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Article type : EAACI Position Paper

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EAACI position paper

Non-invasive and minimally-invasive techniques for the diagnosis and management of allergic diseases

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ALL.14645

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Key words: anaphylaxis, asthma, atopic dermatitis, food allergy, non-invasive diagnosis

Short title: Non-invasive diagnosis approaches in allergy management

Acknowledgements: We particularly thank Maryam Poorafshar, Magnus Molin and Jeronimo Carnes for their advice and experience from the diagnostic and pharmaceutical industry. The authors thank the European Academy of Allergy and Clinical Immunology (EAACI) for financial support of the task force. We also thank EIT Health for their visionary support of innovation projects in this area. EIT Health is a body of the EU and receives funding from the H2020 program. Dr. Pfaar reports grants and personal fees from ALK-Abelló, Allergopharma, Stallergenes Greer, HAL Allergy Holding B.V./HAL Allergie GmbH, Bencard Allergie GmbH/Allergy Therapeutics, Mobile Chamber Experts (a GA2LEN Partner), Anergis S.A., Laboratorios LETI/LETI Pharma, Biotech Tools S.A., Lofarma, grants from Biomay, Circassia, MEDA Pharma/MYLAN, Indoor Biotechnologies, grants from Glaxo Smith Kline, personal fees from Astellas Pharma Global, personal fees from EUFOREA, personal fees from ROXALL, personal fees from NOVARTIS, personal fees from SANOFI AVENTIS, personal fees from Med Update Europe GmbH, personal fees from streamedup! GmbH, outside the submitted work.

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Disclaimer: Dr. Togias' authorship of this report does not constitute endorsement by the US National Institute of Allergy and Infectious Diseases or any other United States government agency.

Conflict of interest statement: Prof. Adcock, Prof. Brockow, Prof. Eyerich, Prof. Garn, Prof. Kowalski, Prof. Ollert, Prof. Untersmayr, Prof. Togias, Dr. Thwaites and Dr. Zissler have nothing to disclose. Prof. Schmidt-Weber and Dr. Jakwerth reports grants from Federal Ministery of Education and Research, grants from European Institute of Innovation & Technology (EIT), during the conduct of the study; Prof. Schmidt-Weber reports from personal fees from Allergopharma and Allergy Therapeutics. Dr. Pfaar reports the following conflicts outside the present publication: grants and personal fees from ALK-Abelló, Allergopharma, Stallergenes Greer, HAL Allergy Holding B.V./HAL Allergie GmbH, Bencard Allergie GmbH/Allergy Therapeutics, Lofarma, Biomay, Circassia, ASIT Biotech Tools S.A., Laboratorios LETI/LETI Pharma, Anergis S.A.; grants from Glaxo Smith Kline and personal fees from MEDA Pharma/MYLAN, Mobile Chamber Experts (a GA2LEN Partner), Indoor Biotechnologies, Astellas Pharma Global, EUFOREA, ROXALL, NOVARTIS, SANOFI AVENTIS, Med Update Europe GmbH, streamedup! GmbH. Prof. Baumann reports grants from Phadia, part of Thermofisher scientific, grants from European Institute of Innovation & Technology (EIT), during the conduct of the study; has a patent pending. Dr. Hansel holds shares of Mucosal Diagnostics. Prof. Biedermann reports grants and personal fees from Phadia-Thermo Fisher during the conduct of the study. Dr. Chaker reports grants for clinical studies and research and other from Allergopharma , ALK Abello, AstraZeneca, Bencard / Allergen Therapeutics, ASIT Biotech, Immunotek, Lofarma, GSK, Novartis, LETI, Roche, Sanofi Genzyme, Zeller and from the European Institute of Technology (EIT); has received travel support from the European Academy of Allergy and Clinical Immunology (EAACI), DGAKI, and SMI, all outside the submitted work.

Abbreviations:

Specific IgE (sIgE)

total IgE (tIgE)

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dried blood spots (DBS)

bronchoalveolar lavage (BAL)

fraction of exhaled nitric oxide (FeNO)

serum periostin (POSTN)

eosinophil cationic protein (ECP)

exhaled breath condensate (EBC)

electronic nose (eNose)

skin prick test (SPT)

cysteinyl leukotrienes (Cys-LTs)

beta-platelet factor 4 (CXCL-4)

b-thromboglobulin, CCL-5 RANTES)

allergen immunotherapy (AIT)

C-reactive protein (CRP)

MicroRNA (miRNA)

transepidermal water loss (TEWL)

nerve growth factor (NGF)

artificial intelligence (AI)

8-hydroxydeoxyguanosine (8-OHdG)

inflammatory bowel disease (IBD)

irritable bowel syndrome (IBS)

eosinophil derived neurotoxin (EDN)

eosinophilic esophagitis (EoE)

matrix-assisted laser desorption/ionization (MALDI)

Leukotriene B4 receptor (LTB4R)

Peptidyl Arginine Deiminase 4 (PADI4)

Interleukin 1 Receptor Type 2 (IL1R2)

Protein Phosphatase 1 Regulatory Subunit 3D (PPP1R3D)

Kelch Like Family Member 2 (KLHL2)

Enoyl-CoA Hydratase Domain Containing 3 (ECHDC3)

C-C Motif Chemokine Ligand 2 also known as MCP1 (CCL-2)

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Abstract:

Allergic diseases of the (upper and lower) airways, the skin as well as the gastrointestinal tract, are on the rise, resulting in impaired quality of life, decreased productivity and increased healthcare costs. As allergic diseases are mostly tissue specific, local sampling methods for respective biomarkers offer the potential for increased sensitivity and specificity. Additionally, local sampling using non-invasive or minimally-invasive methods can be cost-effective and well tolerated, which may even be suitable for primary or home care sampling.

Non- or minimally-invasive local sampling and diagnostics may enable a more thorough endotyping, may help to avoid under- or overdiagnosis, and may provide the possibility to approach precision prevention, due to early diagnosis of these local diseases even before they get systemically manifested and detectable. At the same time, dried blood samples may help to facilitate minimal-invasive primary or home care sampling for classical systemic diagnostic approaches.

This EAACI position paper contains a thorough review of the various technologies in allergy diagnosis available on the market, which analytes or biomarkers are employed, and which samples or matrices can be used. Based on this assessment, EAACIs position is to drive these developments to efficiently identify allergy and possibly later also viral epidemics and take advantage of comprehensive knowledge to initiate preventions and treatments.

