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## Immunological methods for diagnosis and monitoring of IgE-mediated allergy caused by industrial sensitizing agents (IMExAllergy)

Baur, Xaver ; Akdis, Cezmi A ; Budnik, Lygia Therese ; Cruz, Maria Jesus ; Fischer, Axel ; Förster-Ruhrmann, Ulrike ; Göen, Thomas ; Goksel, Ozlem ; Heutelbeck, Astrid R ; Jones, Meinir ; Lux, Harald ; Maestrelli, Piero ; Munoz, Xavier ; Nemery, Benoit ; Schlünssen, Vivi ; Sigsgaard, Torben ; Traidl-Hoffmann, Claudia ; Siegel, Paul

**Abstract:** Industrial sensitizing agents (allergens) in living and working environments play an important role in eliciting type 1 allergic disorders including asthma and allergic rhinitis. Successful management of allergic diseases necessitates identifying their specific causes (ie, identify the causative agent(s) and the route of contact to allergen: airborne, or skin contact) to avoid further exposure. Identification of sensitization by a sensitive and validated measurement of specific IgE is an important step in the diagnosis. However, only a limited number of environmental and occupational allergens are available on the market for use in sIgE testing. Accordingly, specific in-house testing by individual diagnostic and laboratory centers is often required. Currently, different immunological tests are in use at various diagnostic centers that often produce considerably divergent results, mostly due to lack of standardized allergen preparation and standardized procedures as well as inadequate quality control. Our review and meta-analysis exhibited satisfactory performance of sIgE detection test for most high molecular weight (HMW) allergens with a pooled sensitivity of 0.74 and specificity of 0.71. However, for low molecular weight (LMW) allergens, pooled sensitivity is generally lower (0.28) and specificity higher (0.89) than for HMW tests. Major recommendations based on the presented data include diagnostic use of sIgE to HMW allergens. A negative sIgE result for LMW agents does not exclude sensitization. In addition, the requirements for full transparency of the content of allergen preparations with details on standardization and quality control are underlined. Development of standard operating procedures for in-house sIgE assays, and clinical validation, centralized quality control and audits are emphasized. There is also a need for specialized laboratories to provide a custom service for the development of tests for the measurement of putative novel occupational allergens that are not commercially available.

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## REVIEW ARTICLE

# Immunological methods for diagnosis and monitoring of IgE-mediated allergy caused by industrial sensitizing agents (IMExAllergy)

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## Abstract

Industrial sensitizing agents (allergens) in living and working environments play an important role in eliciting type 1 allergic disorders including asthma and allergic rhinitis. Successful management of allergic diseases necessitates identifying their specific

**Abbreviations:** HMW, high molecular weight; LMW, low molecular weight; OA, occupational asthma; OR, occupational rhinitis; RADS, reactive airways dysfunction syndrome; RUDS, reactive upper airway dysfunction syndrome; SIC, specific inhalation challenge; sIgE, specific IgE; WRA, work-related asthma.

Practical guideline from the international DiMoPEX task force, with European Respiratory Society Task Force, European Society for Environmental and Occupational Medicine and the Global Allergy Forum

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causes (ie, identify the causative agent(s) and the route of contact to allergen: airborne, or skin contact) to avoid further exposure. Identification of sensitization by a sensitive and validated measurement of specific IgE is an important step in the diagnosis. However, only a limited number of environmental and occupational allergens are available on the market for use in sIgE testing. Accordingly, specific in-house testing by individual diagnostic and laboratory centers is often required. Currently, different immunological tests are in use at various diagnostic centers that often produce considerably divergent results, mostly due to lack of standardized allergen preparation and standardized procedures as well as inadequate quality control. Our review and meta-analysis exhibited satisfactory performance of sIgE detection test for most high molecular weight (HMW) allergens with a pooled sensitivity of 0.74 and specificity of 0.71. However, for low molecular weight (LMW) allergens, pooled sensitivity is generally lower (0.28) and specificity higher (0.89) than for HMW tests. Major recommendations based on the presented data include diagnostic use of sIgE to HMW allergens. A negative sIgE result for LMW agents does not exclude sensitization. In addition, the requirements for full transparency of the content of allergen preparations with details on standardization and quality control are underlined. Development of standard operating procedures for in-house sIgE assays, and clinical validation, centralized quality control and audits are emphasized. There is also a need for specialized laboratories to provide a custom service for the development of tests for the measurement of putative novel occupational allergens that are not commercially available.

**KEYWORDS**

asthma, IgE, occupational allergies, rhinitis, urticaria

## 1 | BACKGROUND AND OBJECTIVES

Airborne allergens in living and working environments play an important role in eliciting type 1 allergic disorders, including asthma and hay fever. Allergies belong to the so-called noncommunicable diseases which have dramatically risen with industrialization and urbanization worldwide.<sup>1,2</sup> Nearly 600 asthma-causing occupational agents have been identified, of which two thirds are airway sensitizers.<sup>3</sup> Every year new allergic agents are identified at changing industrial or agricultural worksites, for example.<sup>4,5</sup> Some of these agents (chemical/biological) are also general environmental pollutants which can be passively transported by air (sometimes over long distances), and often found in homes. Other industrial sensitizers are used by consumers, for example, genetically engineered enzymes in home detergents, perfume sprays or used for food processing in home kitchens. All these substances contribute to a long list of new sensitizing agents of relevance for both occupational and general environmental medicine and public health.

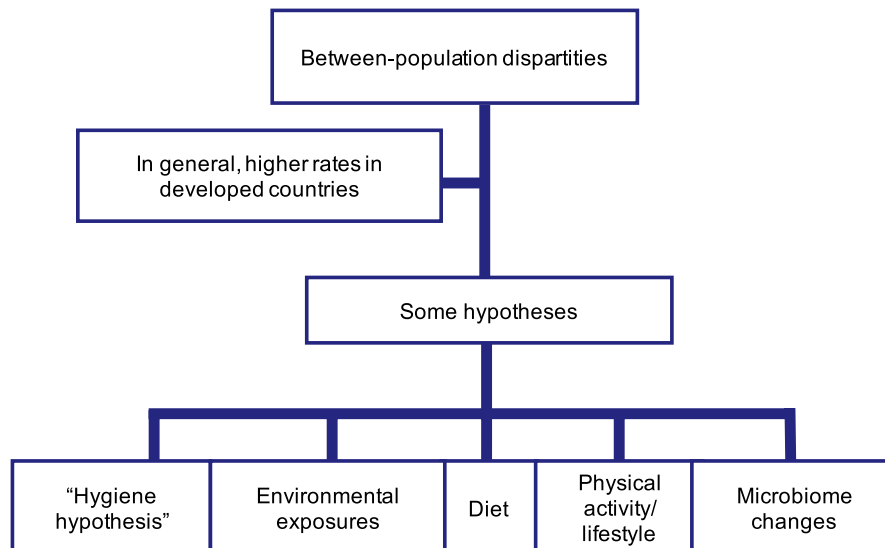
Asthma, the disorder this guideline focuses on represents an increasing global health problem and is now affecting between 8%-10% of the population.<sup>6</sup> The World Health Organization estimates that around 235 million people suffer from asthma worldwide.<sup>7</sup> This

implicates a high socio-economic cost in terms of work and school absenteeism, consumption of resources (drugs, consultations, hospitalizations), and deaths.<sup>8</sup> The increase in asthma figures is partly due to an improvement in diagnostic techniques, but other factors such as environmental pollution (Figure 1) are also relevant in industrialized countries.<sup>9</sup>

