Hevea latex-associated allergies : piecing together the puzzle of the latex IgE reactivity profile

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Running title: Molecular diagnosis of latex allergy.

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Running title: IgE-reactivity profile of latex sensitization

Abstract

Introduction: IgE-mediated *Hevea* latex allergy and associated food-allergies constitute a significant health issue with serious consequences of diagnostic error. Hence, there is need for more reliable confirmatory diagnostics.

Areas covered: Here, we summarize the major limitations of conventional tests using native extracts and describe how piecing together the IgE reactivity profile can benefit correct diagnosis in difficult cases in whom conventional tests yield equivocal or negative results. A diagnostic algorithm integrating traditional sIgE and component resolved diagnosis (CRD) is presented.

Expert opinion: Moreover, it is clear that the discoveries in the field of the *Hevea* latex proteome will contribute to our understandings and accurate approach of sometimes complex cross-reactivity phenomena that extend beyond the "latex-fruit syndrome".

1. Introduction

The term "latex" is often used for the elastic product employed in the composition of rubber articles. Generically, "latex" refers to an aqueous elastomer emulsion, and in the case of natural rubber, the natural latex is drawn from *Hevea brasiliensis* (order Euphorbiales, family Euphorbiacees) as a milky sap. Natural latex is the cytoplasm of specialised plant cells called laticifers and form a tube-like network through the plant, and functions to seal and protect damaged sites. The approximate composition of the liquid natural latex is water (55-65%), *cis*-1,4-polyisoprene rubber (34%), sugars (1.0-2.0%), sterol glycosides (0.1-0.5%), resins (1.5-3.5%), ash (0.5-1.0%) and finally proteins (2-3%) [1]. The latter causing immunoglobulin (Ig)E-mediated latex allergy and associated cross-allergies, particularly to fruits and vegetables but also nuts and cereals (reviewed in [2-5]). Three different fractions can be obtained by high-speed centrifugation of natural latex. There is a white creamy layer of rubber particles at the top. This layer is also called the "rubber phase" and contains approximately 27% of total protein in *Hevea* latex. These proteins are called rubber particle-associated proteins, i.e. the large particle-associated rubber elongation factor (REF) and the small rubber particle protein (SRPP). The bottom fraction (B-serum), containing specialised cell organelles are collectively called "lutoids" and have an approximate total protein percentage of 25%. As shown in table 1, lutoids contains several hydrolases and some pathogenesis-related proteins (i.e., defence proteins). Finally, the yellowish C-serum in between, corresponds to the cytosol from the laticifer cells, and contains about 48% of the total protein [6].

To date, IgE-mediated latex allergy to the constituent proteins of *Hevea* latex is recognized as an international health problem of major importance. Although the exact prevalence of latex sensitization and allergy among the general population is estimated less than 1%, several risk groups such as spina bifida patients and health care workers who are regularly exposed to latex-containing devices have been identified [2, 7, 8]. Besides, several epidemiological surveys have identified IgE-mediated latex allergy as significant cause of anaesthesia-related allergy and anaphylaxis [9, 10] . In these patients, correct

diagnosis of IgE-mediated latex allergy is a prerequisite to avert future potentially life-threatening reactions to latex and potential cross-reactive allergens implicated in latex-associated food allergies. In this review we focus on the potential and limitations of traditional latex-specific IgE (latex-sIgE) quantification and component resolved diagnosis to document IgE-mediated latex allergy.

2. In vitro diagnosis of IgE-mediated latex allergy

In general clinical practice, many physicians rely upon quantification of latex-sIgE antibodies as a primary measure to confirm or discard their clinical suspicion of an IgE-mediated latex allergy. However, correct diagnosis of latex allergy via quantification of latex-sIgE can pose significant difficulties. On several occasions, it has been demonstrated that latex-sIgE results are not absolutely predictive for the clinical outcome. Results of latex-sIgE can be false-negative [11-13] or, much more frequently, false-positive, that is, clinically irrelevant [11-16]. The consequences of false-negative results are obvious, as these entail a risk for life-threatening anaphylaxis upon subsequent exposure. However, over-diagnosis by false-positive results can also have dramatic consequences. For example, during diagnostic work-up of perioperative anaphylaxis, clinical irrelevant results could erroneously lead to the diagnosis of IgE-mediated latex allergy and premature stopping of further testing for the true culprit. Besides, identification of clinically irrelevant latex-sensitization should prevent unnecessary and generally expensive latex avoidance measures. Hence, there is need for additional reliable confirmatory tests.

