

Allergen screening bioassays: recent developments in lab-on-a-chip and lab-on-a-disc systems

Allergies occur when a person's immune system mounts an abnormal response with or without IgE to a normally harmless substance called an allergen. The standard skin-prick test introduces suspected allergens into the skin with lancets in order to trigger allergic reactions. This test is annoying and sometimes life threatening. New tools such as lab-on-a-chip and lab-on-a-disc, which rely on microfabrication, are designed for allergy testing. These systems provide benefits such as short analysis times, enhanced sensitivity, simplified procedures, minimal consumption of sample and reagents and low cost. This article gives a summary of these systems. In particular, a cell-based assay detecting both the IgE- and non-IgE-type triggers through the study of degranulation in a centrifugal microfluidic system is highlighted.

An **allergy** is a hypersensitivity disorder of the immune system. Allergies can be caused by a variety of normally harmless substances called **allergens**, such as pollens, molds, insects, drugs, food and environmental pollutants. 'Allergy' is derived from the Greek words '*allos*' (different or strange) and '*ergos*' (activity or reaction). The word was first used in 1906 by Clemens Freiherr von Pirquet (1874–1929), an Austrian scientist and pediatrician, to describe both the beneficial protection and the hypersensitivity in patients after the second injection of horse serum. von Pirquet also suggested that the word 'allergen' should be used to describe the agent that induced the 'changed reactivity', such as skin rashes or lymph node swellings, which occurred after one or more injections [1]. Although the word 'allergy' is no longer used as originally intended to describe both protection and hypersensitivity, von Pirquet's concept of 'changed reactivity' laid the foundation of modern immunology's understanding of allergies.

Allergic response & its mechanism

An allergy is a serious and often life-threatening health disorder affecting all age groups. The incidence and prevalence of allergic dis-

eases have increased dramatically in recent decades. According to a survey conducted by the WHO in 2004, approximately 22% of the global population suffered from some form of allergic disease [2]. The symptoms of allergies are characterized by tissue- or organ-specific effects, including constriction of airways in the lungs (asthma), reactions in the gut (food allergic reactions), 'wheal-and-flare' reactions in the skin (atopic dermatitis or eczema) and vascular collapse in severe cases (anaphylaxis).

Mechanistically, an allergy is mediated by the soluble factors released from Th2 lymphocytes and the IgE antibody released from B cells in an allergen-specific manner. Th2 cells form a subpopulation of helper T cells that normally provide host protection against extracellular pathogens, such as bacteria and parasites. In pathological conditions, overactivation of Th2 cells often leads to an allergy. In the first phase of an allergy, known as allergic sensitization, uptake of an allergen causes the activation and expansion of allergen-specific Th2 cells for the secretion of interleukins (e.g., IL-4, IL-5 and IL-13), which are used for the IgE antibody production. Among the five antibody classes in the blood, IgE (identified in 1967) has the shortest half-

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Key Terms

Allergy: A hypersensitive response to an antigen called an allergen as a result of the production of the IgE antibody against that allergen in a highly specific manner.

Allergen: A normally harmless substance such as a milk protein that induces an abnormal immune response.

IgE: Cross-linking of the antigen binding sites of IgE antibodies bound to the cell surface Fc receptors on mast cells or basophils by its allergens releases soluble mediators from cellular granules, leading to the development of allergic symptoms.

Lab-on-a-chip: A compact device of several square centimeters in size that brings several laboratory operations in one microarray for various biomedical applications, such as allergy testing.

life (2 days) and lowest concentration – normally 0.1 µg/ml. However, its concentration in the blood can be increased tenfold in patients with an allergy or a parasite infection. The biological events leading to allergies are mediated primarily by the binding of allergens to the antigen-binding sites of the IgE antibody, and the binding of the IgE Fc region to the high-affinity IgE Fc receptors (FcεRI) on the cell surface of mast cells or basophils (Figure 1). An FcεRI not occupied by IgE has a short half-life of 1 day, whereas receptors that are IgE bound appear to be kept for the rest of the life of the cell, and these cells are called sensitized mast cells and basophils (Figure 1) [3].

Within minutes of contact with the same allergen in a subsequent exposure (i.e., after sensitization), the allergens cross-link adjacent IgE molecules that are bound to the FcεRI on the cell surface of the sensitized mast cells and basophils. The cells then degranulate and release both the preformed (e.g., histamine) and newly synthesized mediators (e.g., prostaglandins, leukotrienes and platelet-activating factor) by exocytosis for the early allergic response. These mediators promote vascular permeability, smooth muscle contraction and mucus production, leading to different allergic symptoms (Figure 1) [4].

Despite enormous efforts made to improve our understanding of the molecular mechanism of allergies, this knowledge has not been fully translated into new treatment protocols. Currently, the treatment strategy for allergies involves using cell-permeant small molecules (e.g., R-788, PCI32765 and TF002) to block the signal transduction pathways in the mast cells and basophils after their exposure to allergens. Alternatively, one can eliminate IgE in the blood by using the IgE-specific human monoclonal antibody omalizumab (licensed in the USA) [5]. As only 200–500 FcεRI bindings out of 250,000 receptors/basophils are required for meaningful degranulation and secretion to occur [6], the sensitivity of cells to stimuli becomes the major issue for this treatment approach.

Allergy testing

It is said that an ounce of prevention is worth a pound of cure. Avoiding exposure to known allergens is still the best way to treat an allergy. Therefore, finding out what patients are allergic to is important. Diagnosis of an allergy begins with a comprehensive physical examination and a clinical history. Once symptoms are matched with an allergic disorder, a blood test or skin test for IgE and allergens can be conducted in order to provide confirmation of the diagnosis. Classical allergy tests to identify allergens are the skin-prick test and the patch test. The skin-prick test is a primary diagnostic tool that introduces a number of testing allergens to the skin in order to identify which allergens the patient is sensitive to. A sterile lancet is used to press a drop of solution with the allergen extract through the skin without inducing bleeding to allow the allergen to penetrate into the epidermal layer. A histamine is also used as a positive control to exclude negative findings that are the result of the test subject taking interfering medications, such as glucocorticosteroids. Normally, a wheal with a diameter ≥3 mm will be seen at the site of the positive control and that of the real allergens 15–20 min after pricking [7]. Remarkably, the interpretation of the results of the skin-prick test requires experience. For example, medications such as antihistamines can interfere with the results. A woman's menstrual cycle also affects the outcome of the test [8]. Moreover, life-threatening anaphylaxis occurs from time to time in patients who take the skin-prick test [9]. For this reason, the test should be performed by trained medical practitioners who can provide resuscitation in case of emergent anaphylaxis. Furthermore, the positive reactions in a skin test cause extremely uncomfortable and annoying sensations. These shortcomings indicate the need for new assays for allergy testing.

Unlike the skin-prick test, the patch test does not use lancets. Instead, a suspected allergen is applied to an adhesive tape that is then placed on the skin of the patient for 48 h. For the patch test, 20–30 patches with different test substances (e.g., latex, fragrances or textile dyes) are applied to the upper back of the patient. This test indicates contact dermatitis if the skin shows a rash, thickening or swelling after contact with the testing agent. Unlike the skin-prick test, the patch test only detects contact dermatitis, which is primarily mediated by the delayed-type hypersensitivity through the overactivation of helper T cells, but not IgE [10].