Environmental pollution is a growing public health problem although the exact mechanisms by which it can aggravate asthma are presently not known. Also less exposure to microbial load and diversity in both childhood and adulthood due to the global increase in urbanization has been suggested to contribute to the increase in asthma.<sup>10</sup> Rhinitis is approximately 3 times more prevalent than asthma in the adult population; although symptoms and impairment are generally less life threatening than those in asthma, this disorder also represents a highly relevant socio-economic issue. It is known that there exists a relationship between asthma and rhinitis as well as with respiratory infections.

Current research is aimed at establishing the immunological pathways that determine, not only the phenotypes and endotypes of these diseases,<sup>11,12</sup> but also the presence of certain biomarkers that allow precision medicine to be performed.<sup>13,14</sup> Although there is agreement to distinguish three possible immune responses in the

**FIGURE 1** Asthma between population discrepancies. Figure adapted from: ISAAC (1998), *Lancet* 351:1225-32



development of respiratory allergies, such as the type 2 immune response, the non-type 2 immune response, and the mixed Th2/Th17 immune response, the intimate mechanisms that generate them are still largely unknown. Most of the aforementioned aspects also refer to the less frequent type 1 allergic dermatitis, such as contact urticaria including protein contact urticaria which have to be differentiated from type 4 allergic contact dermatitis, not covered by this guideline.

The diagnostic measurement of specific IgE (sIgE) is often a major step in identifying the precise cause(s) of respiratory allergy. However, only a limited number of allergen-sIgE are commercially available, especially in the occupational field. This necessitates the development and use of in-house tests in specialized laboratories. A further limitation of sIgE measurement is the lack of validated preparations for many allergenic agents. Currently, different immunological tests are available on the market and are in use. The manufacturers of such tests, rarely, if at all, provide detailed data on the allergen preparations used, standardization of the method, and quality controls applied. The various tests often differ considerably and their results are hard to compare. So, a major current problem is lack of transparency in commercial sIgE tests and a lack of generally accepted and applied procedures to assess whether the sensitivity and specificity of these tests are appropriate.

There is an urgent need for an international consensus on technical standards and quality control for allergen preparation to serve as an important resource for physicians and laboratories in diagnostics of exposure-related type 1 respiratory allergic diseases. This document represents international practical statements with the primary objective to provide an overview on available methods required for state-of-the-art clinical diagnostic purposes and for making recommendations for clinical practice. This document focuses on diagnosis of respiratory allergic disorders caused by occupational and/or environmental industrial agents, but may also be applied to related skin allergic disorders. The recommendations include exposure assessment and in-house in vitro testing with commercially

and noncommercially available allergens. A further aim is to present current evidence on sIgE testing in the diagnostics of type 1 allergic disorders with special regard to allergic work-related asthma (WRA) including occupational asthma (OA, where nonoccupational agents do not play a causative role) and allergic work-aggravated (also called allergic work-exaggerated or work-exacerbated) asthma. Recommendations for clinical practice are formulated. This practical statement is intended to provide guidance to clinicians, public health professionals as well as others who interpret the scientific evidence, when new outcomes are reported (eg, policy makers).

Methodological details of this practical guideline are given in the Supporting information (see Supplementary Methods, page S2).

## 2 | CLINICAL PICTURES OF TYPE I ALLERGY DUE TO AIRBORNE OR SKIN EXPOSURES

Type 1 allergy due to airborne or skin exposures is defined clinically as an immediate allergic response caused by a product or raw material (eg, flour) that is present in the living or working environments. For occupational allergy, the agent should be specific to the workplace. Such allergies can affect many target organs, including lower airways, nose, eyes, and skin. The corresponding diseases are allergic asthma, allergic rhinitis, allergic conjunctivitis, allergic contact urticaria/protein contact dermatitis (details are given in the Supporting information, clinical pictures).

There is evidence that type 1 allergies caused by industrial allergens (by either a product or a raw material) are frequently not documented in the literature. Since these cases are left either unrecognized or are not published, the number of causative agents may be higher than that currently found in the list of sensitizers. See example in Supporting information.

Industrial products can also cause allergic reactions in consumers (see Allergen exposure assessment section) but these cases are

rather seldom described and presumably rare. Ingredients such as enzymes in fragrances, cosmetic products, detergents, or food (the latter includes processing at home) are some of the examples.<sup>4,15,16</sup>

### 3 | CAUSAL AGENTS

Agents that cause allergic WRA and/or allergic occupational rhinitis encompass more than 400 natural agents and synthetic chemicals. They are listed in textbooks,<sup>17</sup> review articles<sup>3,18</sup> and websites (eg, <http://www.eaaci.org>; <http://www.csst.qc.ca/en/prevention/reptox/occupational-asthma>; <http://www.aoecdata.org/ExpCodeLookup.aspx>).

Individual publications on allergens causing occupational asthma are listed in the Supporting information (see Table S1).

These agents are categorized into high molecular weight (HMW) and low molecular weight (LMW; <5 kDa) agents. High molecular weight agents are proteins of animal, vegetable, or microbial origin acting through an IgE-mediated mechanism. Low molecular weight agents include organic and inorganic compounds that, may function as haptens and with a few exceptions, are associated with an IgE mechanism in a proportion of affected subjects. Commonly identified agents of OA characterized by a latency period between initial exposure and asthmatic symptoms are diisocyanates, flour, allergens from laboratory animals and insects, enzymes, colophony fluxes, solders, wood dusts, natural rubber latex, acrylates, and glutaraldehyde. Following sensitization, workers with OA may develop an asthmatic attack to very low exposures to the sensitizer. The extent of airway responsiveness may diminish away from exposure, but usually increases with re-exposure to the sensitizer.