3. Principles of Component Resolved Diagnosis

Traditional latex-sIgE assays, are based upon the quantification of serum sIgE directed against crude natural allergen extracts (figure 1). The complexity, variability and instability of natural allergens and the variation between individual sensitization patterns complicate the correct interpretation of sIgE results to crude allergen extracts. Consequently, a positive sIgE against crude extracts should always be interpreted with care as it might merely reflect (cross)sensitization rather than a genuine allergy. For example, for latex, it has been shown that ubiquitous structures such as α-1,3-fucose and β-1,2-xylose bearing cross-reactive carbohydrate determinants (CCD) present on glycoproteins of plants and α-1,3-fucose bearing CCD of hymenoptera venom glycoproteins [14-19] and plant profilins [15-17, 19-23] can elicit a false-positive latex-sIgE results. Therefore, latex-sIgE should not be used in isolation to diagnose IgE-mediated latex allergy. Strategies that can be adopted to detect and circumvent the CCD and profilin issues, are the use of glycan and profilin biomarkers or inhibitors, latex basophil activation tests (BATs) [14], CRD applying non-glycosylated latex-specific components, or BATs with recombinants [24].

The principles as well as major applications of CRD in children and adults are reviewed extensively elsewhere [6, 25-30]. . In contrast to conventional sIgE assays, CRD does not rely upon crude extract preparations obtained from native allergens but on sIgE antibodies directed towards single components

purified from natural sources or produced by recombinant techniques. In other words, CRD involves specific marker components and substructures to study the genuine allergic sensitization of patients to a particular allergen source and sensitization to cross-reactive determinants or components that point to cross-sensitization. These so-called "gatekeeper" tests allow an improved discrimination between genuine allergy and merely clinically irrelevant sensitization and allow the establishment of personalized sensitization profiles. Determining such personalized sensitization profiles creates the opportunity to assess the individual risk of severity of an allergic reaction and to predict the natural course. For example, for latex, CRD has unveiled that health care workers and spina bifida patients display distinct sensitization profiles that are not equally associated with a latex-food syndrome (see below). However, CRD also demonstrates limitations. Not all relevant allergen components are available and it has been demonstrated the technique to be of limited use in determining the clinical relevance of sensitization to homologues of the major birch pollen allergen Bet v 1 [31-33] . Besides, when using these individual components or epitopes for the diagnosis of allergy, the number of tests required to enable a correct diagnosis increases significantly since more than one component needs to be included to allow identification of the entire repertoire of disease relevant peptides and epitopes. The microarray technique for CRD elegantly enables sIgE antibody testing in a multiplex format and allows the simultaneous quantification of many sIgE antibodies. The major advantage of this multiplex technique lies in its potential to study significant numbers of components in parallel, detecting sIgE antibody abundance, functionality, and interaction concerning numerous allergenic determinants using only minute amounts of patients' serum which is particularly important in infants and children.. It is anticipated that CRD by flexible allergen-coated microbead assays, as shown in figure 2, should allow a personalized selection of the components of interest can benefit correct diagnosis in the individual patient [34]. Note that the availability of allergenic components of *Hevea* latex can also benefit sensitivity of the conventional latex-sIgE as has been demonstrated by comparison between a latex-sIgE with and without spiking for the acidic protein of *Hevea brasiliensis* latex (Hev b 5) [35, 36].

