hygienist who is taking the air sample. However, the second computer can also be automatically controlled using radio frequency identification system. The remote control device and the eight-channel cascade impactor are needed for risk assessment, specifically to calculate respiratory deposition and to estimate exposure workers wearing respirators.

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DEVELOPMENT OF A COMPUTER PROGRAM FOR AUTOMATING PERMEATION TESTING DATA ANALYSIS. P. Gao, NIOSH, Pittsburgh, PA; D. Chung, B. Tomasovic, Crystalview Technology Corp., Irvine, CA.

Accurate determination of permeation parameters (including breakthrough time and permeation rate) is essential for selecting suitable chemical protective clothing. Since the data analysis involves a number of equations and experimental factors, experimenter bias and possible calculation errors are critical issues when determining permeation parameters. For instance, significant experimenter bias could result in selecting an inappropriate "steady-state permeation" region for calculating the steady-state permeation rate (SSPR). Accurate calculation of normalized breakthrough time, using a closed loop testing system, requires the use of polynomial curve fitting, polynomial derivatives, and quadratic equations. This calculation is labor intensive and somewhat difficult to do without the necessary computer program. Therefore, a Microsoft Windows compatible program, referred to as "Permeation Calculator," has been developed in an attempt to automate the data analysis. The program imports data files collected by a data acquisition system during a permeation test, which contain time versus concentration or voltage. More information is then entered under the "Additional Data Input" screen, such as the challenge chemical(s), type and thickness of the barrier material, diameter of the permeation cell, instrument settings, sampling flow rate, and dead volume if a MIRAN IR analyzer is used. The program then determines each of the permeation parameters, including breakthrough detection time, normalized breakthrough time, and SSPR for either an open loop or a closed loop test. At the end, the program displays all the permeation parameters together with relevant information as a report file which can be saved and printed. This presentation will provide an overview of the program and describe the strategies, approaches, and algorithms involved in the program development.

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COMPARISON OF REAL-TIME PCR AND DIRECT EXAMINATION ANALYSES USING THE VIACELL AIR SAMPLING CASSETTE. J. Dobranic, C. Cutler, EMSL Analytical Inc., Westmont, NJ.

A study was conducted comparing real-time PCR detection and quantification of mold spores and the traditional direct microscopic examination. ViaCell (Zefon International) spore trap cassettes were utilized in both laboratory and field samples. Initially, three replicate cassettes were sampled in the laboratory with individual pure cultures of *Aspergillus versicolor, Cladosporium cladosporioides*, and *Penicillium* chrysogenum followed by three cassettes with mixtures of these three fungi. The cassettes were first analyzed by direct microscopy then by PCR analysis to determine how comparable were the two techniques. Data will also be presented on a second study comparing three field samples taken in two mold contaminated homes that were again first analyzed by direct microscopy and then by real-time PCR.

The inherent limitations on identifications associated with the direct microscopic examination confines its use. PCR was able to provide much more valuable speciation identification especially of the *Aspergillus/Penicillium* group typically found on spore trap reports. The ViaCell can be effectively used for both direct examinations first, determining spore loads, and to pinpoint circumstances when PCR analysis is required on the same samples.

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SAMPLING MEDIA EFFECTS ON THE CALCULATION OF MOLD SPORE EQUIVALENTS IN QUANTITATIVE PCR. D. Kahane, D. Cox, H. DeLucchi, M. Piercey, Forensic Analytical, Hayward, CA.

Airborne mold samples for quantitative real-time PCR (QPCR) analysis are typically collected on polycarbonate filters. In QPCR analysis, the sample filter is suspended in a buffer solution and a known aliquot of a *Geotrichum candidum* reference spore suspension is added to the sample. The cycle threshold (CT) values for the target species and the *Geotrichum* reference are detected, and the difference in CT values (ΔCT) of the reference and target species is used to calculate the quantity of target species spore equivalents in the air sample. An underlying assumption in the use of the ÄCT method to quantify species is that any condition in the analytical sample that would cause a "shift" in the CT value of *Geotrichum* would also cause the same direction and magnitude of shift in the CT value of any other species. However, we observed that the mere presence of a polycarbonate filter in the buffer solution sample suspension caused a shift in the CT value of the *Geotrichum* but no shift in the CT value of some other mold species. The result of media effects that differ between species has been the systematic underreporting of the number of spore equivalents of some species in samples collected on polycarbonate filters. For example, the result of air samples collected on polycarbonate filters and analyzed for *Cladosporium cladosporioides* were found to have been underreported by a factor of four because of the differing media effects. This paper presents the results of our studies of the media effects of polycarbonate filters and other typical sample collection media on the mold species most commonly analyzed by QPCR. Recommendations are made regarding correcting the results of QPCR analyses by taking into account known media effects.