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Introduction

The prevalence of allergies is continuously increasing, along with associated difficulties in the appropriate treatment of patients [1]. Rapid, precise and pre-symptomatic diagnostic procedures are urgently needed to alleviate this burden. Many allergies are local diseases, and hence systemic diagnostic approaches often fail to detect disease before full manifestation. In contrast, local sampling methods and detection of characteristic biomarkers offer increased sensitivity and specificity, even before disease manifestation. Thus, non-invasive diagnostic approaches would enable earlier patient diagnosis, distinguish infections from allergies, and define endotype-specific prevention or treatment strategies. Additionally, local sampling using non-invasive or minimally-invasive methods can be cost-effective and well tolerated, which may even be suitable for primary or home care sampling. A rich portfolio of potential biomarkers that can be measured in non-invasive samples exists (Figure 1) but is currently not exploited by routine diagnostics. Here, we summarise the main systemic methods and biomarkers in allergic diseases of the airways (allergic rhinitis, asthma), circulation (anaphylaxis), skin (atopic dermatitis) and gastrointestinal tract (food allergy), before we explore possibilities for minimally- or non-invasive sampling methods for allergy management.

Due to the fact, that allergic diseases are mostly tissue specific, local sampling methods using non-invasive or minimally-invasive methods for respective biomarkers offer the potential for increased sensitivity and specificity. It is the goal of this article to illustrate the continually increasing number of biomarkers, and to facilitate the clinical management of allergic diseases in the near future.

This EAACI position paper aimed to anticipate the key challenges and identify opportunities in the future of allergy diagnosis. Ideal diagnostic biomarkers would preferably be based on non- or minimally-invasive sampling (Table 1 and Figure 1), as this enables home and repeated sampling with greater tolerability and reduced costs. Together with telemedicine, early biomarker assessment may allow personalized precision treatment and prevention, and may also enable doctors to avoid unnecessary treatments. To pave the way for this vision, substantial funding in the field is strongly recommended.

Method

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A task force on non-invasive diagnosis of allergy was initiated and approved by the EAACI ExCom and a workshop was announced and held under the title "*Non-invasive diagnostic approaches in allergy*" on the 4th June 2019 at the annual EAACI congress in Lisbon. The meeting was chaired by the last author with the main objective to drive novel developments into clinical applications. The group was split into subgroups, who covered airways, skin, intestinal and systemic manifestation of the disease. The groups generated overviews, which were collated and discussed in detail on a separate manuscript drafting meeting. Following further online meetings for manuscript discussion and improvement of the manuscript, the Position Paper was then finalized by the first author together with last author and presented to the TF for final evaluation and approval.

Classical diagnosis: Minimally invasive options for systemic IgE in allergies

In allergy diagnostics, a main focus is the determination of specific IgE, although a positive IgE test does only imply previous contact and sensitization, but not a concise diagnosis of allergic disease. Multiple technologies are on the market to measure this parameter (Suppl. Tab. 1). *In vitro* detection of allergen-specific IgE (sIgE) against aeroallergens or food allergens and total IgE (tIgE) in human serum or plasma is a very useful tool for the diagnosis of allergic diseases at the clinics and in research. This is particularly helpful at the level of point of care, allowing physicians a quick insight into the sensitization condition of potential allergy patients. The early sensitization pattern to common allergens at the age of 4 years was shown to have a predictive value to the incidence and persistence of asthma and/or rhinitis at 16 years [2]. Although these studies were performed using serum samples, it was shown that in seasonal allergic rhinitis, specific nasal IgE reproduced in 99.7% the presence of the same specific IgE in the serum [3]. Currently, the clinical market for the measurement of sIgE can be divided into fully automated laboratory systems for whole allergens and components, multiplexed systems (microarrays) for component resolved diagnostics, screening tests (allergen mixtures and panels) and point of care systems (Suppl. table 1.). Current multiple simultaneous allergen tests offer simple screening methods, those platforms and tests also have limitations with respect to multiplex capacity, flexibility and analytical performance [4].

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IgE in dried blood spot-based diagnosis

A recent development is the measurement of IgE levels in dried blood spots (DBS) and other capillary blood micro samples. In comparison to venous blood, DBS are considered to be less sensitive to storage and temperature conditions (ambient temperature), as well as transport processes (e.g. by mail), due to the inactivation of degrading enzymes during the drying process. Due to this reason, and as DBS are minimally invasive and require only small amounts of blood (±10 µL), DBS offer the possibility for decentralized sample collection and blood collection from small children and newborns [5] (Table 1). Recent reports showed that the determination of IgE and IgG reactivity to more than 170 allergen molecules has also been possible when using paper-DBS [6]. The paper-dried serum and blood spots could be stored at different temperatures (ie, +37°C, +22°C, +4°C, −20°C) delivering similar test results, indicating the possibility that shipment by mail without the need for cooling or express delivery in different seasons is possible and allows reliable and reproducible detection even of low levels of allergen-specific IgE [6].

Cytokines, Chemokines and RNAs in dried blood spots

In addition to IgE, cytokines and chemokines are detectable in DBS [7]. For example, chemokine levels in DBS were stable after different storage conditions such as room temperature for a week and multiple freeze/thaw cycles [7]. In addition, the DBS-technique allows the identification of viral RNA [8] or microRNA[5]. Whether cytokine, viral RNA and microRNA levels can indeed reliably be tested in DBS has to be further validated and would allow to transfer some of the blood-based biomarkers into a minimally-invasive DBS format.

Non-invasive and minimally-invasive diagnostics of allergic airway diseases

Allergies often start in upper airways and can progress to lower airways at all ages. Asthma can occur even in the first months of life. Therefore, allergic rhinitis and allergic asthma may considerably profit from an earlier accurate local diagnosis, before the disease gets systemically detectable. Current approaches and newer developments for both diseases are summarized in this section.

Classical and novel biomarkers in asthma

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Asthma often starts in childhood and is the most common chronic disease among children worldwide, Biomarker development in asthma started with the observation that asthma comprises allergic eosinophilic, allergic non-eosinophilic, non-allergic (non-)eosinophilic and mixed granulocytic forms. Lung eosinophilia (>300 cells/µL) represents a major cellular biomarker for the diagnosis of asthma endotypes and is associated with increased corticosteroid responsiveness in asthma. However, quantification of lung eosinophils by bronchoalveolar lavage (BAL) and endobronchial biopsy is invasive. Alternatively, sputum induction and sputum eosinophil counting represents a useful biomarker (discussed below) but sputum induction and processing is laborious. Alternative (indirect) minimally-invasive methods and markers for lung eosinophilia include fraction of exhaled nitric oxide (FeNO), total IgE, and serum periostin (POSTN). [9]