4. Component Resolved Diagnosis for IgE-mediated latex allergy

As addressed in the section above, correct serologic diagnosis of IgE-mediated latex allergy by conventional latex-sIgE testing can be seriously be impeded mainly because of the interference of anti-plant/invertebrate CCD and anti-profilin antibodies that can easily be observed in up to one-quarter of patients with a pollen and/or hymenoptera venom allergy [17]. As shown in the table, today 15 components of latex from *Hevea brasiliensis* (Hev b) have been identified and successfully cloned. Some of them have become available for single or multiplex molecular diagnosis of IgE-mediated latex allergy. As a matter of fact, the commercially available component-specific IgE assays for natural latex are non-glycosylated recombinant (r) Hev b 1, 3, 5, 6.01, 6.02, 8, 9 and 11. Particularly, rHev b 5 and 6 and in a lesser extent also rHev b 1 and 3 (both rubber particle-associated proteins) have been shown to be the most important biomarkers to diagnose genuine IgE-mediated latex allergy [37-41]. Sensitization

to Hev b 5 and 6 is primarily found in adult health care workers (HCW) and to a lesser extent also in children suffering from spina bifida (SB) and meningomyelocele. In contrast, sensitization to *Hevea* profilin Hev b 8 (latex profilin) generally, but certainly not always, points to a clinical irrelevant cross-reactivity [16-19, 21, 22]. For example, in an own series, in all patients diagnosis of IgE-mediated latex allergy could be established by the combination rHev b 1, 3, 5 and 6.02. Over three-quarters of our patients were sensitized to rHev b 5 and/or 6.02. Some also displayed sIgE reactivity against rHev b 1 and/or rHev b 3. In contrast, none of the individuals showing a clinically irrelevant sensitization to natural rubber latex demonstrated IgE reactivity to one of these components but three-quarters of them displayed a positive microarray result for rHev b 8 [15]. However, recently we identified some patients with an overt IgE-mediated latex allergy apparently related to monosensitization to Hev b 12, the non-specific lipid transfer protein of latex [42]. The main reason(s) for Hev b 12 monosensitization remain(s) elusive but could to some extent relate to an underlying *Cannabis sativa* allergy [43]. As all available latex components are non-glycosylated proteins, they constitute a helpful instrument to depict clinically irrelevant positive sIgE latex results resulting from a sensitization to plant-derived and invertebrate CCD.

5. Component Resolved Diagnosis for latex-associated food allergy (initially designated as latex-fruit syndrome)

As described above, patients suffering from an IgE-mediated latex allergy can display distinct sensitization profiles and clinical phenotypes, that is, with our without cross-allergies. A large majority of latex allergic patients is sensitized to so-called defence and/or structural proteins. These proteins are quite ubiquitously distributed in plant kingdom and might explain the occurrence of a variety of latex-associated plant food allergies historically designated as “latex-fruit syndrome” mainly involving banana, avocado and chestnut [44]. However, today it appears that the list of cross-reactive plant-derived foods extends far beyond these tropical foods and involves many fruits, vegetables, nuts and cereals [2-5]. Such latex-associated food allergies have been described in about 21-58% of patients with an IgE-mediated latex allergy. In contrast, other patients, mainly children with spina bifida, are sensitized to the rubber particle-associated proteins Hev b 1 (REF) and Hev b 3 (SRPP), which by definition are confined to rubber synthesising plants and will not display a secondary latex-associated food syndrome. Like for all IgE-mediated diseases, the diagnostic approach of latex-associated food allergy starts with a thorough clinical history with a main focus on the latex allergy and potential related cross-reactivities and should further be pieced together using different *in vitro* and *in vivo* tests. As addressed in the section “*in vitro* diagnosis of IgE-mediated latex allergy”, in general clinical practice, most physicians will use quantification of latex-sIgE antibodies as a primary confirmatory diagnostic. However, interpretation of positive latex-sIgE results is not always straightforward and correct diagnosis might require additional testing, mainly because of significant interference of clinically irrelevant anti-plant/invertebrate CCD and anti-profilin sIgE antibodies. Moreover, it has repeatedly been shown these antibodies, together with

