# Laboratory Techniques for Identifying Causes of Allergic Dermatitis

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**KEYWORDS**

• Allergic contact dermatitis • Hazard identification • Patch test • Chemical analysis

**KEY POINTS**

- Validated rapid and sensitive laboratory-based analyses are available to screen chemicals for sensitization potential.
- Laboratory-based chemical analyses can be used to confirm the presence of a patient's putative etiologic allergic contact dermatitis agent, identify unknown allergens, evaluate patch test quality, and test the quality of commercial chemical spot tests.
- Chemical analyses can be complex, time consuming, and costly, which may prohibit its use for routine patient care.

## HAZARD IDENTIFICATION

### *Animal-Based Tests*

Because of the environmental, occupational, and clinical significance of chemical sensitizers that induce allergic contact dermatitis (ACD), the use of rapid and sensitive methods for hazard identification is necessary. A human-based assay known as the human repeat insult patch test (HRIPT) has been used to confirm skin allergy; however, because of ethical concerns and alternate methods, the use of this assay has been eliminated in many countries.[1] Two guinea pig-based assays, the guinea pig maximization test (GPMT) and the Buehler assay, have been used to predict chemical sensitizers. These assays are recommended for use by the Organization for Economic Cooperation and Development (OECD) as test guideline (TG) 406.[2] The GPMT uses intradermal administration of a test chemical combined with or without Freund complete adjuvant followed by topical administration of the test chemical. Two weeks after topical dosing, the animals are challenged by patch test of the flank and the allergic reaction, on the skin is assessed to measure sensitization potential.[3] The Buehler

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assay also uses a guinea pig model, where animals are dermally exposed to the test agent for 6 hours for 3 consecutive weeks. Two weeks after the final patch exposure, the animals are challenged by patch test of the flank for 6 hours[3] to measure the elicitation phase of allergy. Limitations of these assays include limited dose selection range (based on skin irritation threshold) and the use of single induction and challenge concentrations determined from range finding studies, which does not allow for dose response or evaluation of potency, but does provide valuable information for allergen identification.[4] In spite of these limitations, guinea pig-based sensitization assessment assays have significant utility, including a substantial test database, allowing for comparisons to new test agents and information regarding the elicitation phase of allergy.[4]

Currently, the gold standard for hazard identification of dermal sensitizers is the local lymph node assay (LLNA). This assay has been validated among independent laboratories,[5,6] with the United States-based Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)[7] and the European-based European Center for the Validation of Alternative Methods (ECVAM)[1] leading the validation exercises. The LLNA was adopted as TG 429 by the OECD in 2002.[8] The LLNA is based on the concept that repeated dermal exposure to sensitizers causes lymphocyte proliferation in draining lymph nodes (dLNs) proximal to the site of chemical application and that this proliferation can be quantified by using tritiated thymidine ($^3$H), which is incorporated into dLN DNA. **Fig. 1** illustrates the basic protocol of the LLNA. The assay uses female mice (preferably the CBA/Ca or CBA/J strain) at a minimum of 4 mice per group. A test substance is generally considered a sensitizer if it exhibits a dose-responsive increase in dLN proliferation from which the estimated concentration of test substance required to induce a stimulation index of at least 3 (EC3) can be determined. This value indicates a threefold or greater increase in dLN cell proliferation compared with vehicle-control mice. In general, predictive tests