Other allergy tests, such as fluoroenzyme immunoassay (FEIA) or ELISA, are currently conducted in clinical laboratories. They measure the total amount of IgE in the blood. In the FEIA or ELISA, a capturing antibody specific to the Fc region of IgE is immobilized on a solid surface and is used to capture the IgE from

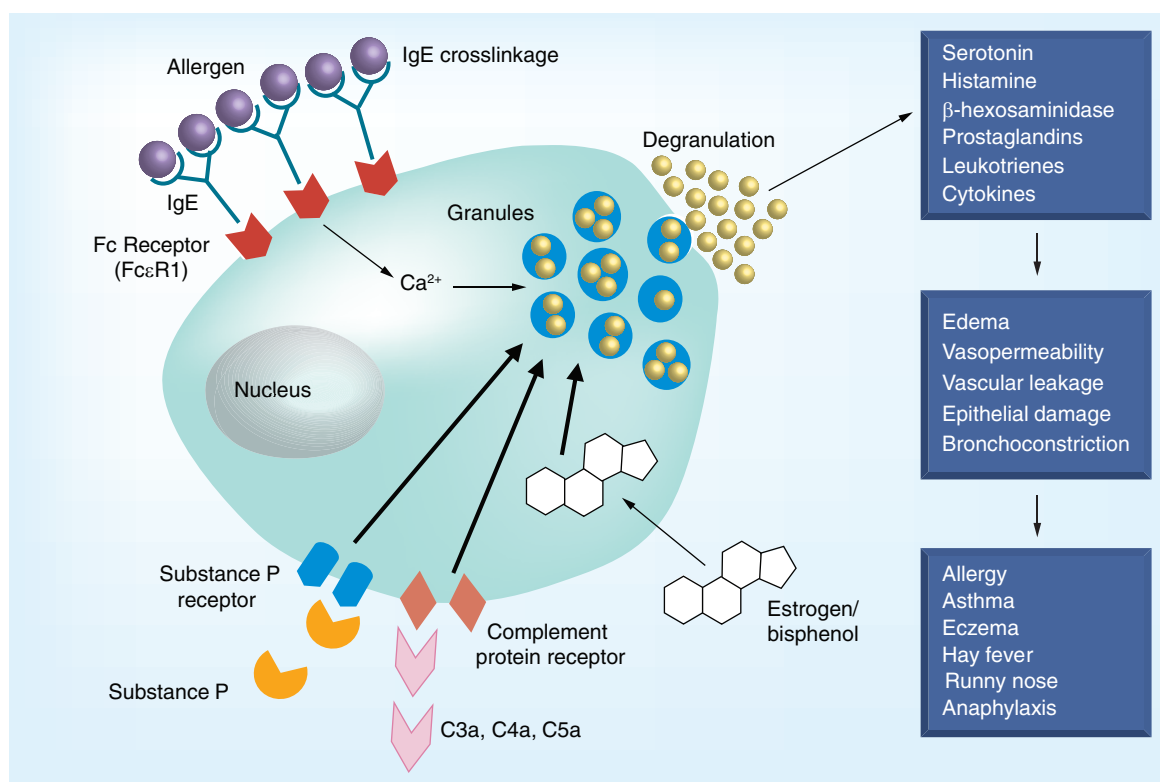


Figure 1. The biochemical pathway of an allergy in basophils and mast cells. Degranulation in mast cells or basophils occurs after cross-linking the IgE that is bound on the cell surface FcεR1 by allergens. Neuropeptide substance P, complement proteins C3a, C4a and C5a, estrogen and environmental estrogen and bisphenol A can also stimulate degranulation in the absence of IgE. The release of allergic mediators such as serotonin and histamine causes edema, increased vascular permeability, smooth muscle contraction and bronchoconstriction, which lead to allergic responses such as allergy, asthma and anaphylaxis.

the sample. After washing, a second reporter antibody, directed to another epitope of IgE, is added linked to an enzyme or a fluorochrome in order to detect the amount of captured IgE. **Figure 2A** illustrates the basic principles for the FEIA or ELISA in the conventional (e.g., 96-well plate) or **lab-on-a-chip** (LOAC) formats. The minimal amount of detectable serum IgE using this approach is usually 0.5–1.0 µg/l. This method is useful for monitoring the effectiveness of omalizumab in lowering the free IgE serum level in treatments for allergic asthma [11].

There are many other assay formats that can be used to determine the level of serum IgE specific to known allergens. Most of these assays are available in a singleplex format that detects the IgE serum level of the allergens extracted from raw biological materials. Over the years, with the advances in recombinant protein and microarray technology, the development of a multiplexed assay that tests the IgE against hundreds of highly purified recombinant allergens simultaneously has become possible [12]. For example, IMMULITE® 2000/2500 (Siemens Healthcare Diagnostics, Erlangen, Germany), ImmunoCAP® 250/1000 (Phadia AB, Uppsala, Sweden) and HYTEC™ (Hycor-Agilent,

IN, USA) are high-throughput serologic IgE assay systems. The core principle used in these assays is to detect the IgE molecules that bind to the allergens immobilized on a solid surface, then the amount of bound IgE is reported by the anti-IgE antibody or aptamer conjugated with fluorochromes [13]. Microarray and bead-based suspension arrays are two commonly used approaches [12,14]. Alternatively, the level of IgE captured by the allergens can be determined by label-free techniques, such as surface plasmon resonance (SPR) in a 1D or 2D manner for signal reporting [15,16]. However, the results of these assays are usually influenced by the amount and quality of the allergen bound to the solid support, the affinity of the IgE antibody to the allergen, the degree of nonspecific binding, the degree of allergen binding by a blocking antibody and the cross-reactivity of the IgE from patients. It is not unusual to see variations in the level of allergen-specific IgE determined by the assays. These make comparison among systems difficult and affect the clinical decision-making process for the patients with allergies [17].

Notably, the serum level of a specific IgE only reflects the free circulating IgE. Its amount may not have any significant correlation with the severity of allergic dis-