sIgE antibodies to the homologues of Bet v 1 (the major allergen from birch (*Betula verucosa*) and non-specific lipid proteins (ns-LTP), to severely hamper correct diagnosis of IgE-mediated plant-derived food allergies. Therefore, the introduction of CRD to establish the individual sensitization profile should not only benefit correct diagnosis of IgE-mediated latex allergy but might, to some extent, help accurate diagnostic and therapeutic management of patients with a clinical suspicion of a latex-associated food syndrome [2-6]. For example, we found sIgE antibodies to the natural extracts of various fruits, vegetables and ficus to be clinically irrelevant in a majority of latex allergic patients [45]. Briefly, as indicated in the diagnostic algorithm (figure 3), allergens with potential importance for IgE-mediated plant-derived food allergies secondary to an IgE-mediated latex allergy are Hev b 5, 6, 7, 8, 11 and 12 [2-6]. Mono-sensitization to Hev b 1, 3 and 4 is unlikely to be associated with cross-reactivity, sensitization to Hev b 9 and 10 can be accompanied by cross-reactivity to moulds [46, 47] and sensitization to Hev b 6 and Hev b 11 with cross-reactivity to ficus species [48]. Sensitization to Hev b 2 and Hev b 13 seems less significant [49].

6. Expert opinion

IgE-mediated latex allergy constitutes a significant medical health problem that requires correct diagnosis for adequate and potentially lifesaving management, that is, avoidance measures. On the other hand, erroneous overdiagnosis of IgE-mediated latex allergy should be avoided, mainly because of the cost of alternative elastomers. Unfortunately, correct diagnosis is not always straight forward and can pose significant difficulties. Today, the most important limitation of the conventional latex-sIgE assays remains the high number of false-positives due to interference by clinically irrelevant anti-CCD and anti-profilin sIgE antibodies. To some extent, these difficulties have been solved by basophil activation experiments [14, 15] but mainly by the characterization and production of an increasing number of native and mainly recombinant allergenic *Hevea* components, with some being available for sIgE assays and more laborious basophil activation experiments. The performance of these component-based diagnostics has been thoroughly explored and quintessence of these studies is clear. The continuous efforts in unravelling the *Hevea l*atex proteome with characterization of relevant allergens and availability of recombinant components (free of profilin and plant/invertebrate glycans) has enabled the development for a more precise and approach of the individual patients. As a matter of fact, CRD offers a more reliable diagnostic and has paved the way to broaden our knowledge in sometimes complex cross-reactivity syndromes.

It is likely the further exploration of the latex proteome to disclose novel (less abundant) allergenic components to benefit precise diagnosis and to shift paradigms about the mechanisms of cross-reactivity syndromes. Whether profiling epitope-specific antibody repertoires will deepen our understandings in the mechanisms behind latex-associated allergies and benefit prediction of severity and phenotypes remains to be established.

186
187
188
189
190

Legends of figures

Figure 1: Source, application and composition of *Hevea* latex.

Generically, "latex" refers to an aqueous elastomer emulsion, and in the case of natural rubber, the natural latex is drawn from *Hevea brasiliensis* (order Euphorbiales, family Euphorbiacees). *Hevea* latex has many applications as the production of dipped thin-film materials such as balloons and gloves. The approximate composition of the natural latex is water (55-65%), *cis*-1,4-polyisoprene rubber (34%), sugars (1.0-2.0%), sterol glycosides (0.1-0.5%), resins (1.5-3.5%), ash (0.5-1.0%) and finally proteins (2-3%) of which 15 have currently been identified and successfully cloned (designated as Hev b 1-15). Sensitization to some components is associated with severe clinical phenotypes whereas sensitization to other components generally results to milder symptoms or is asymptomatic (e.g. Hev b 8 the profilin of *Hevea* latex). Next to the allergenic components *Hevea* latex contains many other proteins of unknown significance.

Figure 2: Principle of measurement of specific IgE (sIgE) antibodies by cytometric bead technique.

In the cytometric bead assay, allergens or components thereof are covalently coupled on beads of the same size and color but with different color intensity. These coated-beads are incubated with patient's serum that contain sIgE antibodies. Subsequently, a fluorochrome-conjugated antihuman-IgE antibody is added. This secondary antibody will bind the antigen-antibody immune complex and can be measured in a flow cytometer. The intensity of the bead determines the antigen/allergen (y-axis), the intensity of the fluorochrome conjugated antibody defines the sIgE concentration.

