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Validation of the Electrophilic Allergen Screening Assay (EASA)

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Introduction

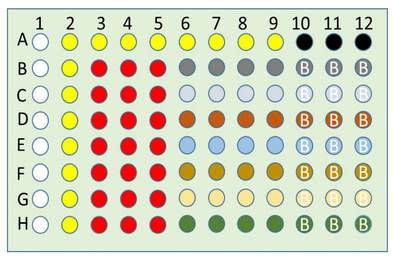
- The electrophilic allergen screening assay (EASA) was developed by the National Institute of Occupational Safety and Health as a cuvette-based assay to identify substances that have the potential to cause allergic contact dermatitis, or skin sensitization (Chipinda et al. 2011, 2014).
- The EASA evaluates a substance's ability to bind nitrobenzenthiol (NBT) or pyridoxylamine (PDA) probes used as surrogates for thiol- or amine-containing skin proteins (Table 1). Skin sensitizers bind with amino acids containing thiol or amine groups to form haptens. Formation of haptens is the initiating event in the skin sensitization adverse outcome pathway.
- The U.S. Consumer Product Safety Commission (CPSC) and the National Institute of Standards and Technology (NIST) converted the EASA into a higher-throughput assay using a 96-well format (Figure 1, Petersen et al. 2022).
- Probe depletion in the EASA is measured by absorbance (NBT) or fluorescence (PDA) spectroscopy. A test substance is considered a sensitizer when it meets the positive depletion criterion for either NBT or PDA and is negative when the depletion fails to meet the positive criterion for both tests (Figure 2).
- Four laboratories participated in a validation study of the EASA:
  - U.S. Food and Drug Administration Center for Devices and Radiological Health
  - Defense Centers for Public Health - Aberdeen
  - Burleson Research Technologies, Inc.
  - CPSC/NIST (lead laboratory)
- The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) assembled a validation management team to oversee the study.
- The validation study tested 20 reference chemicals (Table 2) from the Direct Peptide and Amino Acid Derivative Reactivity Assay (DPRA/ADRA) Performance Standards (OECD 2019), 12 of which were tested three times for the assessment of within-laboratory reproducibility. The performance of the EASA was determined by comparison with local lymph node assay (LLNA) outcomes noted in the performance standards document (OECD 2019).

Table 1. Characteristics of EASA Component Assays

	NBT Absorbance Assay	PDA Fluorescence Assay
Wavelength (nm)	412	324 excitation 398 emission
Measurement times	5, 20, 35, **50 min***	5, 20, 35, **50 min***
Positive control	Benzyl bromide	Glutaraldehyde
Negative control	Solvent without probe	Solvent without probe
Negative response criterion	No statistically significant depletion of probe based on protocol parameters	
Positive response criterion	Statistically significant depletion of probe based on protocol parameters	

*The 50 min time point is used to determine a final positive or negative response.

Figure 1. EASA Plate Layout Map for NBT and PDA Assays

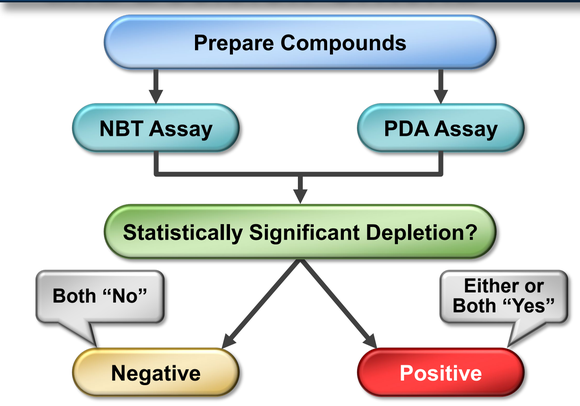


TCs are added starting in columns 6 – 12 in rows B – H, horizontally.
- NC/PC Blank wells (40 µl ACN + 160 µl SS without Probe)
- PC (40 µl PC in ACN + 160 µl SS with Probe)
- NC (ACN) (40 µl ACN + 160 µl SS with Probe)
- TC (40 µl TC in ACN + 160 µl SS with Probe)
- TC Blanks (40 µl TC in ACN + 160 µl SS without Probe)
- Not used– no additions