### 4 | DIAGNOSTIC MEASUREMENT OF sIgE AS THE MAJOR STEP IN IDENTIFYING THE PRECISE CAUSE OF RESPIRATORY ALLERGY

The practical division into HMW and those of LMW allergens is based on the fact that LMW substances cannot induce an IgE-mediated response by themselves; rather they act as incomplete antigens (or haptens), either by binding to host proteins or as reactive agents forming new antigenic sites upon reaction with host molecules.<sup>19,20</sup> High molecular weight allergens are generally polypeptides, proteins, or glycoproteins from animals, plants, bacteria, or fungi, and often have a molecular weight between 20 and 50 kDa. Low molecular weight agents are usually chemical substances with mass <5 kDa.

The distinction between HMW and LMW allergens is important when performing an immunological investigation. It is relatively easy to demonstrate an immune response mediated by IgE in cases of HMW agents and more difficult for most LMW agents. This fact has important repercussions in terms of commercially available tests.

The presence of allergen-sIgE can be investigated by *in vivo* skin tests or by *in vitro* laboratory tests. The procedures are briefly discussed in the Supporting information (see Diagnostic measurement, page S11).

Currently, laboratory methods for the detection of serum antibodies are routine. The most commonly applied techniques are the quantification of total and allergen-specific serum IgE (as a classical biomarker for a given allergen). These procedures have in spite of some limitation a number of advantages over the skin tests because there is no risk to the patient, the results are not influenced by drugs and are more diagnostic for certain groups of patients such as patients with dermographism or atopic dermatitis.

Reaginic antibodies were identified in the 1960s as belonging to a new immunoglobulin class, namely IgE<sup>21</sup> and shortly afterward the radioallergosorbent test (RAST) was developed.<sup>22</sup> The modern sIgE assay is a modification of the RAST assay; using the same basic three-steps, solid phase, noncompetitive binding, immune-metric (labeled antibody) chemistry, combined with a third step, that is, enzyme-substrate color, fluorescence, or luminescence development. Allergen-sIgE antibodies are bound in the first incubation to a solid phase allergen and then bound IgE is detected in a second reaction with labeled anti-human IgE. The magnitude of the response after the final buffer wash is proportional to the quantity of allergen-sIgE antibody in the original test serum. A multipoint total IgE serum calibration curve is used that ranges from 0.1 to 100 kU<sub>A</sub>/l traceable to the World Health Organization human IgE reference preparations (note WHO recently depleted 75/502 preparation is replaced with third standard: 11/234). This allows interpolation of IgE antibody results from any of the hundreds of allergen specificities as long as the allergen-sIgE portions of the assay dilute out in parallel with each other. Recently, an international guidance document has been published on the analytical performance and clinical utility of human IgE.<sup>23</sup> See also Supporting information (page S18).

Currently, there are three singleplex auto analyzers that dominate the clinical market for the measurement of sIgE.<sup>23</sup> These are ImmunoCAP (Thermo Fisher Scientific/ Phadia), Immulite (Siemens Healthcare Diagnostics) and HyTEC88 (Hycor Biomedical, which is being replaced with Falcon). Other IgE assays with a limited market share include Thabest IgE (Visual Diagnostics), Optigen (Hitachi Chemical Diagnostics), and ALLERG-O-LIQ (Fooke laboratories).

Each of the principal auto analyzers has comparable performance parameters, which have to be described and cleared by regulatory agencies. These include excellent intra-assay precision (typically < 15% coefficient of variance), inter-assay reproducibility (generally < 20% coefficient of variance), a limit of quantification of 0.1 kU<sub>A</sub>/l and a reportable assay range of 0.1-100 kU<sub>A</sub>/l.<sup>23</sup>

Studies have compared the three main sIgE assays and all reported different levels of IgE antibody for any given specificity.<sup>24-26</sup> This indicates that it is not currently possible to compare sIgE levels measured using different sIgE assays. This is probably due to the allergen used in the assay, the orientation and structural modifications of the allergen, and differing assay protocols including the solid phase material, slightly different assay calibration, and data computation procedures.<sup>27</sup>

Comparing the analytical precision and accuracy of sIgE assays, performed in different laboratories using various methods, despite standardization to the WHO human IgE reference preparation,

revealed that there were disparate results in four of the six laboratories.<sup>28</sup> This highlights the need for laboratories to participate in quality assurance schemes (eg, CLIA, ISO 17025) and to make those results available to their clients.

Historically, 0.35 kUA/l of allergen-sIgE was considered to be the cutoff point where an individual was considered as a threshold for positivity of the immunoassay, demonstrating sensitization.<sup>29</sup> The current recommendation to clinical laboratories, by a consensus guidance document,<sup>23</sup> is that sIgE levels must be reported as analytical measurements down to the cleared limit of quantitation of the assays, which is usually 0.1 kUA/l. The limit of quantitation within an assay is set to ensure that reported low quantitative results are comfortably above background signal (noise—chemical, instrument and from non-specific IgE binding). Thus, all test results above the limit of quantitation should be regarded as true allergen-sIgE antibodies. Historically, sIgE  $\geq$  0.35 kUA/l was considered to be clinically relevant, however, it is now considered that clinicians should interpret IgE levels in the context of the clinical history, rather than have a defined cutoff point.<sup>29</sup> See Supporting information (page S18) for more details.

It is important to understand that the presence of sIgE is an indicator of sensitization, it is not proof of allergic disease and is only clinically relevant when there are objectively defined allergic symptoms that are temporally associated with a known allergen exposure.<sup>27</sup> A positive IgE test that is concordant with the patient's history and/or a challenge test confirms "allergy" to a particular allergen and can be used as confirmatory evidence of sensitization to manage the patient appropriately and encourage improvement of environmental control measures within the workplace.

#### 4.1 | What is commercially available and suitable?

New allergens are continually being identified within the workplace, for example, horticultural nematode, *Steinernema feltiae*.<sup>30</sup> The list of commercially available occupational allergens is very restricted, for example, Thermo Fisher (Phadia) currently has only 36 ImmunoCAPs for occupational allergens, although they do also provide others such as animal allergens, for example, rat and mouse. The limited availability of commercial assays for the measurement of sIgE is a significant problem within the field of occupational allergy and asthma that necessitates the use of "in-house" assays to measure sIgE to noncommercially available allergens.

There are many allergens and some components available for CAP tests; however, there are only a limited number of occupational HMW allergen components, for ImmunoCAP, for example, there are nine recombinant latex allergens. Availability of allergen components has led to improved diagnosis of sensitization in some cases.<sup>31-34</sup>

#### 4.2 | Occupational allergens are categorized as either high or low molecular weight

sIgE assays are useful to demonstrate IgE-mediated sensitization to HMW allergens (animal and plant proteins) but less so with LMW

allergens, which are predominantly synthetic chemicals. Low molecular weight allergens are too small to be recognized by the immune system without conjugation to a protein, for example, human serum albumin (Table 1; Figure 2; Figure 3).