Figure 3: diagnostic algorithm of *Hevea* latex-associated allergies

Confirmatory testing generally starts with quantification of conventional latex-specific IgE (sIgE) antibodies and/or latex skin prick test (SPT) using native extracts. If these tests yield negative results latex allergy is unlikely. A basophil activation test (BAT) is recommended when history is compelling. If conventional latex-sIgE and/or SPT and/or BAT is/are positive component resolved diagnosis starts with quantification of sIgE to rHev b 5, 6, as well as 1 and 3 to determine clinical significance of conventional latex-sIgE/SPT or BAT and to estimate the risk for a latex-food syndrome. If sIgE to rHev b 1, 3, 5, 6, are negative it is advised to quantify sIgE to *Hevea* latex profilin (rHev b 8) and the glycan biomarker MUXF3. If one of these, or both, is/are positive, a latex allergy is unlikely and sIgE to rHev b 9 and 11 can be quantified, mainly to identify patients at risk for a latex-food syndrome because of sensitization to the class I endochitinase Hev b 11. Note that if a patient tests positive for a recombinant MBP-Hev b component, the clinical relevance has to be confirmed with a negative MBP result, especially if this patient displays a high total IgE value.

Table 1: Allergenic components of latex from *Hevea brasiliensis* (Hev b).									
Allergen	Trivial name	Localization	MW (kDa)	pI	Gly	Predicted physiological role	Glove users	Spina bifida	References
Hev b 1	Rubber elongation factor	Large rubber particles	14.7	5.0	-	Rubber synthesis	Minor	Major	[50]
Hev b 2	β-1,3 glucanase	Lutoids	35.1	9.5	+	Defence-related protein	Minor	Minor	[49, 51, 52]
Hev b 3	Small rubber particle protein	Small rubber particles	22.4	4.8	_	Rubber synthesis	Minor	Major	[53]
Hev b 4	Lecithinase homologue	Lutoids	53-55	4.5	+	Microhelix component	Minor	Minor	[54, 55]
Hev b 5	Acidic latex protein	Cytoplasm	16	3.5	-	Structural protein	Major	Major/minor	[56]
Hev b 6	Hevein and its precursors*	Lutoids	21	5.6	-	Lectin, latex coagulation	Major	Minor	[52, 57-59]
Hev b 7	Patatin homologue (esterase)	Lutoids Cytoplasm	42 44	4.8	+	Defence-related protein	Minor	Minor	[60, 61]
Hev b 8	Profilin	Cytoplasm	15	4.9	-	Cytoskeletal actin binding	Minor	Minor	[62]
Hev b 9	Enolase	Cytoplasm	47.7	5.6	-	Glycolytic enzyme	Minor	Minor	[47]
Hev b 10	Superoxide dismutase (MnSOD)	Mitochondria	26	6.3	-	Enzyme, radical destruction	Minor	Minor	[46, 63]
Hev b 11	Class I endochitinase	Lutoids	33	5.1	-	Defence-related protein	Minor	Minor	[64, 65]
Hev b 12	Nonspecific lipid transfer protein 1	Latex membranes	9.3	10.8	+	Defence-related protein	Minor	Minor	[66, 67]
Hev b 13	Esterase / early nodule specific protein	Lutoids	43	5.0	+	Defence-related protein	Minor	Minor	[49, 68]
Hev b 14	Hevamine (chitinase)	Lutoids	29.5	8.4	_	Defence-related protein	Minor	Minor	[69, 70]
Hev b 15	Serine protease inhibitor	Cytoplasm	7.5	4.8	-	Defence-related protein	Minor	Minor	[71, 72]

Commercially available components for sIgE testing are out in *italics.* * Hev b 6 comprised initially the 21 kDa precursor prohevein (Hev b 6.01), the 4.7 kDa hevein (Hev b 6.02), and the 14 kDa C-domain of prohevein (Hev b 6.03). Actually, the commercial used name Hev b 6 is synonymous with Hev b 6.02. Lutoids = B-serum, Cytoplasm = C serum. Gly: glycosylation. For accession N° see [73].