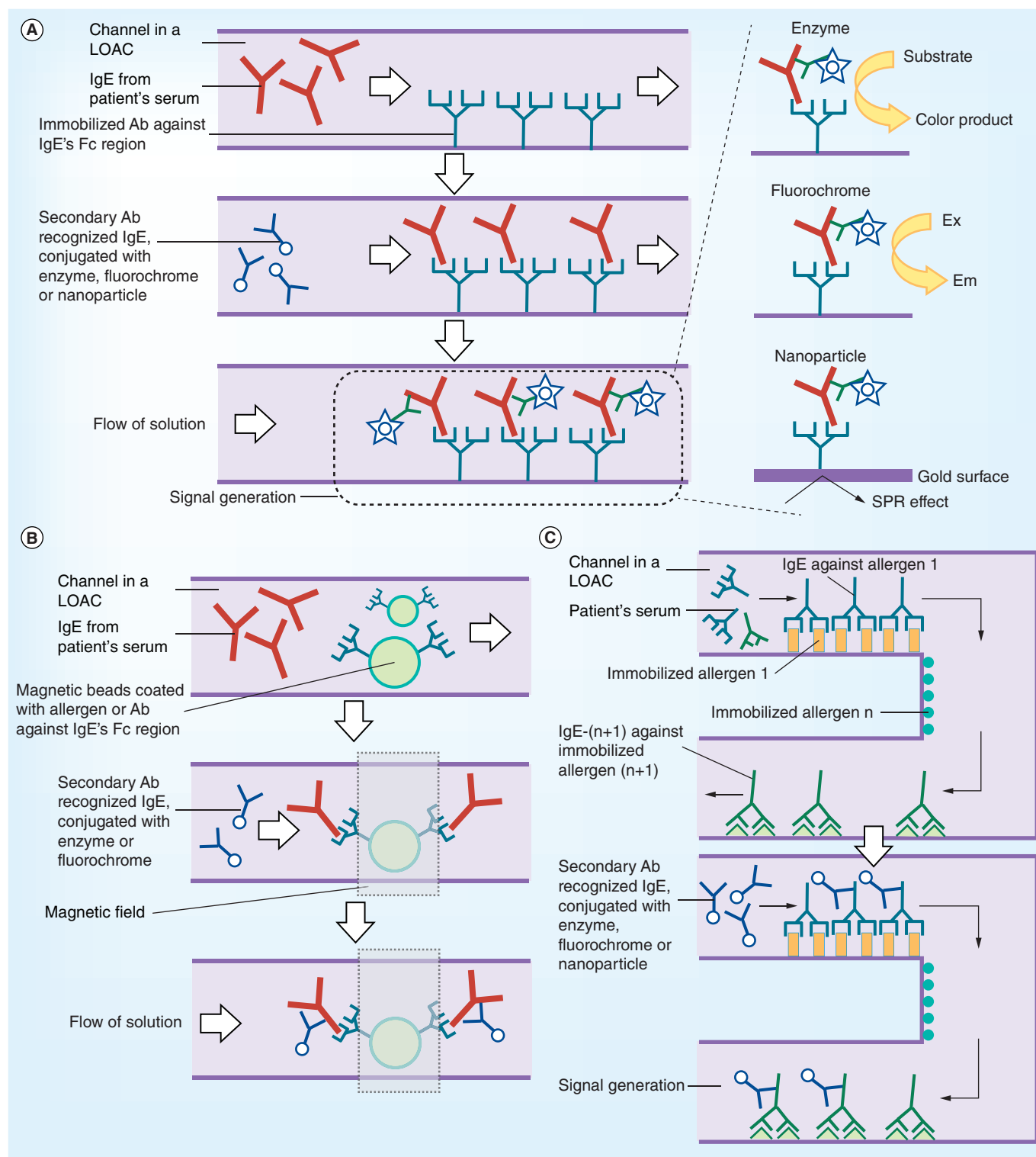


Figure 2. Different strategies in lab-on-a-chip approaches for allergy testing. In a LOAC system, reagents flow unidirectionally for allergy testing. **(A)** An antibody specific for IgE that is immobilized inside the channel captures the IgE from the patient's serum. After washing, the amount of IgE is reported by a second antibody with an enzyme, fluorochrome or nanoparticle. **(B)** An antibody or allergen is coated on magnetic beads to capture the IgE; a second antibody is added and the beads are held by the magnetic field for sensing. **(C)** Known allergens are immobilized in different regions of the channel to identify the IgE's specificity. **(D)** Cytokines for an allergy are tested by antibody–antigen reactions. **(E)** Activation of basophils is tested by cytokine release and the expression of surface markers (e.g., CD203c) or adhesion molecules. LOAC: Lab-on-a-chip.

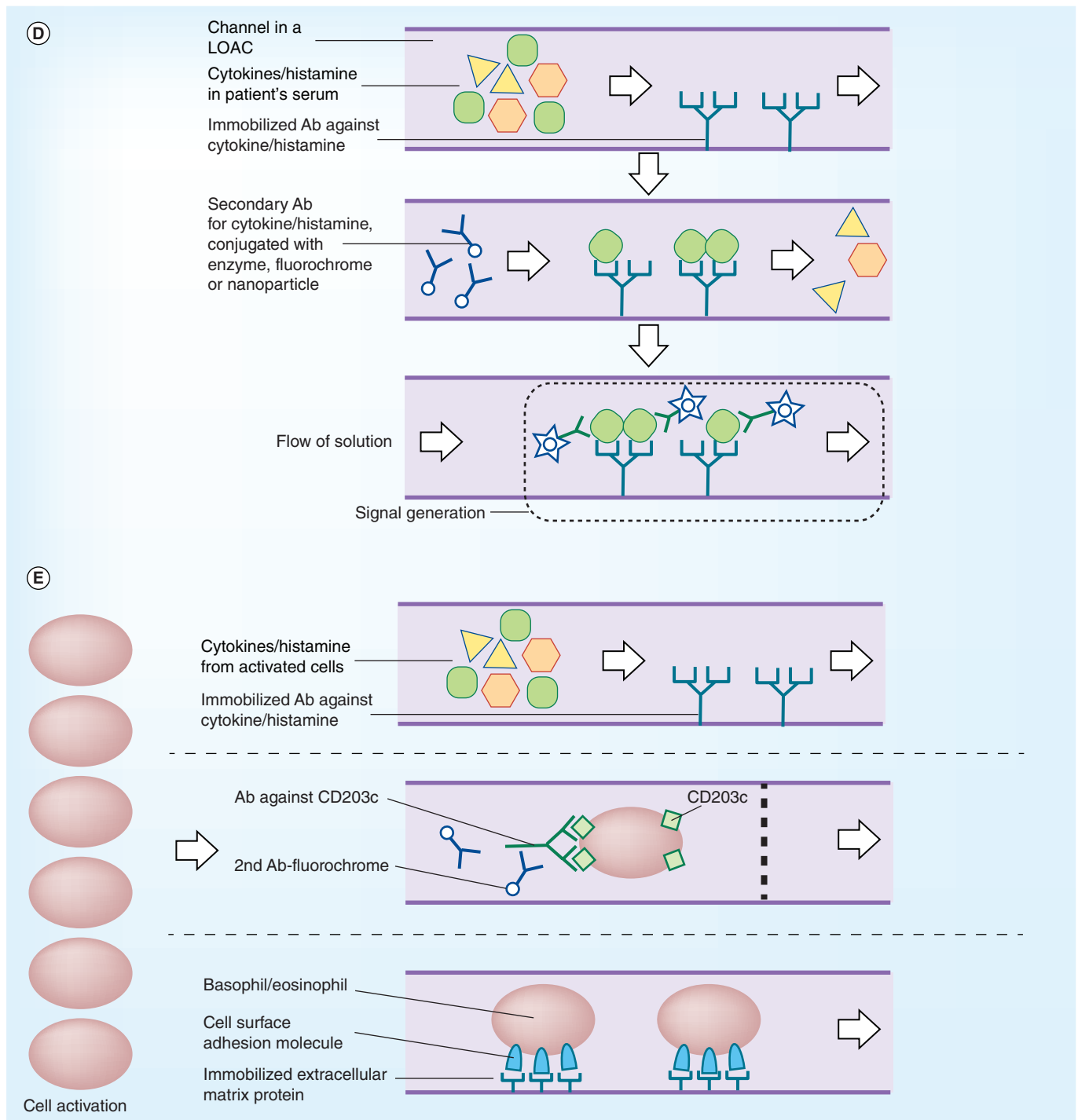


Figure 2. Different strategies in lab-on-a-chip approaches for allergy testing (cont.). In a LOAC system, reagents flow unidirectionally for allergy testing. **(A)** An antibody specific for IgE that is immobilized inside the channel captures the IgE from the patient's serum. After washing, the amount of IgE is reported by a second antibody with an enzyme, fluorochrome or nanoparticle. **(B)** An antibody or allergen is coated on magnetic beads to capture the IgE; a second antibody is added and the beads are held by the magnetic field for sensing. **(C)** Known allergens are immobilized in different regions of the channel to identify the IgE's specificity. **(D)** Cytokines for an allergy are tested by antibody–antigen reactions. **(E)** Activation of basophils is tested by cytokine release and the expression of surface markers (e.g., CD203c) or adhesion molecules. LOAC: Lab-on-a-chip.

eases, such as allergic asthma. The results of recent investigations indicate that increased IgE levels are also

seen in other disorders, such as parasitic infections, viral infections and immunodeficiency diseases [18]. There-

Key Terms

Basophil activation test: An *in vitro* assay designed for the identification of IgE- or non-IgE-type allergy triggers by measuring the degree of degranulation or cell activation in basophils.

Lab-on-a-disc: A microfluidic disc device with chambers, valves and channels that integrates laboratory operations in one platform using centrifugal, inertial and Coriolis forces generated by spinning for fluidic pumping, reagent mixing and metering for detecting disease markers.

fore, measuring the total IgE level in serum is not a specific diagnostic assay for a given allergic disease. For allergen screening, the skin-prick test, the double-blind, placebo-controlled oral food challenge test and the drug provocation test are all *in vivo* tests for the clinical assessment of allergen sensitivity and disease severity [19]. These tests are tedious, costly and have the risk of adverse allergic reactions. Apart from the exposure to allergens, there are many risk factors affecting the degree and prevalence of allergies. An allergic response can also be triggered by nonantigenic stimuli, such as complement proteins, substance P, synthetic polyamines and estrogen, without the involvement of IgE [20,21]. For example, a dramatic increase in the prevalence of childhood allergies in developed countries in the past two decades is a result of environmental pollution [22,23]. For instance, bisphenol A, an environmental estrogen that is a substrate of the plastics used to manufacture baby milk bottles and the aluminium lining of food cans, is believed to increase mast cell degranulation and thus to trigger childhood allergies. Moreover, many small molecules such as heparin [24] and radiocontrast agents [25] are able to directly induce nonreceptor-mediated degranulation. To date, our understanding of this non-IgE type of allergy is poor.