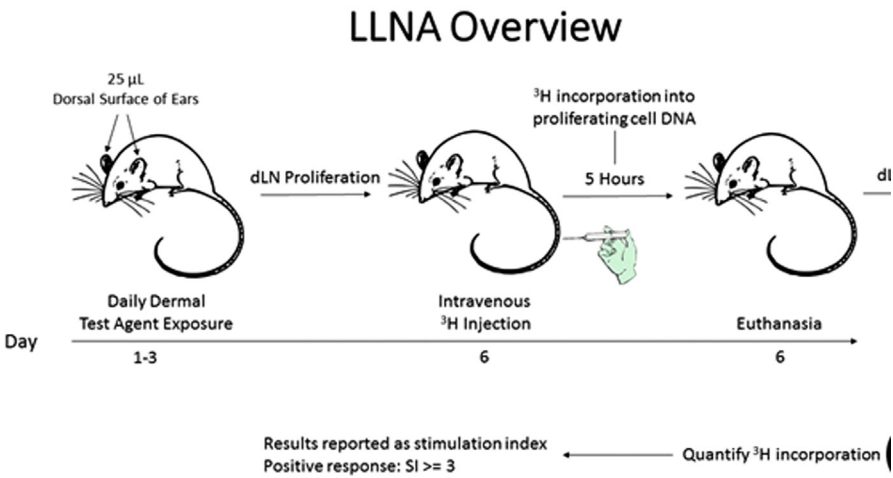


**Fig. 1.** Pictorial overview of the LLNA. Mice are dermally exposed on the dorsal surface of each ear to the vehicle, increasing concentrations of the test agent or a positive control for 3 consecutive days. After 3 days, mice are intravenously injected with $^3$H-thymidine ($^3$H) and euthanized 5 hours later; draining lymph nodes (dLNs) are excised and processed into single cell suspensions. These suspensions are tested for $^3$H incorporation based on disintegration per minute (DPM) readings. Results are expressed as the stimulation index (SI), which is the ratio of dLN allergen treated/control $^3$H incorporation.

exhibit certain limitations and results must be interpreted accordingly. The LLNA is not effective in identifying nickel salts (presumably because of variation in TLR4 signaling between mice and people); it exhibits false positives (specifically regarding nonsensitizing irritants)[9,10] and cannot distinguish between dermal and respiratory sensitizers. Regardless, the LLNA exhibits many advantages over other animal-based sensitization assays, including quick turnover and cost-effectiveness, less animal trauma, an end point directly associated with sensitization, dose response analysis yielding an index of potency (EC3 value), and close correlation between EC3 and human skin sensitization data.[4] There are also 2 nonradioactive modifications to the LLNA, the LLNA: DA (OECD TG 442A)[11] and LLNA: BrdU-ELISA (OECD TG 442B),[12] which assess the lymphocyte proliferation using nonradioactive methods.

### *In Chemico, In Vitro, and In Silico Tests*

Recently, experimental focus has been on the development of nonanimal alternatives to the LLNA and guinea pig methods. The major challenge in the development of in silico, in chemico, and in vitro alternatives for hazard identification of skin sensitizers is the ability to recapitulate the complex in vivo environment of an organism undergoing sensitization. The key chemical and biological events underlying skin sensitization and ACD, which have been extensively studied and are now generally understood,[9,13–16] were harnessed to establish an adverse outcome pathway (AOP) for skin sensitization[17,18] (**Fig. 2**). The mechanistic knowledge of key events (KEs) within the AOP framework enabled the development, validation, and acceptance of in chemico and in vitro methods for hazard identification of skin sensitizers. The clinical manifestation of ACD requires several steps to occur before the involvement of cell types including keratinocytes, Langerhans cells, dendritic cells, and T-lymphocytes.[19] The OECD adopted and developed the AOP framework,[17,18] which is defined as a chain of sequential causally related KEs, starting from a molecular initiating event (MIE) and ending in an adverse outcome (AO).[20] The in chemico and in vitro assays are based on one of the 4 AOP KEs, as illustrated in **Fig. 2**. It is worth noting that physicochemical properties of contact allergens (eg, chemical structure, molecular weight, physical state, pKa, Log Kow, vapor pressure, and water solubility) included in the AOP are important. Skin surface oxidation (for prehaptens), dermal penetration, skin metabolism (for prohaptens), and protein reactivity depend on these properties.