For more details: see http://iuis.org

227

228

Figure 1:

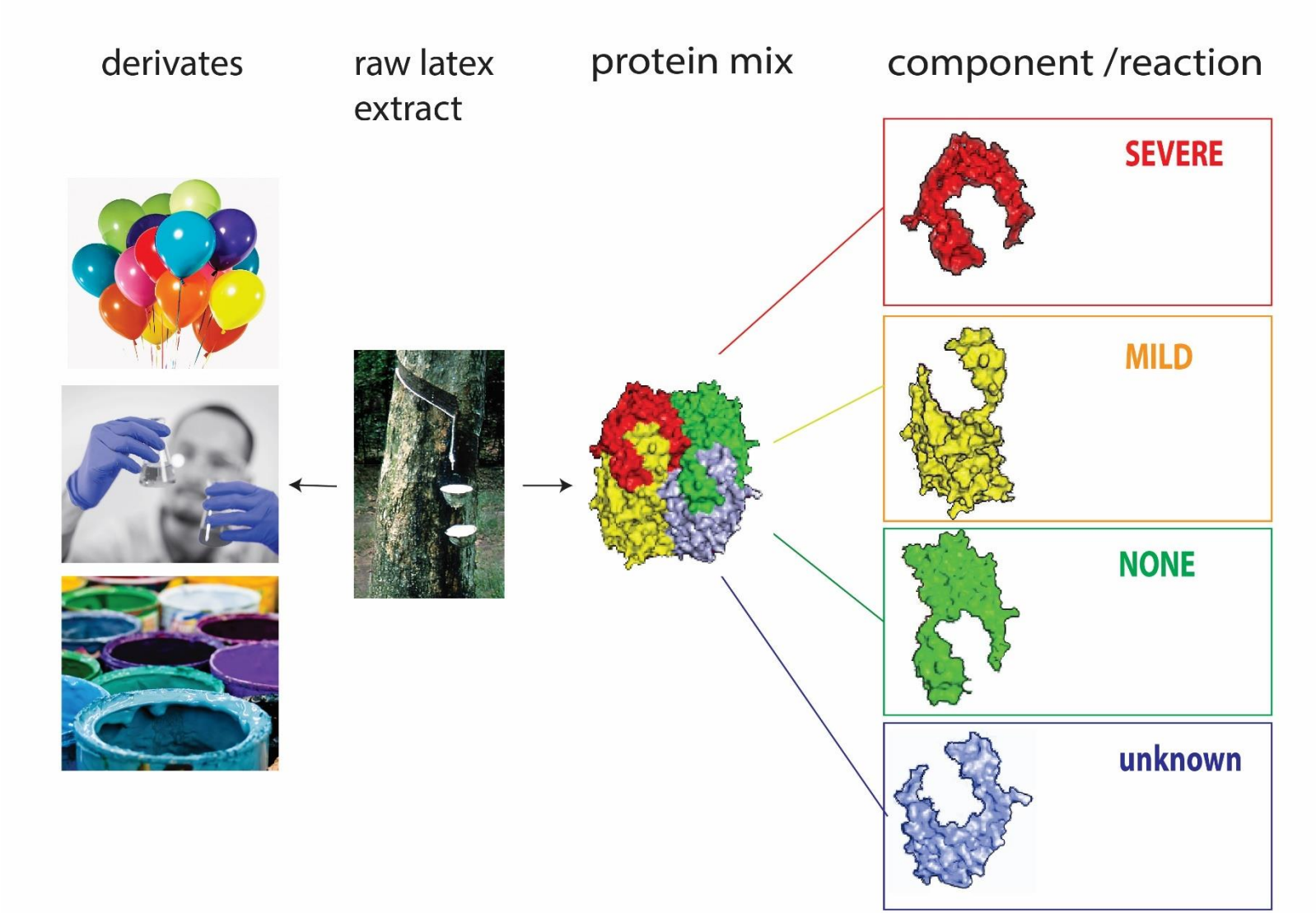
derivates
raw latex extract
protein mix
component /reaction
SEVERE
MILD
NONE
unknown

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Figure 2:

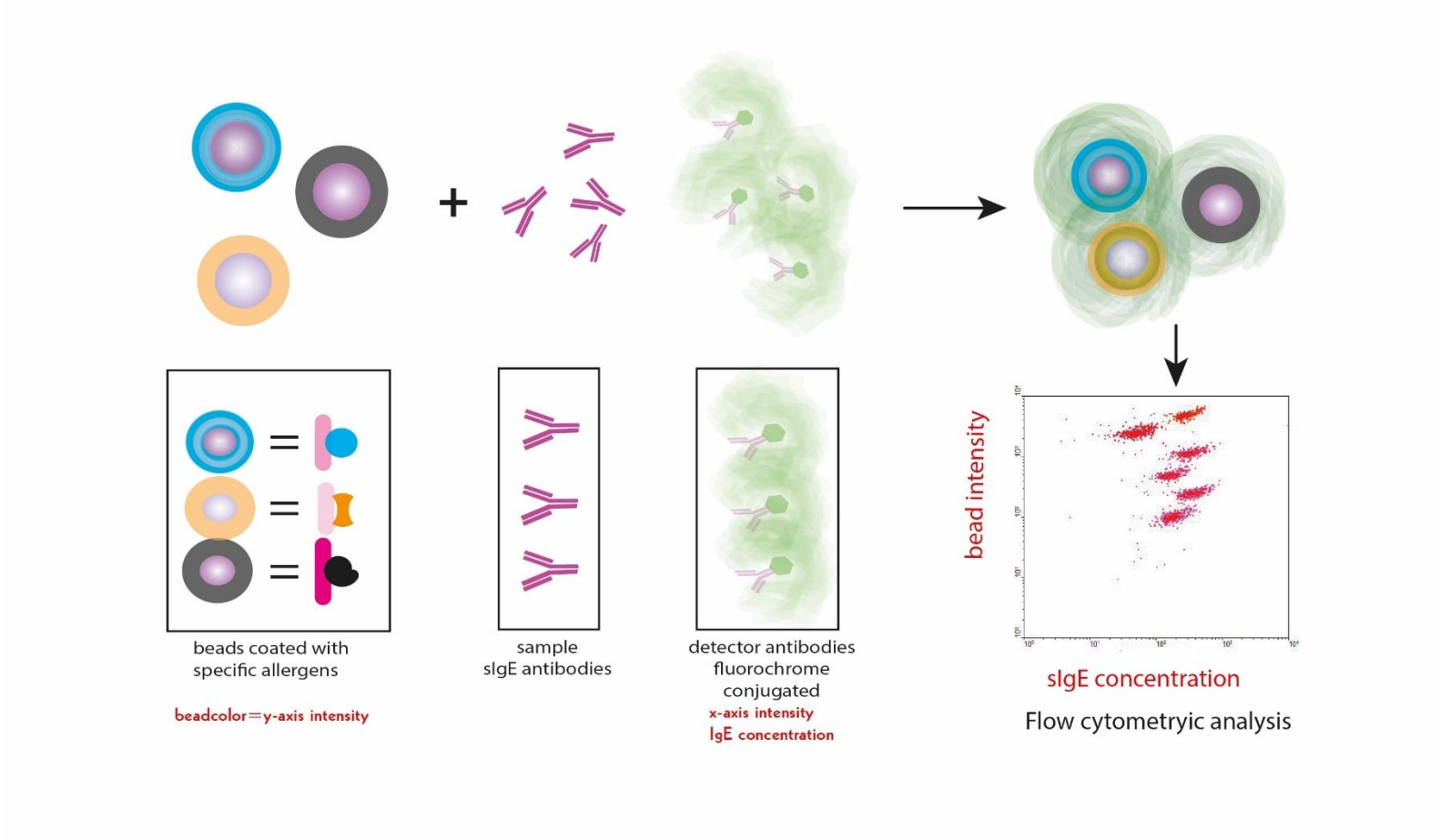
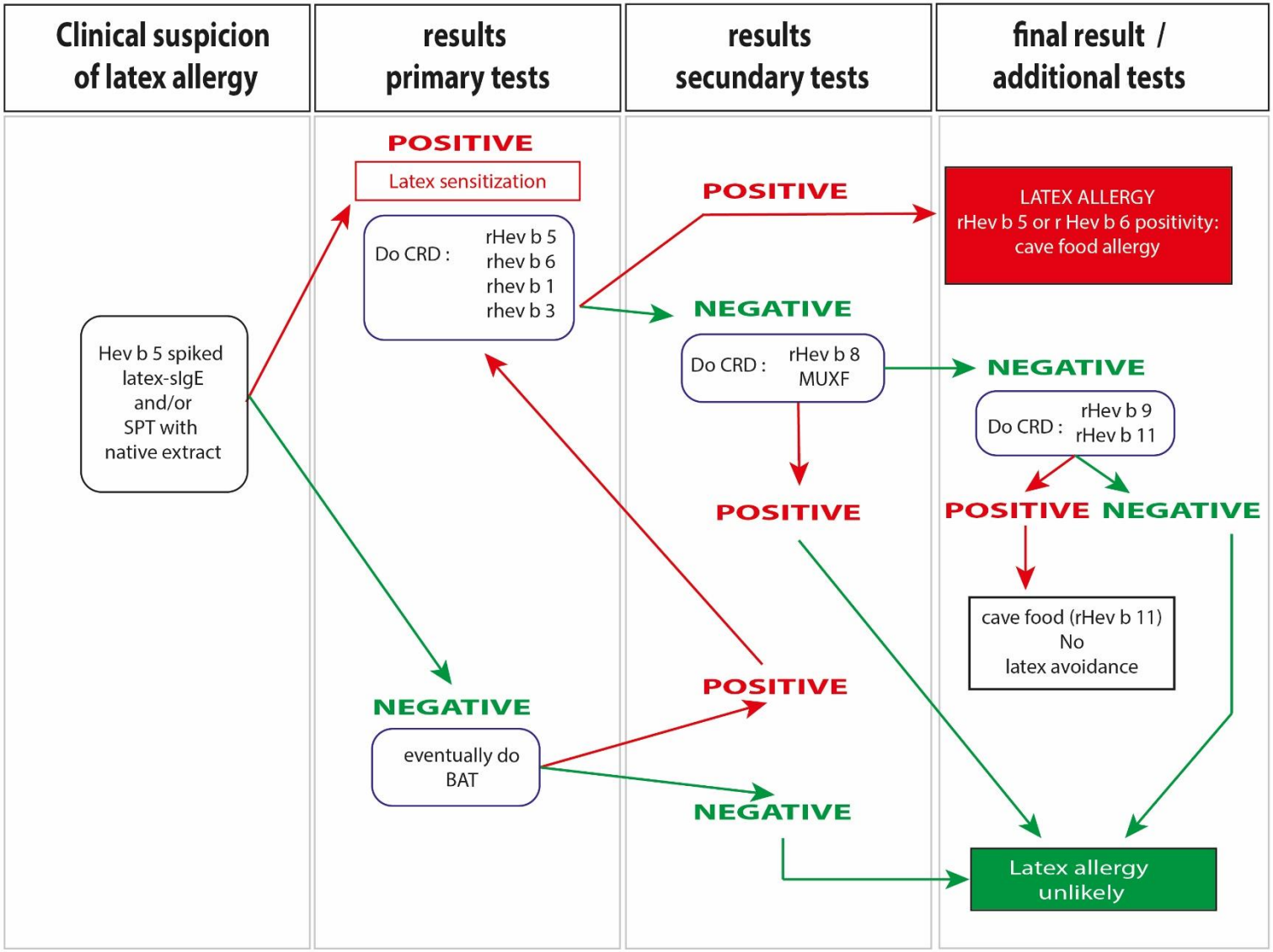

beads coated with
specific allergens
beadcolor=y-axis intensity
sample
sIgE antibodies
detector antibodies
fluorochrome
conjugated
x-axis intensity
IgE concentration
bead intensity
sIgE concentration
Flow cytometryic analysis

Figure 3


Clinical suspicion of latex allergy
results primary tests
results secundary tests
final result / additional tests
Hev b 5 spiked latex-sIgE and/or SPT with native extract
POSITIVE
Latex sensitization
Do CRD : rHev b 5 rhev b 6 rhev b 1 rhev b 3
NEGATIVE
eventually do BAT
POSITIVE
Do CRD : rHev b 8 MUXF
POSITIVE
NEGATIVE
POSITIVE
LATEX ALLERGY rHev b 5 or r Hev b 6 positivity: cave food allergy
NEGATIVE
Do CRD : rHev b 9 rHev b 11
POSITIVE
NEGATIVE
cave food (rHev b 11) No latex avoidance
Latex allergy unlikely

241

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