As stated above, lung eosinophilia (>300 cells/µL) represents a major cellular biomarker for the diagnosis of asthma. To date, the blood eosinophil count as minimally-invasive biomarker for lung eosinophilia shows a modest diagnostic accuracy [9-12], and might therefore be best utilized for either excluding (high sensitivity) or including (high specificity) lung eosinophilia.[10] Moreover, the *blood* eosinophil count is a prerequisite for applying for treatment with anti-IL-5 or anti-IL-5R monoclonal antibodies for eosinophilic asthma. Blood eosinophil counts also characterize various atopic states and diseases, but are subject to significant daily fluctuations, are highly steroid sensitive, and increase after exercise [11]. As new biologics enter the market (e.g. anti-IgE, anti-IL-4Rα, anti-TSLP, IL-33 or anti-macrolides), the need for new and more accurate guiding of asthma therapy is substantial. FeNO represents an established non-invasive parameter that is easy-to-obtain and reflects T-helper cell type 2 (Th2) inflammation of the airways. FeNO may help to discriminate patients with controlled and uncontrolled asthma, predict asthma development and exacerbation, and monitor the efficacy of corticosteroid and biologic therapy. [13]

Eosinophil cationic protein (ECP) is an established clinical biomarker of eosinophil activity in asthma [14]. ECP is stored in specific granules in mature peripheral blood eosinophils, and released after stimuli such as IgG and IL-5, while ECP serum levels are increased in atopic asthma. ECP can also be measured in sputum, nasal secretions /nasal lavage fluid and urine.

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An emerging biomarker in allergic diseases is serum Periostin, a "chronic" type 2 biomarker, in contrast to FeNO and possibly the eosinophil blood count as "acute" type 2 biomarkers (reviewed in [15]).

Recently, analysis of 73 studies identified 190 potential asthma biomarkers (proteomic, transcriptomic, genomic and/or epigenomic), revealing 10 potential biomarkers for asthma in at least 2 of 13 - omics levels[16], while IL-4, IL-13, STAT6 and VEGF-A were associated with asthma at 3 -omics levels. Ideally, these and other biomarkers should be detectable in non- or minimally-invasive samples. The rich list of potential non-cellular biomarkers has been previously summarized [17,18] (and Figure 1). Local sampling, using matrices such as urine, nasal secretions, tears, exhaled breath or exhaled breath condensate (EBC), may support the development of suitable biomarker tests.

Asthma: Exhaled breath

The assessment of exhaled gases *in vivo* or from biofluids using electronic noses (eNose) has developed as a possible non-invasive point-of-care tool for the diagnosis and phenotyping of patients with different respiratory diseases including allergic asthma (Table 1). Some eNOSEs detect specific volatile organic compounds (VOCs) without identifying their molecular entities by utilising multiple cross-reactive sensors (reviewed in [19]). Other eNoses detect the specific molecular entities using LC-MS/MS but this currently requires sending samples to central laboratories. Both types of eNoses enable pattern recognition and the detection of unique breath profiles for individual patients. Clustering on these eNose breath profiles in children and adults and asthmatics has identified differences in atopy [20], inflammatory biomarkers [21] and symptom control asthmatic subjects [22]. There are limited studies investigating the relationship between exhaled VOCs and atopic asthma. VOCs profiles captured by the Cyranose C320 only reported a trend for classification between atopic and non-atopic asthmatics in children [23]. The study was limited by the small sample size (n=31) and potentially better eNOSEs or combinations of eNOSEs would provide better discrimination. The direct detection of viruses in exhaled breath has not been realized and only after sampling of about 300l of air, viral DNA can be captured using various capturing systems [24].

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Brinkman et al recently suggested breath analysis can indeed contribute to the management of severe asthma. The same study that identified and followed up severe asthma phenotypes using metabolomic phenotypes obtained from a composite of eNoses, which were associated with changing inflammatory profiles and oral steroid use of severe asthma [21]. Future studies should further address the stability of eNOSE signatures and their mechanistic link with Gold standard skin prick tests (SPT) or allergen-specific IgE levels.

Induced sputum cell counts and mediators

Hypertonic saline-induced sputum can be used as a source of secreted biomarkers from the lower airways. Sputum cell counts generate reproducible results and can be used as a validated marker of lower airway inflammation, when standardized protocols are applied and thoroughly followed [17,25]. Besides sputum neutrophils, sputum eosinophil numbers are related to disease severity in adults or children [26-29] and distinguish between eosinophilic and neutrophilic asthma [30]. Higher numbers of over 300 eosinophils/µL occur during exacerbations, allergen-induced late-phase responses, or tapering of corticosteroids [31-34]. Sputum eosinophils are a useful tool for disease management, and can be used to improve the prevention of asthma exacerbations [35]. Furthermore, secreted mediators like cysteinyl leukotrienes (Cys-LTs), beta-platelet factor 4 (CXCL-4), b-thromboglobulin, CCL-5 (RANTES), thromboxane or serotonin are directly correlated with severe asthma, and therefore may serve as biomarkers for this condition [36-40]. Sputum Cys-LTs are recognized as central mediators in neonatal and childhood asthma phenotypes and may be particularly promising to monitor the development of asthmatic inflammation in early life [41,42]. The association between sputum markers and asthma control needs further attention and validation.

Allergic rhinitis, chronic rhinosinusitis and biomarkers of the upper airways as proxy for the lung

Allergic rhinitis often starts during childhood. Its early diagnosis is an unmet need, as viral rhinitis or other chronic, often referred to as “idiopathic” rhinitis, can cause similar and equally bothersome symptoms. Non-invasive sampling for distinctive and prognostic biomarkers would

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help to increase acceptance for early testing within high-risk groups (e.g. children with atopic dermatitis or uncharacterized wheeze). A proportion of patients with chronic rhinitis and allergic rhinitis progress to allergic asthma; earlier detection may therefore prove beneficial in allergy *and* asthma management and prevention. Monitoring of allergen immunotherapy (AIT), and preferably prediction of response to treatment, represent clinically meaningful outcomes but are yet not supported by validated biomarkers. Furthermore, especially for the application of biologics in chronic rhinosinusitis with nasal polyps non-invasive sampling can reveal meaningful information about type and severity of inflammation and thus may help to tailor an appropriate treatment strategy.

A comprehensive overview of upper airway biomarkers that are non-invasively accessible in nasal secretions were previously summarized and are shown in the figure (Figure 1)[17,18].

According to the united airway concept, upper and lower airways are in connection to each other and share many similar inflammatory responses. As a practical consequence of this, upper airway biomarkers may be supportive to diagnose processes of the lower airways. Indeed, studies using optimized protocols and selected biomarkers such as IL-24 show a moderate to strong correlation between upper and lower airways using nasal secretions and induced sputa [17]. Moreover, nasal CCL-26 is significantly correlated with sputum CCL-26 (and IL-13 and IL-5) levels, which in turn is linked tightly with airway eosinophilia [17]. Minimally invasive sampling using nasosorption® and sputosorption® have offered some promise in providing surrogate markers of eosinophilic asthma, though these techniques require further study [43]. They are based on adsorptive materials, which allow efficient recovery of analytes (Table 1). Whether nasal secretions may therefore have the potential to replace invasive eosinophil counts, requires verification.