Clearly, a safe and relevant test for the assessment of allergic diseases should measure the degree of activation of mast cells/basophils isolated from patients with specific IgE and allergens. The **basophil activation test** (BAT) is such an assay: it directly measures the basophil responses, rather than simply determining the presence or absence or the amount of IgE in the blood. The BAT is a cell-based, flow cytometric analysis that determines the upregulation of cell surface activation markers upon an *ex vivo* allergen challenge [26,27]. As basophils can be isolated from blood more easily than mast cells in tissues, the BAT has been recently used for the assessment of drug allergies, anaphylaxis and the efficacy of immunotherapy in clinical settings [26,27].

Two predominant approaches can be used to measure the activation of basophils: the release of allergic mediators (e.g., histamine) or the expression of an activation marker on the cell surface. For the second approach, commercial BAT assays are available for the testing of activation markers, such as CD63 (dis-

covered in early 1990) [28] or CD203c (Figure 3) [28]. Basically, CD63 is translocated from the membrane of granules to the plasma membrane during degranulation upon stimulation in basophils. Similarly, the protein translation and expression of CD203c on a cell surface are upregulated upon activation. However, these surface markers have different responses to different stimuli. For example, CD203c seems to be more sensitive than CD63 for the diagnosis of amoxicillin allergy [29], whereas in another study, CD63 had better sensitivity to muscle relaxants [30].

However, the expensive BAT commercial kits usually use IL-3 in order to enhance the assay's sensitivity, yet its addition may distort the testing result profile. In addition, basophils represent only 1% of the circulating leukocytes and only 20,000 basophils can be obtained from 1 ml of blood from a typical donor, with onerous isolation steps. Therefore, more blood samples are required for extensive testing using the conventional approach. Moreover, basophils isolated from patients are preferably assayed within 4 h because their reactivity starts to decline 4 h after blood taking [31].

LOAC for allergy testing

In view of these problems and deficiencies, microfluidic systems for integrated biomedical assays have attracted much attention in recent years [32–37]. In a LOAC, laboratory operations such as reagent storage and release, solution translocation and metering, sample mixing and reaction and signal generation are integrated into a tiny chip ranging in size from a few square millimetres to a few square centimetres. In most of these formats, a micro-injector or -pump is used to generate a unidirectional driving force that allows the reagents, solutions and testing sample, which are loaded in different compartments, to flow through microchannels with valves and to react to each other. In 2002 and 2004, Kurita *et al.* and Matsubara *et al.* independently developed the first generation of LOACs for the detection of histamine released from a rat basophilic cell line [38,39]. In 2009, Ohashi *et al.* built a micro-ELISA platform to measure the IgE level in patients' serum [40]. In 2010, Love and colleagues described a LOAC assay to determine the responses of individual immune cells to allergens. In Love's approach, cells taken from patients were placed into approximately 100,000 individual wells by microengraving. Interleukins such as IL-2, IL-6, IL-17, IFN- γ and TNF- α were determined from individual cells after exposure to allergens [41]. The major advantage of this approach is that it can analyze individual immune cells from patients and measure the response profile to each allergen at the single-cell level. However, the results are difficult to interpret, and it requires expertise to run the assay and draw a conclusion.

With the demand for better healthcare constantly increasing, many strategies using LOACs for allergy testing have been developed in the past few years. **Table 1** summarizes the different existing LOAC formats and strategies for testing allergies. As can be seen, the testing formats can be grouped into three categories, namely the immunoassays, cytokine assays and cell-based assays. In the immunoassays, the IgEs in the sample serum are captured in the reaction chamber by the immobilized antibodies against the Fc region of the IgE. After washing, a secondary antibody (targeting another IgE epitope) is conjugated with an enzyme, fluorochrome or nanoparticle and added in order to react with the captured IgE molecules. The signals for the total amount of IgE can then be measured by light transmittance, fluorescence or SPR, similar to the method used in conventional ELISA/FEIA or SPR assays (**Figure 2A**). In some designs, the IgE-capturing antibody or suspected allergen is immobilized on the surface of magnetic beads. After reaction and washing, the magnetic beads in suspension are held by a magnetic field and the signals are then determined, as shown in **Figure 2A**, by an antibody–antigen reaction (**Figure 2B**). In other LOACs, different allergens of known identity are immobilized in different regions of the microchannel in order to allow the allergen-specific IgE to react. After washing and signal generation, it is possible to identify which allergen(s) the patients is sensitive to (**Figure 2C**). For the cytokine assays, the principle is similar to the ELISA/FEIA/SPR format, except that the IgE-capturing antibody is replaced with the antibody-targeting cytokines for the allergy (e.g., IL-4). The cytokines are then labeled with fluorochrome or recognized by a second antibody (**Figure 2D**). For the cell-based assays, cell activation after challenging with stimuli can be tested by the amount of allergic mediator released (e.g., histamine), activation markers translocated to the cell surface (e.g., CD63 or CD203c) or the number of cells adhered to the extracellular matrix protein (**Figure 2E**).

Lab-on-a-disc (LOAD), or the centrifugal microfluidics platform, is a recent variant of LOAC [52–56]. LOAD alleviates the need for active pumping by introducing centrifugal forces, capillary forces, Euler forces and Coriolis forces. The main benefit is that the forces are present everywhere on the disc for solution translocation, reagent mixing, target separation and for changing the direction of solution movement. This means that sample propulsion is always at the user's disposal. Sample movement can be controlled by varying the spinning speed, spinning direction, valves or rotation-actuated barriers through different combinations of centrifugation force, capillary force, Euler force and Coriolis force. Recent reviews by Nolte [53], Madou

et al. [54] and Gorkin *et al.* [55] offer a comprehensive summary of the device's physics and the applications of centrifugal microfluidics. Simple operations, including flow control using passive capillary valves, single-use active valves based on the melting-wax technique, siphoning, volume metering, mixing and flow switching, have also been demonstrated [53,55]. Applications such as nucleic acid amplification based on PCR [57], loop-mediated isothermal amplification [58], ELISA [59,60], cell counting [61], cell lysing [62], cell staining [63], single-cell entrapment [64,65] and DNA hybridization [66] have been reported. It is evident that LOAD is a viable platform for practical bioassays, including allergy testing. For example, Samsung Advanced Institute of Technology (Suwon, Korea) developed an integrated immunoassay that used LOAD to test for the hepatitis B virus antigen using whole blood in 2009 [60]. In their approach, all of the reagents are preloaded on the disc and the total process can be finished in 30 min. In addition, the detection limit was comparable to that of the conventional ELISA [60]. On the same platform, DNA can be extracted from the whole blood in one step in order to test for infection with the hepatitis B virus [67]. In this connection, recent developments indicate that with an inertial mechanical structure and power coupling, bidirectional flow control within channels of LOAD and on-chip, real-time heating are possible for DNA amplification using PCR [68]. Therefore, LOAD provides a compact setup that enables the full integration of complicated biochemical reactions that require multiple-step fluid handling, including DNA amplification using PCR.

Recently, we developed a LOAD system for allergy testing [51]. Our disc design, with a diameter of 10 cm, contains four identical units for testing allergens (**Figure 4A & inset**). The channels (500- μ m width and 250- μ m depth) and chambers are made of polydimethylsiloxane, which is moulded with an SU-8 master made from photolithography; the siphon valve region is modified with titanium dioxide. The polydimethylsiloxane layer is attached to a flat polymethylmethacrylate (PMMA) disc. This disposable LOAD platform integrates reagent metering, mixing, reaction, analyte selection and signal generation and detection in an automated fashion.