**Table 1** lists the currently available, validated in chemico and in vitro test methods.[21–29] Assays with designated OECD TG went through extensive evaluation and validation exercises conducted by national and international agencies such as ICCVAM, ECVAM, and Japanese Center for the Validation of Alternative Methods (JACVAM) to gauge their performance before OECD adoption. These OECD TGs are not considered stand-alone replacements for the animal tests given the multiple steps and complexity described in the AOP for skin sensitization. The assays need to be combined to be able to encompass the whole AOP framework. As such, an

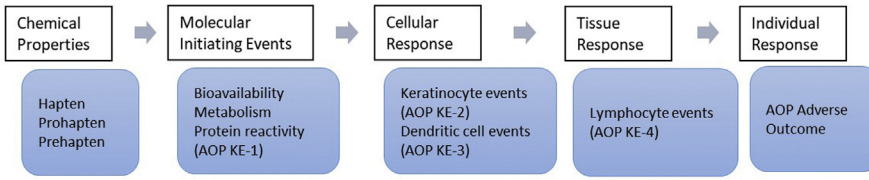


**Fig. 2.** Skin sensitization AOP and validated KEs within the AOP. The AOP was central to the development of in chemico and in vitro tests for low molecular weight (<1 kDa) chemicals.

**Table 1**
**In chemico and in vitro test methods for skin sensitization**

| Adverse Outcome Pathway Phase | In Chemico/Vitro Test Methods | Description |
|---|---|---|
| Dermal bioavailability | *OECD TG 428*[21] (skin absorption) | Skin permeation model representing the stratum corneum and viable skin (epidermis and dermis) where a test substance is applied to the surface of a skin sample separating the 2 chambers of a diffusion cell; the receptor fluid is sampled at intervals and analyzed for the test chemical and/or metabolites to measure the skin permeation |
| AOP KE-1: protein haptenation | *OECD TG 442C*[22] (DPRA) | In chemico test: quantification of the reactivity of test chemicals toward model synthetic peptides containing either lysine or cysteine; cysteine and lysine percent peptide depletion values are used in a prediction model to categorize a substance in 1 of 4 classes of peptide reactivity for supporting the discrimination between skin sensitizers and nonsensitizers |
| AOP KE-2: keratinocyte responses | Activation of biochemical pathways<br>*OECD TG 442D*[23] (ARE-Nrf2 luciferase test method)<br>1. KeratinoSens[23]<br>2. LuSens[23–25] | Perturbation of a cell line containing the luciferase gene under the transcriptional control of a constitutive promoter fused with an antioxidant/electrophile response element (ARE) element from a gene that is known to be up-regulated by contact sensitizers allows quantitative measurement (by luminescence detection) of luciferase gene induction as an indicator of the activity of the Nrf2 transcription factor in cells following exposure to electrophilic test substances; currently, 2 in vitro test methods are covered by this TG: the KeratinoSens assay and the LuSens assay |

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**Table 1**
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| Adverse Outcome Pathway Phase | In Chemico/Vitro Test Methods | Description |
|---|---|---|
| AOP KE-3: dendritic cell responses | Expression of costimulatory and adhesion molecules in dendritic/monocytic cells | |
| | OECD TG 442E[26]<br>1. Human cell line activation test (h-CLAT)[26]<br>2. U937 cell line activation test (U-SENS)[26,27]<br>3. Interleukin-8 (IL-8) reporter gene assay[26,28] | TG covers all 3 tests that are used to support the discrimination between skin sensitizers and nonsensitizers; change in the expression of cell surface marker(s) associated with the process of activation of monocytes and DC following exposure to sensitizers (eg, CD54, CD86) or changes in IL-8 expression, a cytokine associated with the activation of DC, is quantified in these assays; relative fluorescence or luminescence intensity of the treated cells versus controls is calculated and used to support the discrimination between sensitizers and nonsensitizers |
| AOP KE-4: tissue response | 1. The lymphocyte *ex-vivo* transformation test (LTT)[29] | The basis is that exposure in culture of primed memory T-lymphocytes to the relevant antigen will trigger a secondary response that reflects the acquisition of skin sensitization |
| AOP adverse outcome | — | — |

integrated approach to testing and assessment (IATA) is recommended if a decision on whether a test chemical is a sensitizer is to be made based on in vitro testing alone.[30] Combining tests such as the Direct Peptide Reactivity Assay (DPRA, KE-1), KeratinoSens assay (KE-2) and the Human Cell Line Activation Test (h- CLAT, KE-3) leads to test results that are acceptable. IATAs using combinations of these assays have been evaluated for regulatory application, some of which outperform animal tests to predict human skin sensitization.[30–35] It is important to point out that performing these assays requires the use of competent laboratories and scientists. In silico methods focused on predicting chemical reactivity based on the known in vivo reactivity of chemicals bearing structural significance are known as quantitative structural activity relationship (QSARs). There are also numerous in silico expert systems available such as the TImes MEtabolism Simulator platform (TIMES-SS) and Meteor Nexus that are simulators for skin metabolism, autoxidation, and reactivity. Some of these are available within the OECD QSAR Toolbox.[36]