In the context of non-invasive diagnosis, local nasal inflammatory responses to airborne stimuli are particularly important and may be even more sensitive than systemic responses. In support of this assumption, human nasal mucosal C-reactive protein (CRP) responses after the inflammatory stimulus of a 6h-inhalation of ultrafine welding fume particles were found to be not only highly correlated to systemic CRP responses, but also showed increased and earlier reactivity [44]. Moreover, nasal levels of human ST2 and IL-5 were more strongly upregulated than systemic levels after a single nasal allergen challenge [45]. Furthermore, nasal periostin was clearly

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more discriminatory for asthma than systemic periostin, and the same was true for IgE [46]. In addition, potentially pathogenic bacteria were identified by DNA-PCR test in nasal samples of children with chronic cough, enabling specific antibiotic treatment [47]. To enable reliable and reproducible detection of nasal inflammatory responses, standardization of collection materials, elution and laboratory protocols as well as storage and temperature conditions[512] is required [48].

Saliva and tear fluid

Saliva DNA methylation may represent a helpful tool to mirror associations between wheezing and asthma development, as PM20D1 hypermethylation was reported to be associated with early childhood wheezing.[49] Tear sampling is quite difficult, especially if not preceded by a positive conjunctival allergen challenge. Therefore, tear sampling is not within the scope of this position paper

Urine

Urine would represent an ideal matrix for the non-invasive sampling and monitoring of allergic diseases and asthma. Promising preliminary findings from research studies suggest that there may be a place for routine urine sampling in asthma in the future, particularly for the measurement of eicosanoids and their metabolites.

Urinary LTE4 is the stable metabolite of LTC4 and LTD4 and thus represents a biomarker of increased Cys-LT activity [50,51]. Sources of LTE4 include many inflammatory cells types of the upper and lower airways such as eosinophils, mast cells, basophils, macrophages, platelets, and neutrophils [52,53]. Increased urinary LTE4 excretion has been demonstrated in allergen-induced asthma, asthma exacerbations, aspirin challenge, and increased basal excretion in AERD [54-57]. Furthermore, it may represent a biomarker of the inflammatory state in chronic rhinosinusitis, but comorbid asthma and atopy can also contribute to elevated urinary LTE4 levels [58]. Several drugs are able to block Cys-LT synthesis significantly decreasing urinary LTE4 levels [59], whereas corticosteroids do not affect urinary LTE4 [60].

A recent study showed that concentrations of urinary adenosine are complementary to peripheral airway NO in diagnosing pediatric exercise-induced bronchoconstriction reflecting poor asthma control [61]. Another class of mediators that may be measured in the urine is

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eosinophil-derived proteins such as EDN. A recent meta-analysis taking into account 27 pediatric studies conducted over a period of 20 years concluded that over 70% of these studies revealed an association between eosinophil-derived proteins and childhood asthma, thus suggesting a diagnostic role for eosinophil-derived protein measurements [62].

miRNA

MicroRNAs (miRNAs) are small non-coding RNA molecules that are 18–22 nucleotides long and play an important regulatory role in numerous immunological and inflammatory disorders, including allergic diseases (reviewed in [63]). Due to their surprisingly high stability, miRNAs may represent potential biomarkers for allergic diseases. In patients with allergic rhinitis, serum miR-181a levels were reduced, which correlated with disease severity and Th2 cytokines [64]. In addition, local miRNAs in extracellular vesicles isolated from the supernatant of nasal mucus from allergic rhinitis patients showed significant differential expression in comparison to healthy controls [65]. In allergic asthma, a combination of five serum miRNA ratios (miR-21-5p/miR-15a-5p; miR-27a-3p/miR-15a-5p; miR-29c-3p/miR-15a-5p; miR-223-3p/miR-425-5p; miR-15a/miR-342-3p) has been described as a reliable biomarker signature, which correlated well with clinical characteristics such as lung function and therapy regimen [66]. Of note, the use of ratios compensates substantial variation in miRNA levels and contributes to normalization of the result. In addition, single nucleotide polymorphisms in serum pre-miRNAs has been described in connection with the development of allergic asthma [67].

Microbiome

While gut and skin microbiome are obvious biomarker sources for allergy diagnosis (please see below), also the airways are colonized at least in the upper airways (reviewed in [68]), however the diagnostic value is still under investigation.

Non-invasive and minimally-invasive diagnostics of skin diseases

Atopic dermatitis usually begins during the first 6 months of life. It often precedes allergic rhinits and asthma (atopic march) [69], but can also spontaneously resolve. Non-allergic skin diseases other than atopic dermatitis such as systemic sclerosis, SLE, lupus erythematosus and psoriasis

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are beyond the scope of this manuscript. Due to its accessibility, non-invasive diagnosis in skin has a longstanding tradition and even in times of big data and molecular pathology, the eye of a dermatologist is one of the most important diagnostic tools. The visual diagnosis is supported today by several invasive techniques (punch biopsies, histology, lesional gene expression) and more and more non-invasive or minimally invasive methods that are summarized in this section.

Non-invasive methods to assess skin functionality

Type-2 inflammation is associated with barrier dysfunction, but also with alterations in the skin's innate immune function; therefore, the assessment of skin function can play an important role in the diagnosis and clinical management of atopic dermatitis. One hallmark of a disturbed epidermal barrier is *dry skin*, that occurs due to an increased loss of water. This transepidermal water loss (TEWL) can be measured with a Tewameter that detects the rate of evaporation (Table 1). TEWL measurements are often used to determine the grade of barrier disruption in eczema and most importantly to monitor barrier restoration under therapy [70]. *Hydration* of the skin can be also measured using corneometry. Here, the skin serves as dielectric medium and the capacitance is measured as indicator of stratum corneum thickness and water content. Skin hydration represents not an indicator for barrier function, but is an indicator for injury, metabolic alterations and effective topical treatment. Skin vascularization can be measured by laser Doppler perfusion imaging [71]. Currently it is explored whether histamine pharmacodynamic response phenotypes can be distinguished by this technique [72].