In our LOAD system, a human basophil cell line KU-812 carrying human IgE Fc receptors is used in the assay to substitute for a patient's basophils. KU-812 cells are surface nonadherent cells and so they do not stick to the channel surface, thus providing good responses to stimulations in the LOAD system. As reported previously, KU-812 cells showed good time- and dose-dependent responses by measuring the release of histamine and β -hexosaminidase as

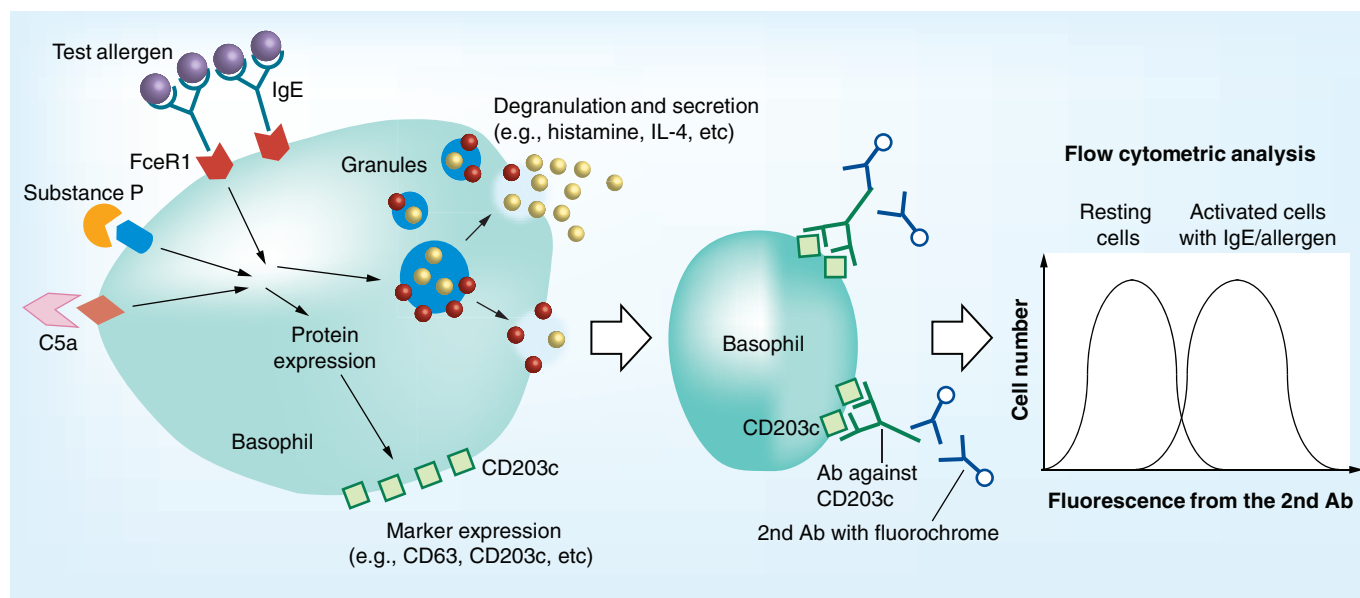


Figure 3. Basophil activation test. Basophils isolated from the patient's blood are stimulated by allergens or other stimuli and their response in terms of the expression of surface activation markers such as CD63 or CD203c is tested at the single-cell level by flow cytometry. Flow cytometric histograms from the resting cells and activated cell population can be compared. With appropriate dilution of the first and second antibody, fluorescence signals will shift to the right-hand side. For simplicity, only CD203c is shown in the assay.

an index of degranulation [69]. In order to reduce the system complexity, a fluorescent reporter – acridine orange (AO) – is used as a mimetic to study degranulation and exocytosis, as described in other studies [70,71]. Mechanistically, AO enters acidic granule compartments as a weak base. The uncharged AO molecules inside the granules are protonated and thus entrapped, and emit red fluorescence. Another feature of AO is its strong green emission when bound to a DNA molecule. Therefore, AO is a versatile reporter for labeling acidic granules in basophils and probing DNA with different light emission colours. When the AO-loaded cells are activated by the stimuli, the AO molecules are released from the granules into the extracellular buffer through degranulation in a Ca^{2+} -dependent manner.

Practically, KU-812 cells loaded with AO in chamber 1 (C1) are mixed with the patient's serum (C2) and the allergen being tested (C3) in the reaction chamber (C5) using sequential spinning (Figure 4B). Supernatant with the AO released from the KU-812 cells after degranulation is transferred into the detection chamber (C6) with the fixed cells from C4 in order to provide DNA for measuring the amount of AO released from the KU-812 cells. After spinning, fixed cells labelled with AO are pulled down at the tip of C6 for sensing. Waste is transferred to the waste chambers (C8 and C9), where

V7 is a siphon valve (Figure 4B). Using the DNA of the fixed HepG2 cells, but not the KU-812 cells, to generate green fluorescence increases the signal-to-noise ratio. Table 2 shows the spinning procedures and Figure 4C shows the detection setup. Using this approach, the difference in the degree of degranulation between the control and the activated KU-182 cells can be determined. Figure 4D shows a kinetic study in real time. As can be seen, the AO fluorescence increased significantly after the challenge of the AO-loaded KU-812 cells with well-known degranulation triggers such as N-formyl-methionyl-leucyl-phenylalanine (fMLP), ionomycin and phorbol 12-myristate 13-acetate (PMA). Taken together, this BAT assay is a reliable, sensitive, cost-effective and simple approach to allergy testing. However, more quantitative tests are required in order to provide good correlations with existing diagnostic tests and assays.

Conclusion

The cell-based microfluidic LOAC and LOAD systems are an emerging biomedical tool for identifying triggers of IgE-mediated and non-IgE-type allergies. With carefully designed pumping strategies or spinning speeds, assays with different testing formats can be executed in a sequence of liquid operations and reactions. The LOAD system mentioned in this article, together with the AO-loaded KU-812 cells, is an easy, fast and cost-efficient cell-based system that provides a method for IgE and non-IgE-type allergy testing for clinical diagnosis and research purposes. We strongly

Key Term

Non-IgE-type allergy: Many environmental pollutants trigger degranulation directly in the mast cells and basophils in the absence of IgE production.

Table 1. Different lab-on-a-chip formats.			
Assay type	Description/format	Features	Ref.
Immunoassay	A personalized food allergen testing platform (iTube) running on a smartphone using a commercially available food allergen testing kit to test the presence or absence of an allergen (e.g., peanuts) by colorimetric assay (Figure 2A)	A fast and point-of-care <i>in vitro</i> diagnostic platform Using a smartphone as a detection device	[42]
	A single-channel microchip using a magnetic field to keep magnetic core-shell nanoparticles coated with allergens in order to capture corresponding IgE for allergy diagnosis by measuring the fluorescence from the second antibody against human IgE (Figure 2B)	With a large surface area-to-volume ratio in the microchannel for immunoreactions, it is reported that this approach improves the analyte capture kinetics by 1000 times compared with conventional methods	[43]
	A micropillar array replacing gel for the separation of protein allergens (e.g., β -lactoglobulin) by isoelectric focusing. Allergens are then transferred to a nitrocellulose membrane for immunoblotting with serum from the allergic patient	This micropillar system coupled with immunoblotting uses a small sample volume for allergen testing Similar to the conventional western blot analysis	[44]
	An automated microfluidic system with five channels that pumps reagents unidirectionally from individual storage chambers into a common reaction chamber where allergen extracts are immobilized at different regions of the channel and the serum-specific IgE is tested by chemiluminescence (Figure 2C)	This system, which is available commercially, shows a good correlation with ImmunoCAP® in the diagnosis of allergen sensitivity according to the patient's clinical history The assay system is similar to the conventional ELISA format	[45,46]
	An automated micro-ELISA system measuring serum IgE with enzymatic reactions on polystyrene microbeads with allergen immobilization under stopped-flow conditions (Figure 2B)	Uses a small sample volume (5 μ l) and has a fast analysis time (12 min)	[47]
Cytokine assay	A chip-based system captures allergy-related cytokines from human biopsies using immobilized receptors. After labeling with fluorescent probes, the target was then separated electrophoretically and detected by a fluorescence detector (Figure 2D)	This system can show the differences in various stages of atopic dermatitis in human patients and can run four to six samples per hour	[48]
Cell-based assay	A microchannel system coated with recombinant human VCAMs in order to study the adhesion profile of resting and GM-CSF-stimulated human eosinophils by microscopic imaging (Figure 2E)	A cell-based assay examines the drug effects in eosinophils under flow conditions	[49,50]
	A centrifuge-based lab-on-a-disc system to examine the allergic responses of live basophils using acridine orange as a fluorescent probe in order to report degranulation for allergen testing (Figure 4)	A cell-based assay examines the IgE allergen or the non-IgE-type degranulation in live basophil cells With the use of house dust mite protein and its corresponding IgE, the efficiency of the lab-on-a-disc system is 80.6%, comparable to other conventional methods	[51]

believe that this trend towards the use of a cell-based LOAC platform and centrifugal microfluidics will continue and more formats will be developed in the field of allergy testing.