### *Current Regulatory Agency-Accepted Test Methods*

Regulatory agencies in the United States and other developed countries have been increasing their general acceptance of the use of in vitro methods to obtain skin sensitization data to support decision making such as chemical registrations.[37] These agencies include the US Environmental Protection Agency (EPA), US Food and Drug Administration (FDA), Consumer Product Safety Commission (CPSC),

Occupational Safety and Health Administration (OSHA), European Chemicals Agency (ECHA), and the European Commission (EU) Cosmetics Regulation (EC) No. 1223/2009. Although all these agencies, except the European Commission, still require animal testing data for product safety evaluations and approvals, the agencies acknowledge the widely accepted AOP for skin sensitization and as such accept submission of in vitro testing data to support weight of evidence (WoE) evaluations.[37,38] In 2013, the Cosmetics Regulation (EC No. 1223/2009) banned animal use in cosmetics testing,[38,39] hence all testing for skin sensitization is now being performed using nonanimal methods such as the OECD TGs described.

## QUALITY CONTROL FOR PATCH TEST ALLERGENS, ALLERGEN STABILITY DATA

At present, the only FDA-approved percutaneous patch test system available is the T.R.U.E TEST. This test consists of 3 different allergen panels comprising a total of 35 allergen and allergen mixes along with a vehicle control contained in the ready-to-use panels. Most patch test allergens and allergen mixes that are not FDA approved are in a petrolatum or water vehicle and supplied in a syringe or dropper bottle. For these preparations, multiple allergens are added to patch test chambers to create patch test panels for diagnostic ACD testing.

Independent of the type of patch test, stability of allergen preparations is critical to prevent false-negative results and irritant reactions caused by product breakdown. Multiple studies pointing to storage instability of specific patch test allergen preparations were previously reviewed by Joy and colleagues[40] and Jou and colleagues[41] Loss of some allergens upon storage or preloading of patch test chambers has been demonstrated qualitatively/semiquantitatively, and/or quantitatively. The qualitative/semiquantitative studies were those that evaluated change of allergic subject patch test reactivity between fresh and stored preparations, while the quantitative test of contact allergen patch test reagents requires complex analytical laboratory analyses. **Table 2** provides a list of allergen stability laboratory studies reviewed by Jou and colleagues[41] along with the patch test allergen matrix and specific findings. Often it can be a challenge to extract the allergen from petrolatum into a solvent suitable for analysis, and extraction protocols can vary considerably depending on the chemical nature of the allergen(s) and the patch test vehicle. It is critical for the analytical laboratory to establish a standard with a known amount of allergen distributed uniformly in petrolatum to develop an extraction method that optimizes allergen recovery. Following development and validation of extraction protocols, quantitation of the allergens within a patch test preparation may be as simple as using a spectrophotometric chemical assay or as complex as requiring the use of chromatographic-mass spectrometric instrumentation.

Issues with allergen stability may be caused by physical and chemical properties of the chemical contact allergen. It has been documented that an allergen's high vapor pressure (volatility) can contribute to loss of the allergen from a patch test reagent, and particularly once the reagent is dispensed into a patch chamber.[42–44] Jou and colleagues[41] recommended that volatile allergens, such as formaldehyde, methyl methacrylate, and fragrances, be stored at lower temperatures in air-tight multidose containers, or sealed single-application containers, and aliquoted and prepared immediately before application. These recommendations would also decrease loss of allergens that are subject to air oxidation. Other possible causes of allergen loss with storage may be from self-polymerization, absorption or reaction with storage container surfaces, and reaction with other chemicals in the preparation. Additionally, loss of allergen may occur during compounding because of volatility[45] or possibly