Another skin parameter, *skin elasticity*, can be measured with a Cutameter that uses negative pressure to aspirate a skin area. This technique is used to evaluate the skin stiffness in scleroderma and systemic sclerosis [70,73]. In addition, *sebum lipids* can be quantified by sebumetry where a semi-transparent tape is applied to the skin that turns transparent after contact with sebum. The level of transparency can then be quantified and indicates the overall lipid content of skin. The exact lipid composition can then be identified by down-stream analyses such as mass spectrometry to provide information on disease-specific lipid alterations [74]. The pH level is another parameter of the skin barrier and is increased in situations of infection [75].

Minimally-invasive techniques to obtain skin samples

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Tape-stripping is a well-established technique to obtain information on the outermost layer of the skin, the stratum corneum. Here, adhesive tapes are applied with a bit of pressure on the skin and removed again to generate an outside-inside view on skin layers (Table 1). The tapes can then be used to analyze mRNA, DNA or proteins and lipids. Whereas the biggest advantage of tape-stripping is its low invasiveness, the disadvantage is the standardization of sampling and the leakage of serum proteins on the tapes in diseases with disrupted epidermal barrier. In addition, human skin is rich in RNAses and molecular diagnostics on expression of disease-specific genes has to be thoroughly validated to avoid negative results due to RNA digestion [76]. In contrast to RNA analysis, protein sampling by tape-stripping is robust. Here, mechanistic insights have been obtained for psoriasis and eczema-associated proteins, anti-microbial peptides and also itch-associated proteins such as neurotrophic nerve growth factor (NGF) and IL-31 [77,78].

Scraping the skin surface with a surgical blade is another method to obtain minimally-invasive skin samples without inducing a scar, but this method has also difficulties in being standardized [79]. Proteins and mRNA can also be obtained furthermore from the interstitial fluid of blisters that have been induced by applying a vacuum (suction blister). Compared to tape-stripping, the amount of proteins and mRNA is much higher in blister fluids [80], however, this method is very time consuming and therefore not suited for clinical routine diagnostics.

Punch biopsies deliver, without any doubt, the maximum of information concerning gene expression, protein, content, tissue architecture and composition of inflammatory infiltrates. However, with diameters of 2-4 mm, local anesthesia and the requirement of sutures, patient's acceptability is low and samples cannot be obtained from children. The development of microbiopsies with a diameter less than 0.5 mm can overcome these disadvantages of common punch biopsies and allows profound RNA and DNA analysis from lesional tissue, even in babies [81]. On the basis of mRNA extracted from the lesional skin biopsies it is possible to reliably distinguish atopic eczema from psoriasis in case where visual anamnesis fails (e.g. on the scalp or hands). Specifically, abundant expression of iNos in the biopsies identifies psoriasis and is low in atopic eczema, whereas CCL27 is low in psoriasis and high in atopic eczema [82,83]. Furthermore, symptoms that occur in multiple skin diseases, such as acanthosis, can be defined on inflammatory mediators such as IL-17 that could be specifically treated with biologics [84].

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New developments in non-invasive skin diagnostics

Probably the most fascinating development in non-invasive skin diagnostics are small microfluidic chips or patches that are applied onto the skin and measure a panel of parameters, such as pH, volume of sweat, lactate, glucose and chloride. Such chips/patches can thereby rapidly deliver information on the level of skin, electrolyte levels, hydration and even markers for cystic fibrosis [85,86]. These chips/patches do not need batteries as they gain their energy from radio waves that are emitted from electronic devices and directly transmit the information to analysis apps. Moreover, electrical impedance spectroscopy has been identified as a rapid and reliable diagnostic tool to detect skin barrier defects, which have been associated with many skin inflammatory disorders such as atopic dermatitis [87]. Together with the aforementioned artificial intelligence (AI) algorithms used for picture diagnosis, these non-invasive techniques will revolutionize diagnostics, not only in skin.

Urine

In atopic dermatitis, aquaporin-2, EDN and LTE4 are elevated in the urine [88,89 90]. Also indicators of oxidation such as nitrate and 8-hydroxydeoxyguanosine (8-OHdG) are elevated in urine of AD patients [91].

miRNA

The serum levels of four miRNAs, including miR-21-5p, were reported to be elevated in experimental allergy models [66]. miRNAs were also found in human breast milk and were associated with early development of AD in the offspring [92]. In addition, miR-203a-3p, measured in the urine of children with AD, can be considered as a biomarker for the degree of inflammation and additional atopic diseases [93]. Moreover, miR-146a-5p [94] and miR-720 [95] were found to be increased in keratinocytes from AD patients and increased serum levels of miR-483-5p [93] and miR-155-5p were described in AD [96]. The latter suppresses CTLA-4 that otherwise has a suppressive effect on T cells. Whether promising systemic miRNAs could also be used in a non- or minimal-invasive manner using DBS, has yet to be explored. The location of some of described miRNAs in breast milk, skin and urine basically paves the way for non-invasive sampling and diagnosis of specific allergic diseases.

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Microbiome

The composition of the microbiota of the skin can also be analyzed by (bacterial) 16S ribosomal DNA [97] or by metagenomics [98]. Today we understand that compositions of the cutaneous microbiota are also determined by the local nature of the skin, and as such cebaceous, oily, moist and dry habitats have been defined [99]. Microbiota of the skin shape the barrier and immunity of the skin; inversely, and barrier and cutaneous immunity determine the composition of the cutaneous microbiota [100]. Early colonization with commensal *Staphylococci* at two months of age lowers the risk of atopic dermatitis at one year, indicating that microbiota analysis has the potential to be used as a biomarkers of disease development [101]. Together with imaging data, functional analysis of the skin, and minimally invasive sample analysis, these analyses offer a holistic view of barrier dysfunction.

Non-invasive and minimally-invasive diagnostics of food allergy, food intolerance and other gastrointestinal diseases

Due to the inaccessibility of the organ, diseases associated with gastrointestinal inflammation are a diagnostic challenge, with the need for invasive diagnostic procedures. In food allergy, which commonly presents in childhood, accurate non-invasive diagnostics and its discrimination from inflammatory or functional bowel diseases would represent a major advantage. Moreover, the physiological conditions of the gastrointestinal tract associated with changing intraluminal pH levels and the presence of a large variety of enzymes and microorganisms impacts on the stability of available potential biomarkers. Liquid biopsies represent a promising approach, thus far especially in advanced cancer diagnosis [102]. Diagnostic and prognostic biomarker characterization is still limited in food allergy [103] and blinded placebo-controlled oral food challenges remain the gold standard for diagnosis of food allergy.