There is a wide spectrum in the ability to detect sIgE for LMW allergens ranging from the possible exception of acid anhydrides with relatively high detection rates (sensitivity 81%) to isocyanates (sensitivity 21%; specificity 94%) and plicatic acid (sensitivity 9.6%).<sup>33</sup>

Recent studies highlighted the difference in sIgE binding according to the conjugate preparation conditions.<sup>35,36</sup> One of the difficulties with measuring sIgE to LMW allergens (including products acting as haptens<sup>37,38</sup>) is that there are no standardized protocols for the preparation of protein-LMW allergen conjugates and, characterization of the resultant protein hapten complex can be technically challenging and complex. There were various approaches to bind LMW agents such as acid anhydrides,<sup>7,39</sup> glutaraldehyde,<sup>40</sup> or isocyanates (for more details see Table S2). The diisocyanates are emblematic of difficulties of elucidating underlying antigenic forms of diisocyanate haptenated proteins. Albumin is the preferred protein carrier, but multiple proteins are potentially haptenated in vivo (Figure 2). Diisocyanates have been reported to covalently bind to albumin, hemoglobin, lung epithelial proteins, tubulin, and keratins.<sup>41-44</sup> Proteomic mass spectrometric studies of in vitro conjugation of diisocyanate to albumin and hemoglobin have demonstrated that diisocyanates can form a variety of complexes with proteins including inter- and intramolecular cross-linked species (see Figure 3).

There has been a debate as to whether the low sensitivity of sIgE assay to diisocyanates may in part be due to heterogeneous pathomechanisms. In a small study of patients with a clinical diagnosis of occupational asthma based on a positive bronchial challenge, there was a striking absence of IL-4 and C $\epsilon$  mRNA observed locally within bronchial mucosa following an active challenge, which is in contrast to patients with asthma to high molecular weight allergens such as grass pollen.<sup>45</sup> This suggests that in some patients, isocyanate induced asthma may be a non-IgE-mediated disease. Confirming this observation in serum of isocyanate patients with occupational asthma is not feasible due to lack of a reliable validated IL-4 assay.<sup>46</sup> Studies also suggest that sIgE against plicatic acid-human serum albumin-conjugate is unlikely to be a causative factor in the pathomechanism of western red cedar asthma, suggesting that this may also be a non-IgE-mediated disease, at least in some patients. Animal models of chemical-induced asthma also suggest that IgE mechanisms are not necessary to elicit respiratory reactions in previously dermally exposed animals.<sup>47,48</sup> Finding sIgE to LMW allergens is confirmatory of sensitization, but a negative sIgE does not rule out that agent as the cause of a WRA.

#### 4.3 | In-house sIgE tests

In-house assays are required, if no commercial tests, are available (ie, in case of a new allergenic protein). In many cases, commercial tests show inconsistent results, though clinical data clearly points to allergen-related symptoms (showing inconsistency of in vivo and in

**TABLE 1** Statements

## Diagnostics

The occupational type 1 allergy of greatest concern is occupational asthma; exposure-related type 1 allergic rhinitis, conjunctivitis, and protein contact dermatitis also play a role

The diagnoses of occupational type 1 respiratory allergies follows an algorithm approach, starting with clinical and qualified occupational history, followed by confirmation of the disease with objective methods and allergy testing (SPT test or sIgE measurement), and, if needed, spirometry monitoring during work shifts or specific nasal challenge or SIC

Commercial sIgE tests generally lack transparent information on the allergen preparation used, applied standardization and quality control

In-house tests mostly do not follow appropriate standardization and quality control; they differ from place to place considerably and do not allow comparison of the results definitively

There are limited commercial occupational allergens available on CAP. This necessitates specialized laboratories to provide bespoke "in-house" assays for novel putative and allergens which are not available on CAP

Negative results from a bespoke in-house assay for putative novel allergens do not necessarily imply a negative test, if there are no positive controls. sIgE tests are only as good as the composition of the allergens used in the assay. Thus, we need to ensure that for any sIgE measurement, we use the appropriate allergens

The laboratory should always assess the performance of sIgE assay by carrying out a clinical audit. The performance of any sIgE test, whether it is RAST or CAP, is very dependent on allergen used and the positive cut off value

CAP, RAST, and other methods for measurement of sIgE can give similar sensitivity to skin prick test when the same allergen is used for both test, for example, 95% for protease and cellulase, 98% for amylase, 99% rat urine

sIgE to HMW allergens provide acceptable sensitivity and specificity and are very useful as a diagnostic test

Specific IgE assays to LMW allergens are more problematic and dependent on the allergen investigated. sIgE measurement to isocyanates is specific but not sensitive, whereas sIgE to acid anhydride could have acceptable sensitivity and specificity, which needs to be assessed with larger cohorts. To date, we are unable to measure sIgE for platinum salts. Thus each low molecular weight allergen must be considered case by case

By use of sIgE tests HMW allergens provide acceptable sensitivity and specificity mostly above 0.7 kUA/l. This is especially shown for extracts from cereals, latex, enzymes, bovine epithelium and bovine dander, molds, insects, and for particular approaches with recombinant allergen components

Some wood extracts and LMW agents such as diisocyanates, acid anhydrides provide much lower sensitivity than confirmed HMW allergens; obviously, this is at least in part due to heterogenous patho-mechanisms including irritant effects

## Prevention

Early diagnosis of respiratory allergies combined with avoidance of the causative allergen is important because it prevents the allergy march from rhinitis to asthma, as well as chronification and deterioration of the disorders

**TABLE 2** (Continued)

## Exposure assessment

Standard chemical air sampling and analysis methods exist for many of the low molecular weight allergens, however, in many cases exposure to these electrophilic chemicals is not just to the monomeric form. Exposure can be to a mixture of monomers, polymers and prepolymeric forms, but analytical methods are mainly only for monitoring the monomeric forms

Exposure assessment for characterizing levels leading to immunological sensitization (vs. asthma elicitation) is extremely difficult and in general lacking. This includes both respiratory and dermal sensitizing events

Biomarkers of exposure have been reported (especially for LMW allergens) in the literature, (eg, allergen metabolites or adducts), but they have not always been used as an exposure monitoring tool

Exposure monitoring for high molecular weight allergens may entail measurement of multiple allergenic proteins, especially from natural products. The specific aeroallergen(s) responsible for disease may vary with the life cycle of that product

Quantitative dermal exposure assessment methods are lacking, which hinders the assessment of the level of dermal sensitization in subsequent asthma development

Early biomarkers of allergic sensitization, in addition to specific IgE are needed to prevent subsequent asthma development

Direct reading instruments with sufficient sensitivity are needed to monitor relevant worker exposure to agents known to cause occupational asthma

vitro diagnostic tests).<sup>49</sup> sIgE antibodies are generally measured with either in-house fluorescence enzyme labeled immunoassay using biotinylated protein coupled to streptavidin CAPs, in-house ELISAs, or in-house immunoblots.