**Table 2**
**Patch test allergen stability**

| Allergen/Vehicle | Instrumentation/Method | Finding | Reference |
|---|---|---|---|
| Thiuram mix/PET: tetramethylthiuram monosulfide (TMTM), tetramethylthiuram disulfide (TMTD), tetraethylthiuram disulfide (TETD) and dipentamethylenethiuram disulfide (PTD) | HPLC-UV/VIS | Thiuram mixes were unstable with individual thiurams reacting with each other to form mixed disulfides | Bergendorff & Hansson,[66] 2001 |
| Balsam of Peru/PET<br>Cobalt chloride/PET<br>Colophony/PET<br>Ethylenediamine/PET<br>Mercaptobenzothiazole/PET<br>Nickel sulfate/PET<br>Potassium dichromate/PET<br>Vioform/PET<br>Disperse yellow 3/PET<br>Formaldehyde/water | TLC or colorimetry | All found to be stable for 6 y in original sealed packaging, at room temperature, except formaldehyde, which had lower than acceptable levels in new and stored samples | Lembo et al,[67] 1993 |
| Methyldibromo glutaronitrile/PET | HPLC-UV | Stable for 1 y of storage at 6–8ºC | Gruvberger et al,[68] 2004 |
| Cinnamal<br>Eugenol<br>Methyl methacrylate (MMA)<br>2-hydroxyethyl methacrylate (2-HEMA)<br>2-hydroxypropylacrylate | HPLC-diode array detector and GC-MS | All except 2-HEMA unstable if stored in patch test chambers at both 4ºC and 22ºC; all were more stable stored in a Vander Bend transport container | Mose et al,[44] 2012 |
| Tixocortol, pivalate, budesonide and hydrocortisone-17-butyrate (Hc-17-B)/pet, or ethanolic solution, or ethanol | HPLC-UV | All stable over a 50-week period except 1% Hc-17-B in ethanol | Isaksson et al,[69] 2000 |
| MMA<br>2-hydroxyethyl methacrylate<br>2-hydroxypropylacrylate (2-HPA)<br>ethylene glycoldimethacrylate<br>triethyleneglycoldiacrylate | HPLC-MS | 2-HPA and MMA predominately had unacceptable levels (<20% of the labeled concentration within the expiration date) | Goon et al,[46] 2011 |

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**Table 2**
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| Allergen/Vehicle | Instrumentation/Method | Finding | Reference |
|---|---|---|---|
| Nickel sulfate/PET<br>Methyl methacrylate/PET<br>Formaldehyde/water<br>Glutaraldehyde/PET | Spectrophotometric measurement<br>GC-MS<br>Spectrophotometric measurement<br>Spectrophotometric measurement | Nickel and formaldehyde were at or above labeled patch test concentrations, but formaldehyde loss occurred with storage; lower than labeled concentrations of methyl methacrylate and glutaraldehyde concentrations were found in commercial patch test preparations independent of expiration date | Siegel et al,[45] 2014 |
| D-Limonene oxidation products/PET | HPLC-UV and GC-MS | Stable for 6 weeks when stored at 6–8ºC Unstable if PET is stabilized with $\alpha$-tocopheryl acetate | Nilsson et al,[70] 1999 |
| Fragrance mix 1/PET components – amyl cinnamal, cinnamal, cinnamyl alcohol, eugenol, geraniol, hydroxycitronella isoeugenol; *E prunastri* extract/PET-sorbitan sesquiol | HPLC-diode array detector or gel permeation chromatography-diode array detector[1] | At 23ºC rapid time-dependent losses were observed for all fragrances in the mix or individually stored in Finn or IQ chambers Storage at 5ºC slowed down the losses | Mowitz et al,[42] 2012 |
| Lyral/Pet | Gel permeation chromatography-UV detector | Losses observed when stored in Finn or IQ chambers above 5ºC at 8 h; minimal loss after 9 d at 5ºC | Hamann et al,[43] 2013 |
| Triglycidyl isocyanurate (TI) powder<br>TI/PET | HPLC-UV detector | Degradation of TI observed in both powder and TI/PET in samples stored, refrigerated for 8 y | Erikstam et al,[71] 2015 |
| 2,4-Toluene diisocyanate (TDI)/PET<br>1,6-hexamethylene diisocyanate (HDI)/PET<br>Isophorone diisocyanate (IPDI)/PET | HPLC-MS | TDI, HDI and IPDI were all found to be stable and at the stated concentration in commercial preparations | Frick-Engfeldt et al,[72] 2005 |