Future perspective

It is estimated that a traditional medical diagnostic cycle involves 56 steps [72]. Most of these steps are conventionally performed by trained personnel using expensive equipment. However, errors are frequently made and it

takes a long time to complete the cycle. In this context, LOAC diagnostic devices have the potential and tool-set to improve the situation so that the test turnaround time and number of steps can be shortened and reduced, respectively. This will increase the chance of a successful outcome because therapeutic interventions can be executed earlier. The concept of the LOAC is to miniaturize clinical laboratory tests, integrating them into a tiny microfluidic device using a small amount of samples and expensive reagents. This semiautonomous platform

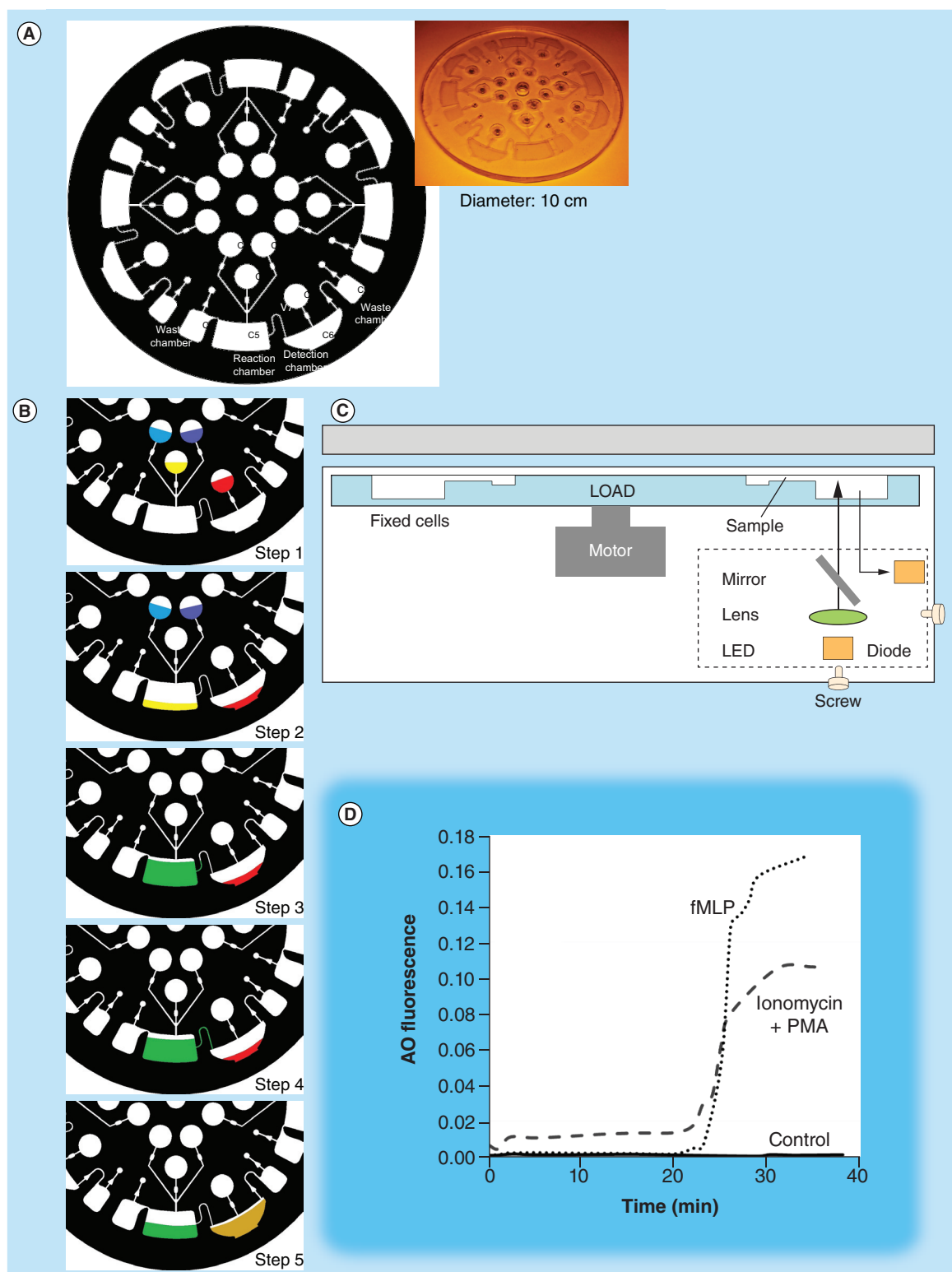


Figure 4. The basic lab-on-a-disc design for allergy testing. (A) LOAD design and its photograph. (B) Spinning procedures for sensing. (C) LOAD reader for signal detection. Details are available in [51]. (D) Degree of basophil degranulation determined by LOAD using the AO released from the KU-812 cells and the DNA from the glutaraldehyde-fixed HepG2 cells after the challenge of the KU-812 cells with fMLP (1 μ M), ionomycin (1 μ M) and PMA (20 nM) or the medium alone. Dotted line: fMLP; dashed line: ionomycin + PMA; solid line: control (no allergen). AO: Acridine orange; LOAD: Lab-on-a-disc; fMLP: *N*-formyl-methionyl-leucyl-phenylalanine; PMA: Phorbol 12-myristate 13-acetate.

Table 2. Reagents and spinning procedures in lab-on-a-disc for allergy testing.	
Procedure [†]	Reagents/spinning conditions
Step 1	The following reagents were loaded into chambers 1–4 (C1–C4): C1: AO-loaded KU-812 cells (40×10^6 cells/ml, 10 μ l) C2: Phosphate-buffered saline (10 μ l) C3: Medium alone or stimulus (e.g., ionomycin [1 μ M] plus PMA [20 nM] or fMLP [1 μ M], 10 μ l) C4: Glutaraldehyde-fixed HepG2 cells (40×10^6 cells/ml, 10 μ l)
Step 2	Rotation speed was set at 500 rpm ($6 \times g$) for 20 s. Acceleration speed was generated from the motor AO-loaded KU-812 cells in C1 entered the reaction chamber (C5) Glutaraldehyde-fixed HepG2 cells entered the detection chamber (C6) at 500 rpm ($10 \times g$)
Step 3	Rotation speed was set at 1100 rpm ($24 \times g$) for 20 s Stimulus and phosphate-buffered saline entered the reaction chamber (C5)
Step 4	Rotation speed was set at 800 rpm ($28 \times g$) for 20 s, then 200 rpm ($2 \times g$) for 10 s Siphon valve was filled with solution
Step 5	Rotation speed was set at 800 rpm ($28 \times g$) until the end of the assay Supernatant in C5 was transferred to C6 in order to label the DNA of the HepG2 cells Signals were acquired in a real-time manner at 1-min time intervals
[†] Procedures (steps 1–5) correspond to the panels shown in Figure 4B . AO: Acridine orange; fMLP: <i>N</i> -formyl-methionyl-leucyl-phenylalanine; PMA: Phorbol 12-myristate 13-acetate.	

would consist of different modules, such as chambers, channels, valves and surface properties, in order to provide answers and solutions for patients in a more timely and cost-effective manner. However, a number of challenges still need to be addressed. For example, further improvement in chip geometries and sensing techniques are required in order to improve the assay sensitivity and reliability, with the emphasis on high-throughput, multiplexed, and yet simple operation platforms for personalized medicine. In this regard, LOAD is one potential solution because all of the steps can be controlled simply by spinning and it is easy to couple it with some other components for local heating, magnetic field generation and wireless data communication. We expect that a subset of the LOAC or LOAD will be widely adopted for its

convenience, reliability and highly adaptable utility in healthcare and point-of-care systems.