There is a need for establishment of a publicly funded laboratory working with this sophisticated in-house sIgE issue. For more details see additional Supporting information.

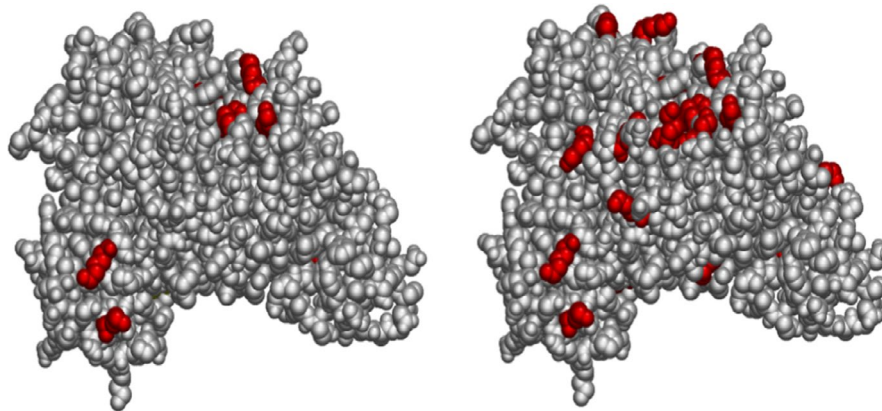
**4.4 | The need for assay standardization**

At present there are no standard operating procedures for conducting "in-house" assays.<sup>33</sup> Current FDA guidance regulations, regarding fluorescence enzyme labeled immune-assay based methods, state that the source and stability of allergen-specific control sera should be specified. It is, however, to be emphasized that all immunological methods (in-house and commercial) have to be validated routinely with control serum samples and additional standard set points (two analytic standards, one with low concentration and the other with high concentration, are used as set points).<sup>4</sup> For validation of the assays, a minimum of the following controls need to be included: tests with pooled sera from sensitized and nonsensitized subjects, biotin and HSA-control samples. The measured day-to-day precision and variability in series measurements should follow good laboratory practice rules.

For further details, techniques, and analyses of mediators see Supporting information.

(Continues)





**FIGURE 2** Chemical haptentation of albumin upon dermal exposure to diisocyanate (from Hettick and Spiegel, International Journal of Mass Spectrometry 309, 168-75, 2017). The figure displays diisocyanate haptentation sites on human serum albumin (left = MDI, right = TDI). Electrophilic chemical allergens can bind to multiple nucleophilic sites on self-proteins. Diisocyanates can also self-polymerize, thus multiple potential neo-antigens may be produced following chemical exposure. Left: serum albumin Lys residues haptentated by MDI. Right: serum albumin Lys residues haptentated by TDI. Serum albumin and skin keratins have been identified by mass spectrometry as targets of haptentation upon dermal exposure. Which residues are haptentated depends on: Identity of the isocyanate (electrophilic reactivity and size); Accessibility of the site (sterics); Chemical composition of the residue (primary amines); pH of the microenvironment and/or pKa of the sidechain ( $-NH_2$  reactive, not  $-NH_3^+$ ); Concentration of the isocyanate (less kinetically favorable observed at higher concentration). "Dilysine" motifs are preferred conjugation sites in vitro and in vivo: Lys413-Lys414 and Lys524-525 of serum albumin are conjugated in skin and lung of mouse model; These sequence motifs are conserved in humans; Enhanced reactivity most likely due to suppressed pKa of second Lys residue. For more information, see: Hettick, J et al *Xenobiotica*. 2017 Jul 21:1-11; Nayak, A et al *Toxicol Sci*. 2014 Aug 1;140(2):327-37; Hettick, J et al *Anal Biochem*. 2012 Feb 15;421(2):706-11; Wisniewski, A et al *Anal Biochem*. 2010 May 15;400(2):251-8

#### 4.5 | Early biomarkers of allergic sensitization, mediators, and cellular tests used in diagnostics

Allergy is an immune mediated, inflammatory response involving different types of cells that release a multitude of inflammatory mediators.<sup>50</sup> The mast cells act as effector cells releasing mediators such as histamine and tryptase among others upon antigen- and IgE-dependent activation, but also by several other mechanisms. During cell activation, eosinophils secrete eosinophil cationic protein (ECP), while basophils release the content of their granules after a process of activation dependent on the antigen.

For diagnostic details see the Supporting information.

### 5 | OUTCOME OF THE META-ANALYSIS AND REVIEW PERTINENT QUESTIONS

#### 5.1 | What are the sIgE test performances for high molecular weight allergens?

The aforementioned meta-analysis of studies including asthmatic subjects exposed to various occupational HMW agents found that specific IgE determination provided a sensitivity of 74% [95% CI 66%-80%], as compared with specific inhalation challenge or serial peak flow measurements, while the specificity of these tests was 71% [95% CI 63%-77%].<sup>33</sup> The results are in the range of a previous analysis based on fewer studies.<sup>51</sup>

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for a cohort of bakers were calculated at a cutoff value of 0.35 kUA/l for wheat and rye flour sIgE. Specificity

was 68% and 62%, PPV 74% and 82% and NPV was 82% and 71%, respectively, whereas sensitivity was 87% for each of these flours.<sup>52</sup>

For natural rubber latex (NRL), the sensitivity, specificity, PPV, and NPV of a sIgE level  $\geq 0.35$  kUA/l as compared to the result of SICs were 94%, 48%, 86%, and 71%, respectively.<sup>31</sup>

Results of our new meta-analysis for sensitivities of single HMW allergens ranged from 72% [95% CI 42%-90%] for cellulase, 0.79 [95% CI 65%-88%] for wheat, 0.84 [95% CI 65%-94%] for rye to 0.88 [95% CI 42%-99%] for latex.<sup>33</sup>

