Validation of the Electrophilic Allergen Screening Assay (EASA) to Detect Substances that Impact the Initial Key Event in the Adverse Outcome Pathway for Skin Sensitization

J. Gordon[1], J. Truax[2], A. Borrel[2], E. Reinke[2], V. Adams[3], D. Rua[4], E. Petersen[5], R. Uhl[1], D.G. Allen[2], N. Kleinstreuer[6], J. Strickland[2]

[1]CPSC, Rockville, MD, United States; [2]Inotiv, RTP, NC, United States; [3]Defense Public Health-Aberdeen, Aberdeen Proving Ground, MD, United States; [4]FDA/CDRH, Silver Spring, MD, United States; [5]NIST, Gaithersburg, MD, United States; [6]NIH/NIEHS/DTT/NICEATM, RTP, NC, United States

The Electrophilic Allergen Screening Assay (EASA) was originally developed by the National Institute of Occupational Safety and Health as a cuvette-based assay to identify substances that have the potential to cause allergic contact dermatitis. Chemical binding to skin proteins is the initial key event of the adverse outcome pathway for skin sensitization. In the EASA, substances were tested for their ability to bind to nitrobenzenthiol (NBT) or pyridoxylamine (PDA) probes as surrogates for thiol- or amine-based proteins. Probe depletion was measured by absorbance or fluorescence using spectrometers. A test substance is positive when it meets the positive depletion criterion for either NBT or PDA, but negative results are recorded only when the depletion fails to meet the positive criterion for both tests. EASA was subsequently modified by the U.S. Consumer Product Safety Commission (CPSC) and the National Institute of Standards and Technology (NIST) into a higher-throughput assay using a 96-well format through a measurement science approach.

The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) assembled a validation management team to oversee the validation of EASA that has now been completed by three of four laboratories: U.S. Food and Drug Administration Center for Drug Evaluation and Research (FDA), Defense Public Health Center-Aberdeen (DoD), and CPSC/NIST (lead laboratory). A prevalidation transferability assessment using positive and negative control tests totaling 10 plates each for the NBT and PDA probes established acceptance criteria for the validation study. Acceptance criteria were based on performance of the positive and negative controls (solvent and probe) and the blanks (solvent only).

For the validation study, each laboratory evaluated 20 coded chemicals based on the Organisation for Economic Co-operation and Development (OECD) performance standards for the direct peptide reactivity assay and amino acid derivative reactivity assay test methods. Of these, 12 chemicals were tested three times to evaluate intralaboratory reproducibility. The performance of the EASA was determined by comparison with local lymph node assay outcomes. This preliminary assessment shows that accuracy, sensitivity, and specificity for EASA were 85%, 85%, and 86% for DoD, 79%, 83%, and 71% for CPSC/NIST and 80%, 85%, and 71% for FDA. Within-laboratory reproducibility was 94% for CPSC/NIST, 84% for DoD and 86% for FDA. These results suggest that the EASA may be a useful non-animal alternative to identify potential skin sensitizers.

This project was funded in whole or in part with federal funds from the NIEHS, NIH under contract No. HHSN27220150000010C. The views expressed above do not necessarily represent the official positions of any federal agency.

Keywords: chemical allergy, chemical hazard assessment, alternatives to animal testing