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WHEN YOU REALLY NEED TO KNOW: FUNGAL SPECIES IDENTIFICATION USING DNA SEQUENCING AND ANALYSIS. F. Wu, S. Huang, Aemtek Inc., Fremont, CA.

Sampling of fungi is often an integral part of indoor environmental quality assessment. Currently, spore trap, Andersen sampler, and dust check methods are widely used for fungal sampling. Subsequently, direct microscopy, culture morphology, and QPCR are used for fungal identification and analysis, respectively. Each of the methods has advantages and limitations and offers various degrees of resolution. In many cases, one or a combination of the methods mentioned above is sufficient. However, there are situations where none of the methods is adequate. The purpose of this presentation is to introduce DNA sequencing and analysis as an alternative for species identification. Comparative DNA sequence technology obtains and utilizes nucleic acid sequence information to achieve the most accurate and reproducible fungal identifications available. The procedure includes DNA extraction, PCR amplification, sequencing of the PCR products, and separation using automated DNA sequencer. Currently, the most widely used fungal sequences are ITS regions, 5.8S, and the subunits of ribosomal DNA. Mycological research in recent years has demonstrated that the ITS regions offer high level of interspecific specificity and low intraspecific variation in many groups of fungi. The sequence data is compared to databases to find the closest match. In addition to commercial databases, nucleic acid sequence data are regularly compiled in several publicly available databases. GenBank is the best known and most widely used. It currently has over 750,000 entries for fungal nucleic acids and represents the largest comparative data sets ever collected. The DNA sequencing technology works well with bulk samples of fungal colony and most of culturable samples. Viability or sporulation of the fungi is not required. Although this method is not for every investigation, it can be applied to situations where confirmative identification of unknown fungi is necessary to address health related issues.

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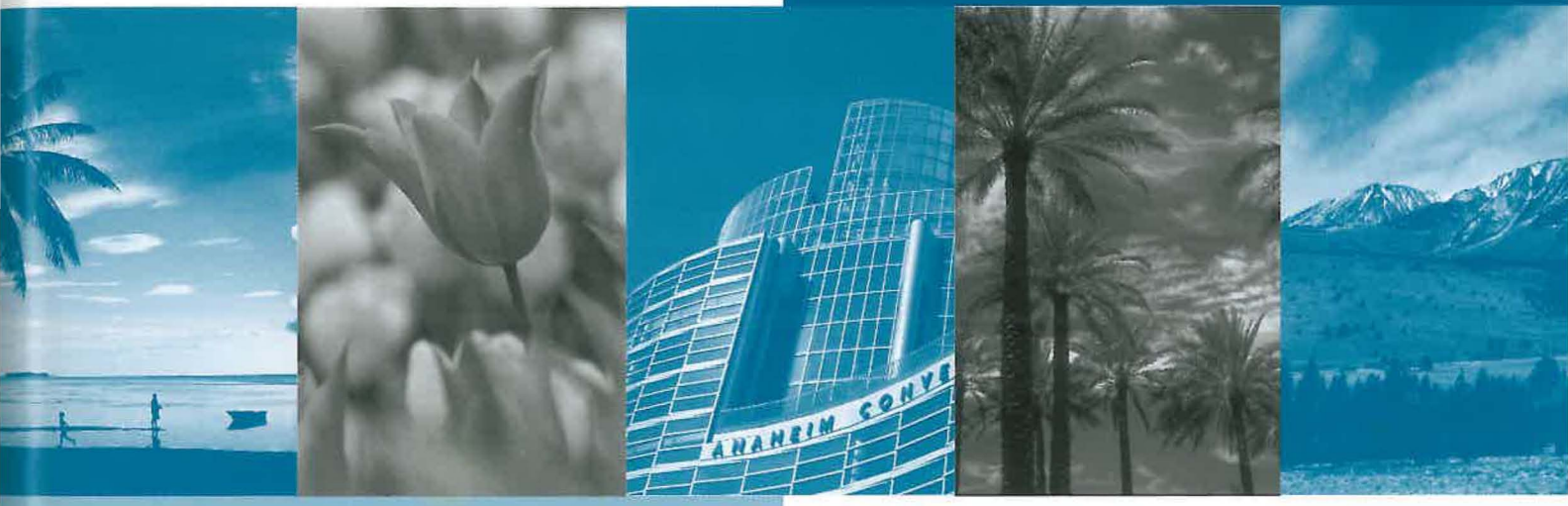

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