#### 5.2 | What are the test performances for allergenic components?

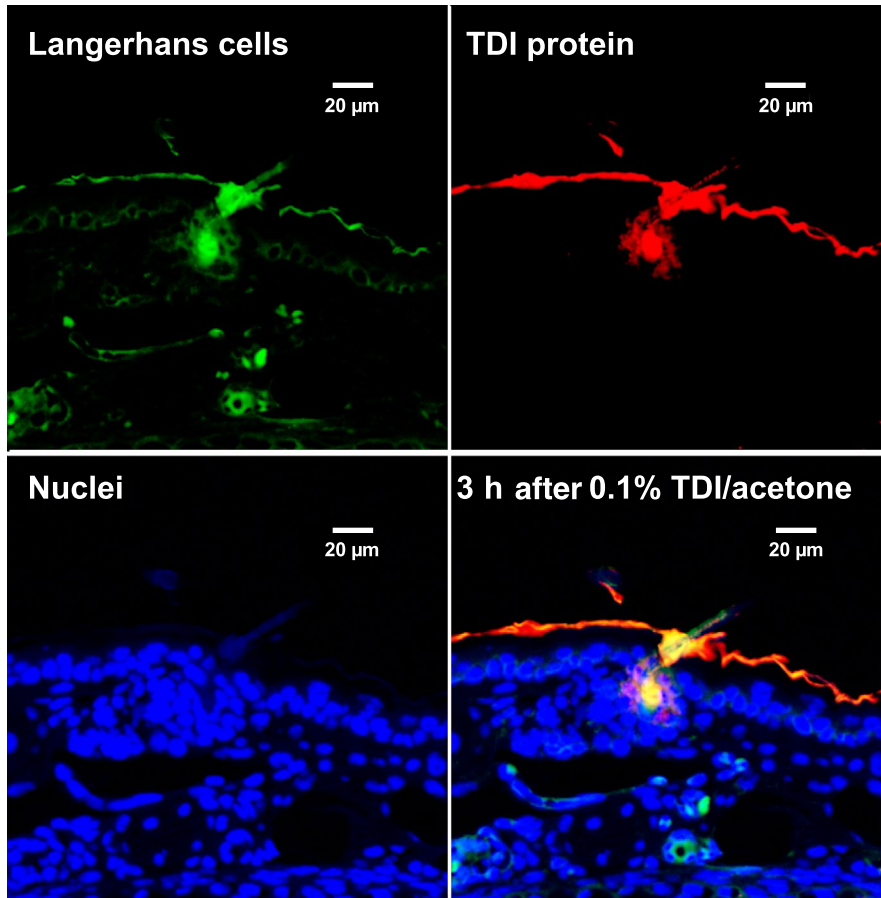
Using commercial ImmunoCAP analysis, sensitization to green coffee beans was found in 2/18 (11%) of workers with symptoms of allergic rhinitis and/or conjunctivitis under coffee dust exposure. ELISA screening showed sIgE reactivity to rCof a 1, rCof a 2, and rCof a 3 in a total of 8 out of 18 (44%) sera of symptomatic coffee workers.<sup>53</sup>

The specificity of measuring sIgE to a panel of *Triticum aestivum* components, (Tri a) 27, 28, 29.02, 32, and 39 was 97% with a sensitivity of 70% for wheat flour allergy among bakers.<sup>54</sup>

Twenty-one (48.8%) of the 43 sera with negative results to commercial wheat flour ImmunoCAP (f4) yielded positive results, with at least 1 of 6 newly identified wheat recombinant proteins.<sup>55</sup>

However, for routine diagnosis of baker's allergy, allergen-sIgE tests with whole wheat and rye flour extracts were shown to be of superior diagnostic sensitivity, when compared to components.<sup>54</sup>

Vandenplas et al<sup>31</sup> found that the sum of sIgE concentrations against the recombinant allergens of *Hevea brasiliensis* (rHev b5) and



**FIGURE 3** Immunochemical co-localization in mouse skin of 2,4-TDI haptenated proteins (albumin, and cuticular and cytoskeletal keratins) along with antigen presenting cells (from: Nayak et al *Tox Sci*: 140(2) 327-337, 2014). Dermal LMW chemical sensitization is often used with subsequent respiratory challenge to model LMW chemical asthma. TDI was observed to rapidly haptenate dermal proteins, especially in the outer root sheath of the hair follicle, and recruit antigen-presenting cells (CD11b APCs, CD207 Langerhans cells and CD103+CD207+ Langerhans cells) with subsequent transport to local draining lymph nodes. Confocal microscopic images of Langerhans cells (top left), TDI haptenated tissue (top right), cell nuclei (bottom left) and overlay of Langerhans cells, nuclei, and TDI haptenated tissue (bottom right)

r Hev b 6.01 or 6.02 yielded a higher predictive value (>95%) for positive SIC similar to that provided by the level of sIgE against the whole latex extract, but with higher sensitivity (79%) and diagnostic efficiency (0.67) as compared with whole latex-sIgE (49% and 0.41, respectively). Combining positive sIgE results for the NRL extract and the recombinant allergen components rHev b 5, 6.01, 6.02, and 11 provided similar AUC (0.84-0.85) and Youden index (0.61-0.65) values (data not detailed), but did not improve the diagnostic performance. Nevertheless, measurement of sIgE antibodies against the tested panel of recombinant latex allergen components did not improve the negative predictive value of immunological testing as none of the subjects with positive SIC and a negative IgE against the whole latex extract showed IgE reactivity to allergen components.

Supplementation of natural extracts with recombinant components ("spiking") has already entered daily practice in the field of occupational allergy laboratory analyses. Latex component Hev b 5 added to the natural extract raised sensitivity from 76% to 90% without lowering specificity. The method became commercially available with a CAP test.<sup>56</sup>

### 5.3 | What are the sIgE test performances for low molecular weight allergens?

For LMW the preparation conditions can greatly affect the degree of haptenation and test performance. There are no well accepted

standard methods for reacting LMW agents to albumin, or for characterizing the resultant haptenated protein.

Lux et al<sup>33</sup> reported a sensitivity of 28% [95% CI 18%-40%] with a specificity of 89% [95% CI 77%-95%] for occupational asthma caused by various LMW allergens. In a previous publication with a smaller study group, similar data with sensitivity of 31% and specificity of 97% were reported.<sup>51</sup>

Elevated sIgE by the ELISA method to diisocyanate conjugated with human serum albumin had a sensitivity of 31% and a specificity of 97%, in bronchial provocation test positive patients.<sup>57</sup> Tee et al<sup>58</sup> reported a sensitivity of 28% and a specificity of 92% with a RAST ratio cutoff point of 2.0 in patients who had a positive bronchial challenge test to isocyanates. Baur found a sensitivity of 14%,<sup>59</sup> and later 20% with comparable specificity (personal communication). Meta-analysis provided 21% [95% CI 14%-31%] and 94% [95% CI 88%-97%] for pooled pairs of sensitivity and specificity.<sup>33</sup> Pooled sensitivities of single diisocyanates HDI, TDI, and HDI ranged from 21%-42%. Sensitivity for acid anhydrides was exceptionally high with 81% [95% CI 46%-95%], but the estimation was based on few results<sup>33</sup>.