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Executive summary

- The incidence and prevalence of allergic diseases have increased dramatically in recent decades.
- Identifying the IgE and non-IgE-type triggers is a crucial step in allergy prevention and treatment.
- Lab-on-a-chip and lab-on-a-disc systems are emerging as powerful tools for allergy testing.
- Cell-based assays (e.g., the basophil degranulation test) can determine both IgE-mediated and non-IgE types of allergies.
- Hardware (lab-on-a-chip/lab-on-a-disc) and software (e.g., immunoassay or cell-based assay) developments will continue to refine and simplify the process of allergy testing.

References

Papers of special note have been highlighted as: • of interest;
 •• of considerable interest.

- 1 Kay AB. 100 years of 'allergy': can von Pirquet's word be rescued? *Clin. Exp. Allergy* 36(5), 555–559 (2006).
- 2 Warner JO, Kaliner MA, Crisci CD *et al.* Allergy practice worldwide: a report by the world allergy organization specialty and training council. *Int. Arch. Allergy Immunol.* 139(2), 166–174 (2006).
- 3 MacGlashan DW Jr. Endocytosis, recycling, and degradation of unoccupied Fc epsilonRI in human basophils. *J. Leukoc. Biol.* 82(4), 1003–1010 (2007).
- 4 Gould HJ, Sutton BJ. IgE in allergy and asthma today. *Nat. Rev. Immunol.* 8(3), 205–217 (2008).
- **Critical review showing how IgE exerts its effect in allergies.**
- 5 Pelaia G, Vatrella A, Maselli R. The potential of biologics for the treatment of asthma. *Nat. Rev. Drug Discov.* 11(12), 958–972 (2012).
- **Comprehensive review showing the potential treatment of asthma using omalizumab.**
- 6 MacGlashan DW Jr. IgE-dependent signaling as a therapeutic target for allergies. *Trends Pharmacol. Sci.* 33(9), 502–509 (2012).
- 7 Heinzerling L, Mari A, Bergmann KC *et al.* The skin prick test – European standards. *Clin. Transl. Allergy* 3(1), 3 (2013).
- 8 Kalogeromitros D, Katsarou A, Armenaka M *et al.* Influence of the menstrual cycle on skin-prick test reactions to histamine, morphine and allergen. *Clin. Exp. Allergy* 25(5), 461–466 (1995).
- 9 Lin MS, Tanner T, Lynn J *et al.* Nonfatal systemic allergic reactions induced by skin testing and immunotherapy. *Ann. Allergy* 71(6), 557–562 (1993).
- 10 Fuiano N, Incorvaia C. The atopy patch test: is it time to redefine its significance? *Ann. Allergy Asthma Immunol.* 106(4), 278–282 (2011).
- 11 Hamilton RG. Accuracy of US Food and Drug Administration-cleared IgE antibody assays in the presence of anti-IgE (omalizumab). *J. Allergy Clin. Immunol.* 117(4), 759–766 (2006).
- 12 Scala E, Alessandri C, Palazzo P *et al.* IgE recognition patterns of profilin, PR-10, and tropomyosin panallergens tested in 3,113 allergic patients by allergen microarray-based technology. *PLoS ONE* 6(9), e24912 (2011).
- 13 Hu K, Yang H, Zhou J *et al.* Aptasensor for amplified IgE sensing based on fluorescence quenching by graphene oxide. *Luminescence* 28(5), 662–666 (2013).
- 14 King EM, Vailes LD, Tsay A *et al.* Simultaneous detection of total and allergen-specific IgE by using purified allergens in a fluorescent multiplex array. *J. Allergy Clin. Immunol.* 120(5), 1126–1131 (2007).
- 15 Wong CL, Ho HP, Yu TT *et al.* Two-dimensional biosensor arrays based on surface plasmon resonance phase imaging. *Appl. Opt.* 46(12), 2325–2332 (2007).
- 16 Ho HP, Law WC, Wu SY *et al.* Phase-sensitive surface plasmon resonance biosensor using the photoelastic modulation technique. *Sens. Actuators B Chem.* 114(1), 80–84 (2006).
- 17 Wang J, Godbold JH, Sampson HA. Correlation of serum allergy (IgE) tests performed by different assay systems. *J. Allergy Clin. Immunol.* 121(5), 1219–1224 (2008).
- 18 Pien GC, Orange JS. Evaluation and clinical interpretation of hypergammaglobulinemia E: differentiating atopy from immunodeficiency. *Ann. Allergy Asthma Immunol.* 100(4), 392–395 (2008).
- 19 Westwood GS, Huang SW, Keyhani NO. Allergens of the entomopathogenic fungus *Beauveria bassiana*. *Clin. Mol. Allergy* 3(1), 1 (2005).
- 20 Galli SJ, Nakae S, Tsai M. Mast cells in the development of adaptive immune responses. *Nat. Immunol.* 6(2), 135–142 (2005).
- 21 van den Berge M, Heijink HI, van Oosterhout AJ *et al.* The role of female sex hormones in the development and severity of allergic and non-allergic asthma. *Clin. Exp. Allergy* 39(10), 1477–1481 (2009).
- 22 Saxon A, Diaz-Sanchez D. Air pollution and allergy: you are what you breathe. *Nat. Immunol.* 6(3), 223–226 (2005).
- 23 Narita S, Goldblum RM, Watson CS *et al.* Environmental estrogens induce mast cell degranulation and enhance IgE-mediated release of allergic mediators. *Environ. Health Perspect.* 115(1), 48–52 (2007).
- 24 Warkentin TE, Greinacher A. Heparin-induced anaphylactic and anaphylactoid reactions: two distinct but overlapping syndromes. *Expert Opin. Drug Saf.* 8(2), 129–144 (2009).
- 25 Salas M, Gomez F, Fernandez TD *et al.* Diagnosis of immediate hypersensitivity reactions to radiocontrast media. *Allergy* 68(9), 1203–1206 (2013).
- 26 Chirumbolo S. Basophil activation test in allergy: time for an update? *Int. Arch. Allergy Immunol.* 158 (2), 99–114 (2012).
- 27 Kleine-Tebbe J, Erdmann S, Knol EF *et al.* Diagnostic tests based on human basophils: potentials, pitfalls and perspectives. *Int. Arch. Allergy Immunol.* 141(1), 79–90 (2006).
- 28 Sturm EM, Kranzelbinder B, Heinemann A *et al.* CD203c-based basophil activation test in allergy diagnosis: characteristics and differences to CD63 upregulation. *Cytometry B Clin. Cytom.* 78(5), 308–318 (2010).
- **Good paper showing the use of CD203c for allergy testing.**
- 29 Abuaf N, Rostane H, Rajoely B *et al.* Comparison of two basophil activation markers CD63 and CD203c in the diagnosis of amoxicillin allergy. *Clin. Exp. Allergy* 38(6), 921–928 (2008).
- 30 Sudheer PS, Hall JE, Read GF *et al.* Flow cytometric investigation of peri-anaesthetic anaphylaxis using CD63 and CD203c. *Anaesthesia* 60(3), 251–256 (2005).
- 31 Sturm GJ, Kranzelbinder B, Sturm EM *et al.* The basophil activation test in the diagnosis of allergy: technical issues and critical factors. *Allergy* 64(9), 1319–1326 (2009).
- 32 Mark D, Haeberle S, Roth G *et al.* Microfluidic lab-on-a-chip platforms: requirements, characteristics and applications. *Chem. Soc. Rev.* 39(3), 1153–1182 (2010).