Petersen et al. 2022

NC = negative control, PC = positive control, ACN = acetonitrile,
TC = test chemical, SS = solvent system

Figure 2. EASA Workflow and Decision Criteria



Conclusions

- According to performance criteria established by the Organisation for Economic Co-operation and Development (OECD; OECD 2019), in vitro skin sensitization assays should concur with LLNA results with sensitivity, specificity, and accuracy of at least 80%. Within- and between-lab reproducibility should be at least 80%.
- Although the EASA did not meet the acceptance criteria established by the OECD for similar assays in every participating laboratory (Table 3), the EASA may be useful for identifying potential skin sensitizers.
- The validation report is in preparation. The report will undergo peer review upon acceptance by the validation management team. Results will also be reported in the peer-reviewed literature.
- This method may be proposed to OECD as an addition to OECD Test Guideline 442C.

Table 3. EASA Performance by Laboratory

Lab #	Balanced Accuracy	Sensitivity	Specificity	Within Lab Reproducibility	Between Lab Reproducibility
1	76%	85%	67%	94%	96%
2	82%	92%	71%	100%	
3	84%	85%	83%	97%	
4	84%	85%	83%	94%	
Mean	**82%**	**87%**	**76%**	**96%**	

Balanced accuracy: the average of sensitivity and specificity.
Sensitivity: proportion of all positive chemicals correctly classified.
Specificity: proportion of all negative chemicals correctly classified.

Table 2. EASA Results by Laboratory with Comparison to Reference Data

Test Chemical	EASA Outcomes[1]				LLNA Outcomes	DPRA/ADRA Outcomes[3]
	Lab 1	Lab 2	Lab 3	Lab 4		
Lauryl gallate	Pos	Pos	Pos	Pos	Pos	Pos/Pos
Chloramine T trihydrate	Pos	Pos	Pos	Pos	Pos	Pos/Pos
Metol (4-methyl amino phenol)	Pos	Pos	Pos	Pos	Pos	Pos/Pos
2-Mercaptobenzothiazole	Pos	Pos	Pos	Pos	Pos	Pos/Pos
Benzyl salicylate	Pos	Pos	Pos	Pos	Pos	Pos-Neg/Pos
Cinnamaldehyde	Pos	Pos	Pos	Pos	Pos	Pos/Pos
Imidazolidinyl urea	Pos	Pos	Pos	Pos	Pos	Pos/Pos
Ethyl acrylate	Neg	Neg	Neg	Neg	Pos	Pos/Pos
Salicylic acid	Inc[2]	Neg	Inc[2]	Inc[2]	Neg	Pos-Neg/Neg
Benzyl alcohol	Pos	Pos	Neg	Neg	Neg	Pos-Neg/Neg
Glycerol	Neg	Neg	Neg	Neg	Neg	Neg/Neg
Isopropanol	Neg	Neg	Neg	Neg	Neg	Neg/Neg
Benzoquinone	Pos	Pos	Pos	Pos	Pos	Pos/Pos
Dihydroeugenol	Neg	Pos	Neg	Neg	Pos	Pos-Neg/Pos-Neg
Palmitoyl chloride	Pos	Pos	Pos	Pos	Pos	Pos/Pos
Farnesol	Pos	Pos	Pos	Pos	Pos	Pos/Pos
Dimethyl isophthalate	Neg	Neg	Neg	Neg	Neg	Neg/Neg
Methyl salicylate	Pos	Pos	Pos	Pos	Neg	Pos-Neg/Neg
4-Aminobenzoic acid	Neg	Neg	Neg	Neg	Neg	Neg/Neg
Benzyl cinnamate	Pos	Pos	Pos	Pos	Pos	Neg/Neg

[1] EASA NBT and PDA outcomes were used to determine the EASA call as described in Figure 2. RED indicates a positive call, BLUE indicates a negative call, and BLACK indicates an inconclusive call.
[2] Inc = Inconclusive; the substance tested negative at concentrations lower than that specified in the protocol.
[3] OECD 2019. Pos-Neg indicates that some laboratories produced a positive result while others produced a negative result.

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A summary of NICEATM and ICCVAM activities at the 12th World Congress is available online at https://ntp.niehs.nih.gov/go/niceatm-wc12.

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