## 6 | ALLERGEN EXPOSURE ASSESSMENT

An important aspect in the management and diagnosis of type 1 allergic disorders is the knowledge of the environmental levels of

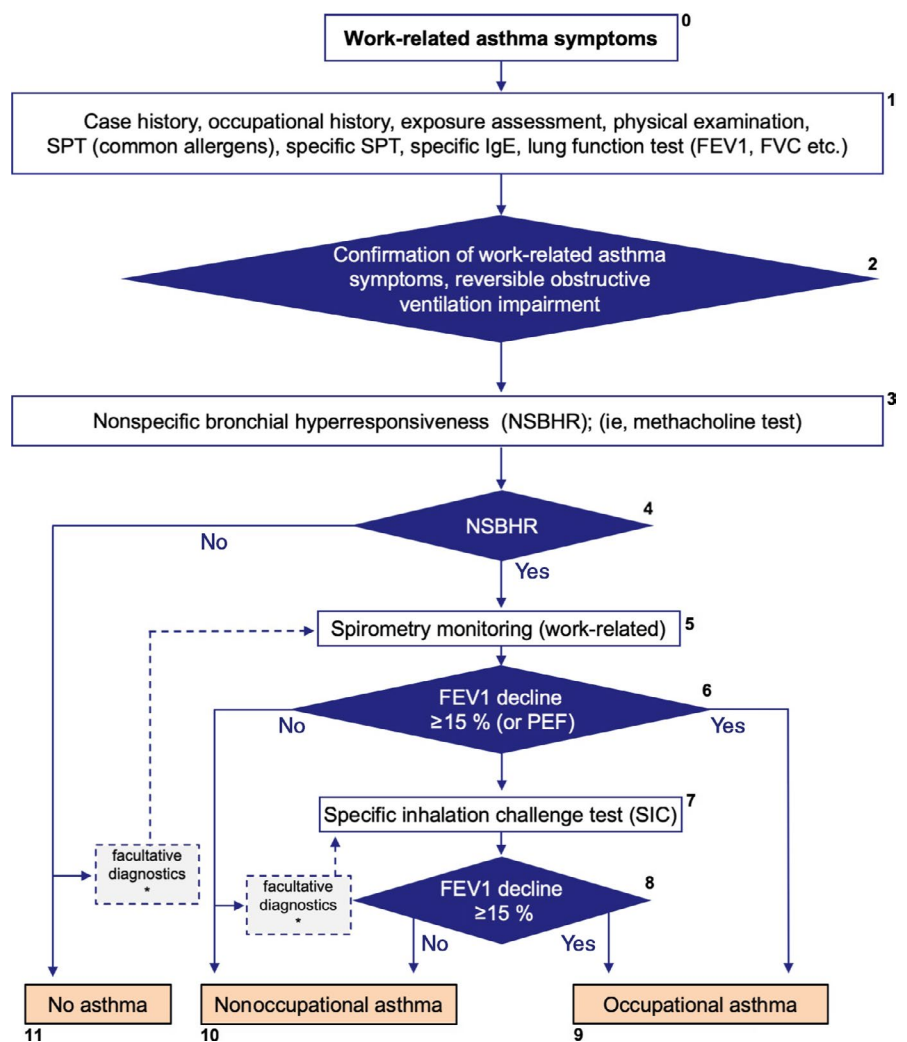
antigens. This involves an understanding of the total environment including both the external environment and also the interior of the houses or work areas. The quantification of substances in the environment has various applications that can be of help in the diagnosis of these disorders. Quantitative and qualitative measures of past and present allergen exposure are especially important in identifying the sensitizing agent(s), to estimate dose response relationships and for preventing the elicitation of asthmatic episodes. Exposure assessment can be multifaceted including the use of questionnaires, job exposure matrices, direct sampling of the air and on skin, and biomonitoring.

## 6.1 | Air sampling methods and databases

Heederik et al<sup>60</sup> thoroughly addressed the topic of workplace exposure sampling, analyses, assessment strategies, and data interpretation. For asthma, inhalable dust sampling is most commonly measured and recommended in order to collect the portion of the aerosol that can be inhaled by the worker and deposited in any part of the respiratory tract (see Supporting information, Figure S1).

For air monitoring to allergens, it is important to consider that generally HMW allergen and many LMW exposures occur in the

workplace as dust or aerosols (the latter may also occur as vapors) which dictates the type of corresponding sampling procedures. Established and validated procedures for the sampling of dust are available from NIOSH, the MAK commission, and other working groups (NIOSH Manual of Analytical Methods 5th edition<sup>61</sup>; the MAK-Collection for Occupational Health and Safety Part III: Air Monitoring Methods (DFG).<sup>62</sup> Further collection of air monitoring methods is provided by the Health and Safety Executive (UK)<sup>63</sup>. In the United States, the National Institute for Occupational Safety and Health's Manual of Analytical Methods (NMAM; 4th and 5th addition) is available on line <https://www.cdc.gov/niosh/docs/2003-154/default.html>; <https://www.cdc.gov/niosh/nmam/default.html>.<sup>61</sup> In addition to specific methods, guidance chapters are available in the NMAM to assist in developing sampling strategies and methods development. Particularly, the following chapters of the NIOSH Manual of Analytical Methods may be helpful as guiding documents: Purpose and Scope, Development and Evaluation of Methods, Measurement Uncertainty and NIOSH Method Accuracy Range, General Considerations for Sampling Airborne Contaminants, Factors Affecting Aerosol Sampling, Sampling and Characterization of Bioaerosols, Filter Pore Size and Aerosol Sample Collection, Measurement of Fibers, and Sampling



**FIGURE 4** Diagnostic approach in case of suspected occupational (environmental-related) asthma. For details see text in the Supporting information. \*Please note that there are rare cases of work-related asthma without NSBHR or absence of an obstructive ventilation pattern during work. If the case history is supportive of work-related asthma additional (facultative) diagnostics is recommended as indicated in Figure 4. It should be taken into consideration that false-negative outcomes of the NSBHR test, spirometric monitoring and the SIC test may occur due to medication or latency periods of several weeks or more since last exposure. The SIC may also be false negative if not performed with the correct agent. If this has to be assumed in a case whose history is strongly supportive of asthma, repetition of the individual diagnostic tests has to be considered

**TABLE 2** Recommendations

## General diagnostic aspects

Diagnostics of respiratory and skin type 1 disorders should start with a qualified clinical and occupational case history, followed by non- or less-invasive methods, that is, clinical status, functional, and allergy tests (prick and/or sIgE) and finally, if needed for prevention or therapy, by serial lung function measurement and/or specific nasal challenge or SIC

In case of suspected allergic OA, but unclear diagnostic findings, serial lung function measurement according to standardized protocol (2 weeks work, 2 weeks off work) are recommended. SIC is only recommended in suspected occupational asthma cases if serial lung function measurement is not possible and all diagnostic tests do not provide a clear diagnosis but diagnosis is needed for far-reaching preventive or therapeutic measures