| | | | |
|---|---|---|---|
| diphenylmethane-4,4′-diisocyanate(MDI)/PET | HPLC-MS | MDI concentrations were lower than the labeled amount in commercial patch test preparations | Frick et al,[73] 2005 |
| MDI, and polymeric (pMDI))/PET | HPLC-MS | Time and temperature dependent instability of both MDI. pMDI loss also observed except when stored in he freezer | Frick-Engfeldt et al,[74] 2007 |

because of compounding errors.[46] Because petrolatum is a solid at room temperature with a melting point of 70 to 80°C, the compounding process may require melting petrolatum or extensive mixing to obtain a uniform distribution of the allergen.

## CHEMISTRY LABORATORY DERMATOLOGIST SUPPORT

The potential role of laboratory chemistry in clinical ACD diagnosis includes confirmation of etiologic allergens in patients' products, unknown allergen identification, and development and evaluation of allergen spot tests. These activities differ from the traditional clinical laboratory testing, and impediments exist in implementation of chemical laboratory analyses for individual patient diagnosis and counseling. De Groot[47] surveyed the journals, *Contact Dermatitis* and *Dermatitis,* between 2008 and 2015, and found 172 new contact allergens that were identified by patch testing. This is likely an underestimate of new contact allergen exposures, as not all cases are reported in the literature, patch testing of potential causative materials is often not performed, and the specific etiologic chemical agent goes unidentified. At present, identification and possible quantification of allergens from products associated with ACD have been mostly limited to research studies and case reports of new, novel allergens because of the complexity and cost of such chemical analyses.

Dermatologists identify contact allergens to which their patients react through clinical history and the use of dermal patch testing. They can verify if a product (eg, personal care products or clothing) used by the patient is associated with the patient's allergy through product patch testing. However, it can be more problematic confirming the presence of the patch test-positive allergen in the suspect material. Siegel and colleagues[48] found that the patient's ability to identify the glove source of their ACD was directly related to the severity of their patch test reaction to the rubber allergen, and inversely related to the number of different rubber glove types in use by the patient. It was concluded that in the absence of chemical analysis of a patient's possible ACD-causative gloves, all of the patient's rubber gloves need to be considered as potential sources of the contact allergen.

Product content labels or even safety data sheets (SDSs) are not always reliable for allergen identification. Multiple studies have demonstrated the presence of undeclared allergens or absence of declared allergens by product chemical analyses for the suspect allergens. For example, undeclared isothiazolinones have been documented in several reports of products such as gel face mask,[49] emulsifying oil,[50] wall paints,[51,52] and dish soap[53]; undeclared dehydroabietic acid in neoprene surgical gloves[54]; and undeclared formaldehyde/formaldehyde releaser in personal products,[55–57] baby wipes,[58] and tattoo ink.[59] The presence of these undeclared contact allergens may be due to several reasons including presence in the raw materials used in the product production, mislabeling, or contamination from machinery treated with biocides.[53]

Reliable labeling and SDSs would be the most expedient and cost-effective tool to confirm allergen clinical relevance of a patient's patch test result. However, a manufacturer may consider an ingredient as proprietary and be reluctant to provide more specific product content or confirm the presence of a specific chemical. In such cases, chemical analysis to assess if the material contains a suspect allergen(s) would provide strong evidence of clinical relevance to the allergen patch test results; however, obstacles to widespread contact allergen product testing exist. Methodological considerations for confirmation of etiologic allergen content of patient products and identification of unknown allergens was recently reviewed by Siegel and colleagues,[60] as well as by Gruberger and colleagues[61] Although confirmation of the presence of a

specific contact allergen(s) in a suspect material does not need to involve a quantitative analysis, the extraction and measurement procedures should be sufficiently robust to avoid false-negative or false-positive analytical results. The product matrices can also vary substantially from product to product, which may require substantial method modifications to extract the contact allergen from the product into a solvent that is compatible with the analytical chemical detection method. Chemical detection methods can range from a simple spot test to highly sophisticated chromatographic mass spectrometric analyses. The more sophisticated instrumentation may add a greater level of confidence in the allergen identification confirmation, but this can also significantly increase the cost of the analysis. If quantitative analysis of the amount of the allergen in the product is desired, additional steps would be required to evaluate the extraction recovery and the precision and accuracy of the measurement method.