Intestinal biomarkers

For non-invasive gastrointestinal diagnostics, stool samples containing inflammatory mediators, specific antibodies or markers associated with disease mechanisms may be used. Even though IgE is known to be present in stool samples of food sensitized patients [104], its value in food allergy diagnosis remains to be determined. In gastroenterological routine evaluation of patients with

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inflammatory bowel disease (IBD), fecal calprotectin (S100A8/S100A9 dimer), which is indicative for neutrophils, plays an essential role for monitoring treatment efficacy and remission of inflammation. Of interest, fecal calprotectin levels were repeatedly reported to be elevated in food allergic children [105,106], but their diagnostic value as a biomarker in IgE-mediated food allergy still needs to be confirmed. Calprotectin is an antimicrobial peptide of the S100 family and is expressed by a large number of cells including monocytes and granulocytes and therefore represents a general inflammation marker (reviewed in [107]). Calprotectin measurements were suggested to be utilized for discrimination between allergic and non-allergic intestinal inflammation. For example, in non-IgE mediated cow's milk allergy, fecal calprotectin levels were significantly elevated in children with positive oral food challenge results even under elimination diet suggesting an ongoing intestinal low-grade inflammation [108]. In irritable bowel syndrome (IBS) patients with food hypersensitivities showing a positive oral food challenge after elimination diet, ECP and tryptase levels in fecal samples were found to be significantly higher compared to non-responders in challenge tests [109]. As serial serum ECP measurement has proven diagnostic value in eosinophilic esophagitis (EoE) [110], it is tempting to speculate that serial fecal testing could be an non-invasive alternative since ECP was described to be relative stable even under conditions of gastric acids [111]. In infant non-IgE mediated cow's milk allergy, fecal levels of another eosinophilic granule component, the eosinophil derived neurotoxin (EDN), showed the highest disease specificity (besides intestinal permeability tests) compared to all other determined parameters [112]. This knowledge might be useful for future definition of a minimally-invasive diagnostic panel for eosinophilic gastrointestinal diseases. For EoE sponge or string sample collection, as well as breath condensates are new methods under investigation that could replace endoscopic evaluations in the future [113]. The fraction of FeNO was proposed to prospectively correlate with peanut oral food challenge outcome if assessed together with Ara h2-specific IgE antibodies [114]. This correlation shows a high reproducibility in repeated measurements even after 1 year intervals [115]. However, another study performed in cow's milk allergic children did not observe a difference regarding FeNO levels in children with positive oral food challenge tests and in children without clinical reactions [116].

Although non-invasive biomarkers for food allergy are still in the early phase, the need to replace the troublesome oral food challenge is very high.

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Urine

Food allergy has been associated with an apparent defect of the intestinal barrier function, which may in turn be associated with the flux of immunologically intact allergens across the intestinal epithelium. The carbohydrate uptake quantified in urine for measurement of intestinal permeability has high relevance in food allergy (and in IBD, IBS and colon cancer) [117]. Taken together, promising urinary biomarker candidates are already known and require further validation for allergic diseases.

miRNA

While various miRNAs play a role in diabetes mellitus, there are hardly any miRNA studies on food allergy or food intolerance. No differences in the miRNA expression profile of mast cells were found in a single publication on peanut allergy [118]. However, (extracellular) blood levels of miR-150 and miR-342-3p [119], but also of miR-106b-5p, miR-26a-5p and miR-29b-3p [120] were increased in IBS patients. miR-150-3p and -5p are said to be involved in inflammatory processes, whereas miR-342-3p plays a role in intestinal motility and the control of smooth muscle cells [119]. In addition, miR-490-5p plays a role in the mast cells in patients with the same clinical picture [121].

Microbiome

Specific fecal microbiota and metabolite signatures were suggested as early diagnostic markers [122] thus far in colon cancer. Preliminary specific microbial signatures have also been suggested for the diagnosis of food allergy [123] potentially enabling non-invasive diagnosis. For further investigation of microbial pattern, large-scale studies considering standardized pre-analytic sample preparation are required.

Non-invasive and minimally-invasive diagnostics of systemic allergies and anaphylaxis

Anaphylaxis, the most severe manifestation of an IgE-mediated allergic reaction, is an acute emergency [124]. The diagnosis is often straightforward based on clinical manifestations and exposure to a potential triggering agent. However, the diagnosis may be difficult when presentation is atypical (e.g. skin symptoms are absent in about 20% of patients), clinical

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development is more gradual, and/or for health care workers who are not familiar with the presentation [125]. Thus, there is a high need for validated biomarkers that can be used to identify individuals at high risk for anaphylaxis, as well as for rapid confirmation of the diagnosis upon presentation to an urgent care facility.

Current biomarkers of anaphylaxis

Biomarkers in the blood, urine or in other tissue fluids are already available for anaphylaxis such as plasma histamine (or its metabolite, methylhistamine in urine) and total serum mast cell tryptase [126]. The rationale to use these mediators for diagnosis is based on the fact that tryptase and histamine are released from cellular granules upon activation [127]. The available diagnostic tryptase assay measures all forms of circulating tryptase. After anaphylaxis, levels are increased from 15-30 min to several hours after onset. As mature tryptase has to diffuse from the tissue to the blood, elevation of serum tryptase may not be detectable during the first 15 to 30 minutes [128].

Need for novel biomarkers and experimental biomarkers in anaphylaxis

The tryptase determination is the current gold standard for anaphylaxis diagnosis however, with limited sensitivity. Therefore, sequential analysis is necessary in mild forms of food anaphylaxis, where levels are low with the caveat that baseline samples are not available[129]. The assay is laborious, has no immediate result and difficult to realize in 'point of care' settings.

In the field of metabolomics and systems biology, novel early biomarkers of anaphylaxis may be identifiable using highly sensitive techniques such as matrix-assisted laser desorption/ionization (MALDI) in studies with well-phenotyped patients. Also, peripheral blood cell transcriptomics have been analyzed in patients with positive oral food challenges and potential signatures of six genes (LTB4R, PADI4, IL1R2, PPP1R3D, KLHL2, and ECHDC3) have been identified [130].

Urine

After anaphylaxis increased urinary concentrations of mast cell carboxypeptidase A3, chymase, cytokines, platelet-activating factor, CCL-2, and prostaglandins or sulfidoleukotrienes were found [131,132]. A multiplex approach in patients with anaphylaxis may identify further biomarker candidates using a small amount of serum and urine.

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miRNA or microbiome

Currently both miRNA or microbiome were not explored as biomarker for anaphylaxis risks. However, miRNA-155 was shown to control mast cell activation and anaphylaxis in a mouse model [133] and may therefore offer future options of diagnostic characterization of anaphylaxis.