Measurement of sIgE to suspected causative environmental allergens by use of a standardized specific and sensitive method is recommended within the diagnostic setup in order to identify the causative agent

The manufacturers of commercial sIgE are requested to provide full transparency of the allergen preparations they use along with details of standardization and quality control

For in-house sIgE assays there should be standard operating procedures which should include batch to characterization of allergen for in-house s IgE assays (including measurement of protein content, electrophoresis and immunoblotting). Development of assay should include testing of at least 20 negative sera of subjects not exposed to individual allergen (however, their total IgE should cover a range up to 1000 IU/ml) and at least three sera of subjects suffering from IgE sensitization. The sIgE assay should be standardized against the gold standard 3rd World Health Organization (WHO) International Reference Reagent (IRR) for serum IgE (75/502;5000 IU/ampoule). (Note: this may be difficult for putative novel allergens). [http://www.who.int/biologicals/BS\\_2220\\_Candidate\\_Preparation.pdf](http://www.who.int/biologicals/BS_2220_Candidate_Preparation.pdf)

There is a need for specialized laboratories to provide a bespoke service for the measurement of putative novel occupational allergens and also those allergens not available commercially

Determination of sIgE to high molecular weight allergens is recommended as a valuable diagnostic tool within the diagnostic setup algorithm in bronchial asthma

The presence of sIgE to low molecular weight agents such as diisocyanates and acid anhydrides should be regarded as strong evidence for relevant sensitization, whereas negative sIgE must be regarded with caution due to frequent false negative results

Laboratories should always carry out clinical audits (systematic and independent examination whether processes, requirements and rules meet required standards) on their sIgE assays which will determine whether commercially available assays or "in-house" assay provides the best diagnostic specific IgE assay

Commercially available sIgE assays can be recommended for most allergens (although there are exceptions eg, acid anhydride, isocyanates) as a standardized method to measure sIgE; however, its relatively high costs have to be considered and limit its broad application

## Nasal diagnostic aspects

The diagnosis of allergic rhinitis including occupational rhinitis is based on history, clinical findings and confirmation by sIgE to relevant allergens

**TABLE 2** (Continued)

The nasal endoscopy is indicated to evaluate anatomical or infectious diseases

The nasal challenge test is recommended to diagnose allergic rhinitis, local allergic rhinitis, nasal hyperreactivity, or occupational rhinitis

Specific nasal allergen challenges should be performed according to the standardized protocol of the recently published EAACI Position paper<sup>65</sup> Diagnostic tests such as assessment of nonspecific nasal hyperresponsiveness or specific nasal allergen challenges are a key aspect of specialized clinical centers

## Skin testing

Patients suspected to suffer from immediate-type allergy to occupational/environmental allergens should be appropriately screened for possibly causative agents, and, where possible, discontinued on medications that interfere with test results, accentuate systemic allergic reactions or render patients less responsive to treatment with epinephrine.

Even though SPT is safe with no reported fatalities, a physician or other healthcare professional and emergency equipment should be immediately available when such tests are performed

SPT should be performed with extra caution during the respective allergy season when the patient has allergic symptoms, or when baseline tryptase levels are elevated.<sup>66,67</sup> The latter is a risk factor for anaphylaxis and mastocytosis

Relative contraindications for SPT include pregnancy, in view of a remote possibility of inducing a systemic allergic reaction that could induce uterine contractions or necessitate the use of epinephrine (thought to cause constriction of the umbilical artery)

SPTs are difficult to perform or to interpret in patients with severe eczema, dermatographism, or who are taking antihistamines or other medications such as certain antidepressants or calcineurin inhibitors

The degree of skin test reactivity can be decreased in subjects with chronic illnesses such as renal failure, or cancer. Furthermore, chronic or acute UV-B radiation of the skin in the test area may reduce the wheal size from SPT

It is difficult or impossible to develop stable test extracts for certain allergens, in particular, certain foods, for example, for skin testing to uncooked fruits and vegetables. A prick-to-prick technique is utilized, that is, first pricking the fresh food with the lancet and then pricking the skin, to test for sensitization to such allergens when clinical allergy is suspected, in particular, oral allergy syndrome. Dry foods, for example, nuts or cereal, can be tested in saline and also utilized using the prick-to-prick technique

## Exposure assessment

"At-risk" worksites exposure monitoring of the specific allergenic components should be routinely conducted and employ direct reading continuous (personal) monitors whenever possible

Biomarkers of exposure should be standardized and used to supplement environmental monitoring

and Analysis of Soluble Metal Compounds. The Occupational Safety and Health Administration (OSHA) has a similar database at <https://www.osha.gov/dts/sltc/methods/index.html>. While these databases are not specific for chemicals that cause asthma, they contain many methods for the more common ones. Methods

(Continues)

harmonization for occupational exposure monitoring among stakeholders including government entities and consensus standard organizations is recognized as important in “leveraging current and future applied research, as well as technology transfer endeavors, within the discipline of occupational hygiene chemical and biochemical sampling and analysis.”<sup>64</sup> Assessing exposure to low molecular weight agents is mostly performed with help of ambient- and biomonitoring methods.<sup>60</sup>

For details see<sup>60</sup> and Supporting information (page S23).

## 7 | INTEGRATED DIAGNOSTIC APPROACH FOR OCCUPATIONAL AND ENVIRONMENTAL INDUSTRIAL ALLERGENS

Details are given in Figure 4 and in the Figure S2.

## 8 | STATEMENTS AND RECOMMENDATIONS

Based on the aforementioned data and results, we make the following statements and recommendations (Table 1, Table 2).

It is important that manufacturers of commercially available sIgE tests communicate their data on diagnostic allergen preparation, standardization, quality control, including background definition, and inform whether their method provides quantitative results by referring to the WHO IgE standard. Development of in-house methods for sIgE testing necessitates expertise with such methods and is rather expensive and laborious. It would be helpful if in-house assays could aspire to aforementioned prerequisites for commercially available tests, although in reality it is difficult to be able to include a WHO IgE standard reference. Regarding diagnostics we also stress exposure assessment by a questionnaire combined with measurement of the allergen load in the specific environment; this allows the risk of allergic disorders to be estimated. Surveillance of high risk subjects (eg, those with high level of sIgE antibodies) and individuals in “at-risk” sites, especially workplaces, are recommended in order to take appropriate preventive measures and to optimize medication.

For a broader view of this items see Supporting information, section *Points to be emphasized and prospective*.

### CONFLICT OF INTEREST

None of the authors has a financial or any other conflict of interest to disclose. The findings and conclusions shown 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, Centers for Disease Control and Prevention (NIOSH/CDC). Mention of any company or product does not constitute endorsement by either NIOSH/CDC or any other participating institution.

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#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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