9Spot tests are simple qualitative or semiquantitative tests that use small amounts of sample, reagents, and test steps to yield fast results that usually consist of a color change. There are commercially available spot test kits for only a few chemical allergens, and most have not been validated for the various matrices/products associated with ACD reactions. The authors have recently reported on the utility of various kits for detection of formaldehyde[56] and isothiazolinones[53] marketed for water analyses. The chemical detection-based formaldehyde test strip kits were found to have utility in detecting formaldehyde from the consumer products tested, while the enzyme-based kit was unreliable. The accuracy of the isothiazolinone test kit was very poor for detection of isothiazolinones in dish soaps and personal care products. Test kits are also available for several metals including nickel, cobalt, and hexavalent chromium.

Identification of an unknown contact allergen in the absence of an a priori postulated chemical structure can be complex and costly. Bruze and colleagues[62] developed a thin layer chromatographic patch test that can aid in separation of a product's chemical components and segregation of the contact allergens. If the contact allergen is compatible with gas chromatographic-electron impact-mass spectral analyses (GC-EI-MS) and the resultant electron impact ion spectra matches that from an MS library, a tentative chemical identification can be made. Chemical identity should be confirmed against a chemical standard when one is available. Many chemical contact allergens cannot be assayed directly using GC-EI-MS or have a spectrum that is not in an MS library. For these instances, further analyses using ultrahigh performance liquid chromatography with tandem mass spectrometry (UHPLC-MS-MS) can be used to obtain the chemical's mass, isotopic distribution, and daughter ion fragments to determine a potential molecular formula. Two-dimensional proton nuclear magnetic resonance could be conducted; however, this technique requires milligram quantities of a pure substance to tentatively identify the chemical structure. Confirmation of the tentatively identified chemical would be performed by demonstration of identical chromatographic retention times and mass spectral ion chromatographs to that of a pure chemical standard. Such analyses may be prohibitively expensive, labor intensive and require high levels of expertise for routine implementation in patient diagnosis and counseling.

Identification of unknown allergens has been reported for synthetic and natural extracts used in the fragrance industry. Chaintreau and colleagues[63] reported a GC-MS method for quantification of 24 fragrance contact allergens; however, there remains unidentified fragrance contact allergens especially in natural extract fragrances. Oak moss extract is one such natural extract that is used in perfumes. It is a complex chemical mixture of greater than 170 compounds[64] and a cause of ACD. Although most fragrances are amenable to GC-MS analyses, the number of chemical components in the extract can present a challenge in the identification of the allergenic

components. This is common with all chemical substances of unknown or variable composition, complex reaction products and biological materials (UVCBs). Oak moss extract is regarded as a UVCB substance. Bernard and colleagues[65] fractionated and subfractionated oak moss extracted using gel permeation and silica gel column chromatography, respectively, with all fractions testing positive in oak moss-allergic subjects. GC-MS analyses identified multiple potential allergens, but only a standard of chloroatranol/atranol elicited positive patch test reactions. This study demonstrates the complexity of identification of unknown specific chemical allergens from complex mixtures.

## CLINICS CARE POINTS

- Laboratory-based analyses are available to identify the skin sensitization hazard of potential chemical allergens.
- Commercially available patch test reagent storage instability may be a potential cause of a false-negative finding. Storage of volatile and labile reagents at lower temperatures can decrease allergen loss from patch test reagents.
- Few reliable chemical spot tests are available to clinicians to confirm that a patch test-positive chemical is in the product eliciting the allergic contact dermatitis. Laboratory-based chemical allergen identification can be complex and is generally unavailable for patient care.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest. The authors alone are responsible for the content of this article. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the National Institute for Occupational Safety and Health or the Centers for Disease Control and Prevention.

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