Outlook and EAACI position

The article highlights multiple opportunities that allow non- or minimally-invasive diagnosis of allergic diseases. The Covid-19 pandemic illustrates that the distinction of allergic diseases from other diseases such as viral infections is a key demand in medicine. As viral airway diseases such as Covid-19 affects the airway epithelium and thus the same organ and the same cells as it is the case in allergic airway diseases, these could potentially also be diagnosed with the same technology, which deserves further research. Most of the recently developed techniques along with the steadily increasing number of cytokine, chemokine, lipid mediator and miRNA biomarkers shows that the diagnosis and management of allergic diseases could be revolutionized in the coming years. In addition, the systematic use of multiple omic technologies combined with machine learning or artificial intelligence (reviewed in [134 135 136]) paves the way for further opportunities of non-invasive diagnosis in allergy. Examples for progress in this area are the discovery of DNA-methylation pattern in food allergy [137], or IgE pattern in large cohorts [138]. Moreover the assessment of multi-omics analysis in the U-BIOPRED cohort [139] and even in multi-cohort analysis of [140] have demonstrated the potential of computer-aided omic analysis. These future biomarkers are important drivers for precision medicine in the field of allergic diseases. To demonstrate the robustness of each biomarker and their predictive power, the task force strongly recommends support of research and innovation investments for sampling of well-defined patient cohorts to enable head-to-head testing, in order to make biomarker tests available for precision prevention and treatment of allergic diseases.

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Figure Legend

Figure 1 summarizes the five main matrices of non-invasive diagnosis. Excretion such as urine and faeces are promising sources for food allergy diagnosis, while breath exhalates and nasal secretions (mucosal lining fluids) are key for airway diseases. Serum and microbiopsies are more universal matrices, however usually more invasive to obtain than the other matrices. In the inner circle, the cellular sources of biomarker are depicted, while the outer circle shows typical analytes implicated for the respective matrices. An excerpt of biomarkers discussed in the article is shown.

Table 1 Legend

The table 1 is summarizing the key methodologies used for the none- and minimally-invasive diagnosis of allergic diseases.

Suppl. table 1 Legend

The table displays current technology platforms available for diagnosis of allergy.

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Indication	Device / Tool or methodology	Description
Allergic airway diseases: Allergic rhinitis, Asthma	**Dried blood spots**	Dried blood spots (DBS) allow sampling of capillary blood micro samples in very small volumes (±10 µL).
	Nasal absorption sampling	Nasal absorption sampling allows the collection of nasal lining fluids (±40 µL) and determination of epithelial cytokines in a standardized manner.
	Nitric oxide analyzers	Nitric oxide analyzers for the determination of exhaled fraction nitric oxide (FeNO) level.
	CT-imaging	CT-imaging technology allows to identify intra- or extra-thoracic airway thickening.
	eNoses	eNOSEs detect specific volatile organic compounds (VOCs) without identifying their molecular entities. Other eNoses detect the specific molecular entities using LC-MS/MS but this currently requires sending samples to central laboratories.
Skin (Atopic dermatitis)	**Tewameter**	TEWL measurements are often used to determine the grade of barrier disruption in eczema by determination of the rate of evaporation.
	Cutameter	Cutameter measure *skin elasticity* to evaluate the skin stiffness in scleroderma and systemic sclerosis.
	Sebumetry	Sebumetry is using a semi-transparent tape which is applied to the skin that turns transparent after contact with sebum for the quantification of the overall lipid content of skin. Potential down-stream analyses such as MS to determine disease-specific lipid alterations.
	Corneometry	Corneometry allows the measurement of the hydration of the skin as indicator of stratum corneum thickness and water content as an indicator for injury, metabolic alterations and effective topical treatment.
	Microfluidic chips or patches	Microfluidic chips or patches on the skin allow the measurement of pH, volume of sweat, lactate, glucose and electrolyte levels.
	Tape-stripping	Tape-stripping is a well-established technique to obtain information on the outermost layer of the skin, the stratum

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		corneum. The tapes can then be used to analyze mRNA, DNA or proteins and lipids.
	Scraping the skin surface	minimally-invasive skin samples without inducing a scar
	Electrical impedance spectroscopy	Electrical impedance spectroscopy allows the detection of skin barrier defects, which have been associated with many skin inflammatory disorders such as atopic dermatitis.
	Suction blister	Proteins and mRNA can also be obtained furthermore from the interstitial fluid of blisters that have been induced by applying a vacuum (suction blister).
Food allergy	**Urine**	Excretion for monitoring of allergic diseases and asthma by measurement of e.g. eicosanoids and their metabolites.

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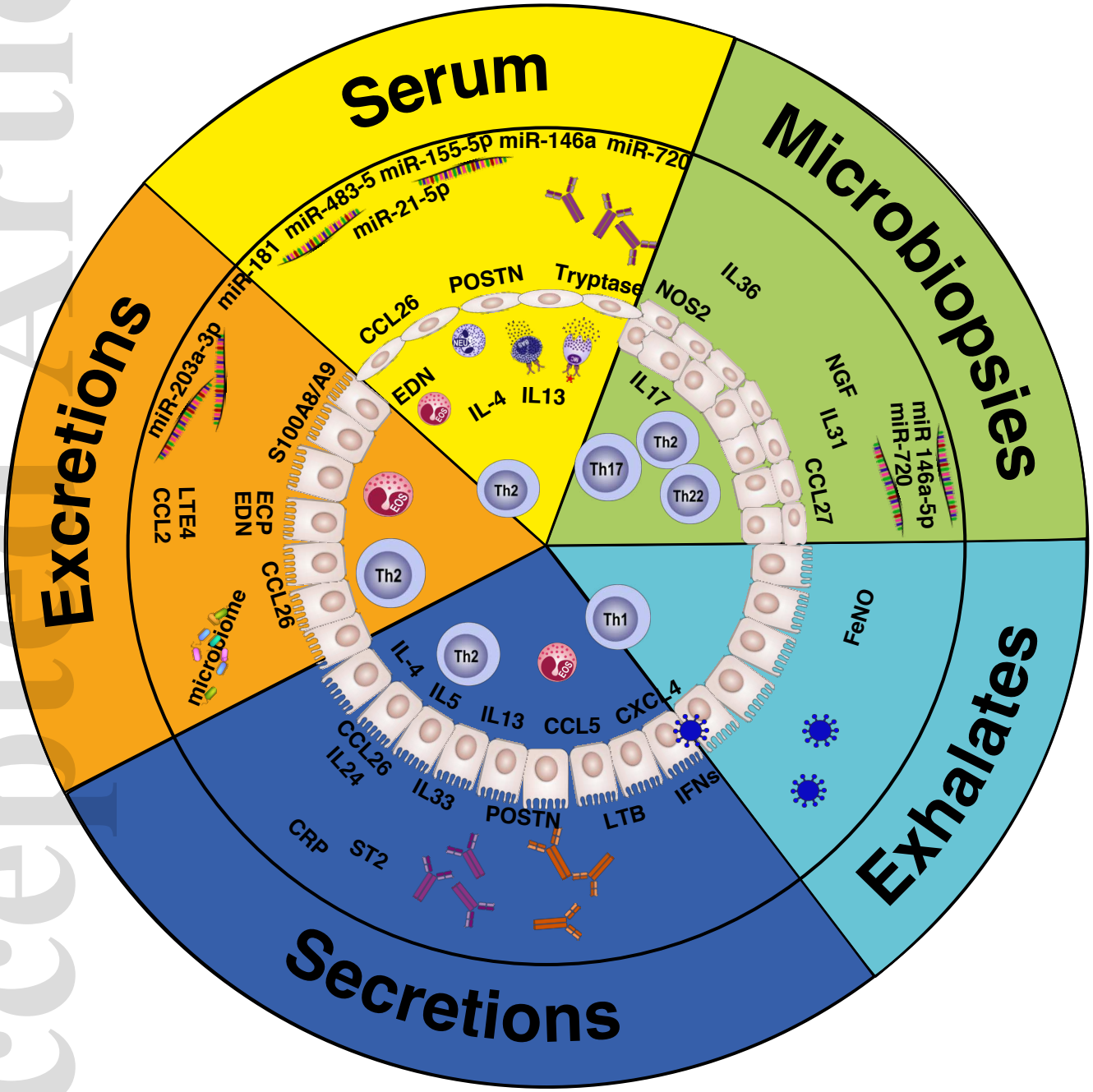