- 33 Srinivasan V, Pamula VK, Fair RB. An integrated digital microfluidic lab-on-a-chip for clinical diagnostics on human physiological fluids. *Lab Chip* 4(4), 310–315 (2004).
- 34 Chin CD, Linder V, Sia SK. Lab-on-a-chip devices for global health: past studies and future opportunities. *Lab Chip* 7(1), 41–57 (2007).
- 35 Haeberle S, Zengerle R. Microfluidic platforms for lab-on-a-chip applications. *Lab Chip* 7(9), 1094–1110 (2007).
- 36 Franke TA, Wixforth A. Microfluidics for miniaturized laboratories on a chip. *Chemphyschem* 9(15), 2140–2156 (2008).
- 37 Yeo LY, Chang HC, Chan PP *et al.* Microfluidic devices for bioapplications. *Small* 7(1), 12–48 (2011).
- 38 Kurita R, Hayashi K, Horiuchi T *et al.* Differential measurement with a microfluidic device for the highly selective continuous measurement of histamine released from rat basophilic leukemia cells (RBL-2H3). *Lab Chip* 2(1), 34–38 (2002).
- 39 Matsubara Y, Murakami Y, Kobayashi M *et al.* Application of on-chip cell cultures for the detection of allergic response. *Biosens. Bioelectron.* 19(7), 741–747 (2004).
- 40 Ohashi T, Mawatari K, Sato K *et al.* A micro-ELISA system for the rapid and sensitive measurement of total and specific immunoglobulin E and clinical application to allergy diagnosis. *Lab Chip* 9(7), 991–995 (2009).
- 41 Han Q, Bradshaw EM, Nilsson B *et al.* Multidimensional analysis of the frequencies and rates of cytokine secretion from single cells by quantitative microengraving. *Lab Chip* 10(11), 1391–1400 (2010).
- 42 Coskun AF, Wong J, Khodadadi D *et al.* A personalized food allergen testing platform on a cellphone. *Lab Chip* 13(4), 636–640 (2013).
- 43 Teste B, Malloggi F, Siaugue JM *et al.* Microchip integrating magnetic nanoparticles for allergy diagnosis. *Lab Chip* 11(24), 4207–4013 (2011).
- 44 Li HY, Dauriac V, Thibert V *et al.* Micropillar array chips toward new immunodiagnosis. *Lab Chip* 10(19), 2597–2604 (2010).
- 45 Shyr SD, Jan RL, Webster JR *et al.* Determination of multiple allergen-specific IgE by microfluidic immunoassay cartridge in clinical settings. *Pediatr. Allergy Immunol.* 21(4 Pt 1), 623–633 (2010).
- 46 Tai LW, Tseng KY, Wang ST *et al.* An automated microfluidic-based immunoassay cartridge for allergen screening and other multiplexed assays. *Anal. Biochem.* 391(2), 98–105 (2009).
- 47 Ohashi T, Mawatari K, Sato K *et al.* A micro-ELISA system for the rapid and sensitive measurement of total and specific immunoglobulin E and clinical application to allergy diagnosis. *Lab Chip* 9(7), 991–995 (2009).
- 48 Phillips TM, Kalish H, Wellner E. Receptor affinity CE for measuring bioactive inflammatory cytokines in human skin biopsies. *Electrophoresis* 30(22), 3947–3954 (2009).
- 49 Robinson AJ, Kashanin D, O'Dowd F *et al.* Fluvastatin and lovastatin inhibit granulocyte macrophage-colony stimulating factor-stimulated human eosinophil adhesion to inter-cellular adhesion molecule-1 under flow conditions. *Clin. Exp. Allergy* 39(12), 1866–1874 (2009).
- 50 Williams VD, Kashanin D, Shvets IV *et al.* Microfluidic enabling platform for cell-based assays. *J. Lab. Automation.* 7, 135–141 (2002).
- 51 Chen QL, Cheung KL, Kong SK *et al.* An integrated lab-on-a-disc for automated cell-based allergen screening bioassays. *Talanta* 97, 48–54 (2012).
- **First paper to show the use of lab-on-a-disc for allergy testing.**
- 52 Mark D, Haeberle S, Roth G *et al.* Microfluidic lab-on-a-chip platforms: requirements, characteristics and applications. *Chem. Soc. Rev.* 39(3), 1153–1182 (2010).
- 53 Nolte DD. Invited review article: review of centrifugal microfluidic and bio-optical disks. *Rev. Sci. Instrum.* 80(10), 101101 (2009).
- 54 Madou M, Zoval J, Jia G *et al.* Lab on a CD. *Annu. Rev. Biomed. Eng.* 8, 601–628 (2006).
- 55 Gorkin R, Park J, Siegrist J *et al.* Centrifugal microfluidics for biomedical applications. *Lab Chip* 10(14), 1758–1773 (2010).
- 56 Glass NR, Shilton RJ, Chan PP *et al.* Miniaturized lab-on-a-disc (miniLOAD). *Small* 8(12), 1881–1888 (2012).
- 57 Focke M, Stumpf F, Faltin B *et al.* Microstructuring of polymer films for sensitive genotyping by real-time PCR on a centrifugal microfluidic platform. *Lab Chip* 10(19), 2519–2526 (2010).
- 58 Asiello PJ, Baeumner AJ. Miniaturized isothermal nucleic acid amplification, a review. *Lab Chip* 11(8), 1420–1430 (2011).
- 59 Noroozi Z, Kido H, Peytavi R *et al.* A multiplexed immunoassay system based upon reciprocating centrifugal microfluidics. *Rev. Sci. Instrum.* 82(6), 064303 (2011).
- 60 Lee BS, Lee JN, Park JM *et al.* A fully automated immunoassay from whole blood on a disc. *Lab Chip* 9(11), 1548–1555 (2009).
- **In-depth paper showing how to use lab-on-a-disc for immunoassaying with whole blood.**
- 61 Imaad SM, Lord N, Kulsharova G *et al.* Microparticle and cell counting with digital microfluidic compact disc using standard CD drive. *Lab Chip* 11(8), 1448–1456 (2011).
- 62 Haeberle S, Brenner T, Zengerle R *et al.* Centrifugal extraction of plasma from whole blood on a rotating disk. *Lab Chip* 6(6), 776–781 (2006).
- 63 Chen H Li X, Wang L *et al.* A rotating microfluidic array chip for staining assays. *Talanta* 81(4–5), 1203–1208 (2010).
- 64 Furutani S, Nagai H, Takamura Y *et al.* Compact disk (CD)-shaped device for single cell isolation and PCR of a specific gene in the isolated cell. *Anal. Bioanal. Chem.* 398(7–8), 2997–3004 (2010).
- 65 Lee SW, Kang JY, Lee IH *et al.* Single-cell assay on CD-like lab chip using centrifugal massive single-cell trap. *Sens. Actuators A Phys.* 143(1), 64–69 (2008).
- 66 Wang L, Li PC. Optimization of a microfluidic microarray device for the fast discrimination of fungal pathogenic DNA. *Anal. Biochem.* 400(2), 282–288 (2010).

- 67 Cho YK, Lee JG, Park JM *et al.* One-step pathogen specific DNA extraction from whole blood on a centrifugal microfluidic device. *Lab Chip* 7(5), 565–573 (2007).
- 68 Wang G, Ho HP, Chen Q *et al.* A lab-in-a-droplet bioassay strategy for centrifugal microfluidics with density difference pumping, power to disc and bidirectional flow control. *Lab Chip* 13(18), 3698–3706 (2013).
- **First paper to show the on-disc power of PCR.**
- 69 Cheung KL, Chen H, Chen Q *et al.* CTAB-coated gold nanorods elicit allergic response through degranulation and cell death in human basophils. *Nanoscale* 4(15), 4447–4449 (2012).
- 70 Ueki S, Melo RC, Ghiran I *et al.* Eosinophil extracellular DNA trap cell death mediates lytic release of free secretion-competent eosinophil granules in humans. *Blood* 121(11), 2074–2083 (2013).
- 71 Jaiswal JK, Fix M, Takano T *et al.* Resolving vesicle fusion from lysis to monitor calcium-triggered lysosomal exocytosis in astrocytes. *Proc. Natl Acad. Sci. USA* 104(35), 14151–14156 (2007).
- 72 Price CP, St John A, Hicks JM. Point-of-care testing: what, why, when and where? In: *Point-of-Care Testing*. Price CP, St John A, Hicks JM (Eds). American Association for Clinical Chemistry Press, Washington, DC, USA, 3–10 (2004).