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Suppl. Table 1

Fully automated laboratory systems (single tests)			
	Products	Technology Overview	Assay type
Hycor Biomedical Inc.	HYTEC 288™	288 tests per run (>500 allergens available), random access analyzer	Automated solid phase, colorimetric (EIA)
	NOVEOS™	150 allergens on board, 700-1200 tests per run, batch analyzer	Automated liquid phase, chemiluminescence (CLIA)
Siemens Healthineers	3gAllergy™, IMMULITE™ 2000 XPi Immunoassay System	>450 allergen extracts incl. 60 allergen components. 200 tests/hour. Continuous random access analyzer.	Automated chemiluminescence immunoassay (CLIA)
Thermo Fisher Scientific	ImmunoCAP™, Phadia™ Laboratory Systems	>500 allergen extracts incl. >100 allergen components. From 60–240 tests/hour. Continuous random access and batch analyzers.	Automated fluorescence enzyme immunoassay (FEIA)
Microarray tests (molecular allergology)			
Macro Array Diagnostics GmbH	ALEX^2	Microarray-based assay based on bead technology. >120 allergen extracts and 170 allergen components.	Solid-phase immunoassay
Thermo Fisher Scientific	ImmunoCAP™ ISAC	Microarray-based immunoassay. Semi-quantitative assay for sIgE to 112 allergen components.	Fluorescence immunoassay (FIA)
Screening tests (molecular allergology)			
Thermo Fisher Scientific	Phadiatop™, Phadiatop Infant	Semi-quantitative assay for identifying atopic individuals. Mixtures of allergens for assaying in children and adults; and infants.	Automated FEIA
Euroimmun AG	Euroline Atopy Screen	54 allergens per assay. Semi-quantitative.	EIA (line blot)
	Allercoat 6 ELISA	25 allergens per assay. Quantitative determination of sIgE. >600 allergens and allergen mixtures.	ELISA
	Defined Partial Allergen Diagnostics (DPA-Dx)	53 allergen components per assay.	EIA (line blot)
Hitachi Chemical Diagnostics	CLA Allergen-Specific IgE Assay	36 allergens. 3 panels. Manual assay.	EIA (cellulose threads)
	OPTIGEN Allergen-Specific IgE Assay	36 allergens. 3 panels. Semi-automated assay.	EIA (multi-well)
LG Chem	AdvanSure AlloScreen	20-31 allergens per panel. 5 panels. Semi-quantitative assay.	EIA (line blot)
Proteome Tech Inc.	PROTIA Allergy-Q	134 allergens. Quantitative assay.	EIA (line blot)
R-Biopharm AG	RIDASCREEN Spec. IgE	42 allergens. Semi-quantitative assay. Cellulose discs.	EIA (discs)
Biocheck GmbH	POLYCHECK	6–30 allergens per panel/assay. 44 panels.	EIA (line blot)
HOB Biotech Group	BioLINE Allergy	20 allergen per panel. 10 panels. Semi-quantitative assay.	EIA (line blot)
Immunodiagnostic Systems Holdings PLC	IDS Allergy	60 allergens. Quantitative assay.	EIA
Immunolab GmbH	Immuno^{LINE}	20 allergen per panel. 10 panels. Semi-quantitative assay.	EIA (line blot)
Arlington Scientific, Inc.	IVT Allergy 3 Screen	10 allergens per panel. 8 panels. Qualitative assay.	EIA (line blot)
Mediwiss Analytic GmbH	AllergyScreen	20 allergens per assay. Quantitative sIgE assay.	EIA (line blot)
	AlleisaScreen	30, 44 or 60 allergens per assay. Quantitative sIgE assay.	EIA (line blot)
Point of care			
Abionic SA	abioSCOPE	Total IgE and specific IgE to 5 perennial allergens. Results in 5 mins.	Nanofluidic technology
Bluejay Diagnostics	Allereye Tear Total IgE Test	Total IgE in tear fluid. Results within 10 minutes.	Strip format
DiaSys Diagnostic Systems GmbH	QDx Allerg	12 allergens per test. Rapid semi-quantitative ELISA test. Results in 30 minutes.	ELISA
Diagn. Systeme & TechnologienGmbh	FastCheckPOC 20	20 allergens. Semiquantitative test. Results in 30 minutes. For capillary blood.	Lateral flow
Dr. Fooke Laboratorien GmbH	ALFA (Allergy Lateral Flow Assay)	8 allergens. Qualitative test for whole blood, serum or plasma. Results in 20 minutes.	Lateral flow
Human GmbH	Hexagon IgE	Total IgE test for serum. Results in 15 minutes.	Lateral flow
Microtrace	MyCare kit	Tests for sIgE to dust mite, dog and cat. Results in 10 minutes.	Lateral flow
Novartis	Niji	Total IgE test using capillary blood. Results in 12 minutes.	Lateral flow
Omega Diagnostics Group PLC	Allergodip	9 allergens per panel. Semi-quantitative test for serum or plasma.	EIA (dipstick)
Thermo Fisher Scientific	ImmunoCAP Rapid	10 allergens. Semiquantitative test. Results within 20 minutes. Wheeze/rhinitis and asthma/rhinitis panels.	Lateral flow