Open access • Journal Article • DOI:10.1016/J.JAIP.2018.01.004

# In Vitro Diagnosis of Immediate Drug Hypersensitivity During Anesthesia: A Review of the Literature. — Source link

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**Institutions:** University of Antwerp

**Published on:** 01 Jul 2018 - The Journal of Allergy and Clinical Immunology: In Practice (Elsevier)

**Topics:** Basophil activation and Basophil

# In vitro diagnosis of immediate drug hypersensitivity during anesthesia : a review of the literature

# *In vitro* diagnosis of immediate drug hypersensitivity during anaesthesia: a review of the literature

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Key words: allergy, anaesthesia, basophil activation, drugs, flow cytometry, immediate drug hypersensitivity reaction (IDHR), specific IgE (sIgE), beta-lactam, penicillin, MRGPRX2, neuromuscular blocking agents (NMBA), opiates, tryptase

The authors declare not having a conflict of interest nor financial support.

List of abbreviations:

α-gal: galactose-α(1,3)-galactose

BAT: basophil activation test

DPT: drug provocation test

FcεRI: high affinity receptor for sIgE

Hev b: *Hevea brasiliensis*

IHR: immediate hypersensitivity reactions

NMBA: neuromuscular blocking agents

PAPPC: p-aminophenyl phosphoryl choline

sIgE: specific IgE antibodies

THIQ: tetrahydroisoquinoline


## Abstract

Quantification of specific IgE (sIgE) antibodies constitutes an important measure to document anaesthesia-related immediate hypersensitivity reactions (IHR). However, only a few drug-specific assays are available and their predictive value is not known. In cases of non-IgE-mediated IHR, diagnosis might benefit from cellular tests such as basophil mediator release tests and basophil activation tests (BAT).

To review the potential and limitations of quantification of sIgE, mediator release and BAT in anaesthesia-related IHR a literature search was conducted using the key-words allergy, basophil activation, CD63, CD203c, diagnosis, drugs, hypersensitivity, flow cytometry, MRGPRX2, specific IgE antibodies, leukotrienes, histamine and tryptase; this was complemented by the authors' experience.

The drugs and compounds that have predominantly been studied are neuromuscular blocking agents (NMBA), β-lactams, latex and chlorhexidine. For sIgE, sensitivity and specificity varies between 38.5-92% and 92-100% for NMBA respectively and between 0-85% and 52-100% for

β-lactams. Specific IgE to morphine should not be used in isolation to diagnose IHR to NMBA nor opiates. sIgE for latex, and in difficult cases molecular diagnosis with quantification of sIgE to *Hevea* components constitute reliable diagnostics.

For drugs, sensitivity of BAT varies between 50 and 60%, specificity reaches 80-90%. Basophil mediator release tests seem to be abandoned and supplanted by BAT.

## Introduction

The gold standard to ascertain correct diagnosis of immediate hypersensitivity reactions (IHR) to drugs is a controlled drug provocation test (DPT) with the culprit compound(s). However, DPTs entail a risk of severe, life-threatening complications and can be contraindicated (e.g., patients having suffered from life-threatening reactions) or impossible (e.g., full-dose DPT in hypersensitivity to curarizing neuromuscular blocking agents (NMBA)). Moreover, the predictive value of DPTs is not known and DPTs might yield false negative results [1]. Therefore, the diagnostic approach of anaesthesia-related IgE-mediated IHR generally starts with history taking, thorough review of the anaesthetic/surgical notes complemented with skin testing and/or *in vitro* quantification of specific IgE (sIgE) antibodies. However, only a few drug-sIgE assays are available, and most of them have not been clinically validated. Furthermore, IHR might not *per se* involve IgE/FcεRI-cross-linking, but may also result from alternative pathways such as an off-target occupation of the Mas-related G-protein receptor MRGPRX2 [2, 3] that cannot be detected by a sIgE antibody assay. The development and validation of cellular tests such as basophil activation tests (BAT) would, to some extent, be promising in such cases.

The objective of this article is to review the literature on the value of serum tryptase, histamine, commercially available drug-sIgE assays and basophil activation tests such as mediator release tests and BAT in the diagnosis of anaesthesia-related IHR. Emphasis is put on some misconceptions, shortcomings, and unmet needs. As with any subject still beset by many questions, alternative interpretations, hypotheses, or explanations expressed here may not find universal acceptance.

## Quantification of serum tryptase

Although quantification of peak and baseline serum tryptase do not contribute to the identification of the culprit, serum tryptase has proven to be extremely valuable in diagnosing anaesthesia-related IHR, mainly to confirm mast cell degranulation and/or to rule out or confirm (clonal) mast cell disorders [4] and mast cell activation syndromes [5]. Currently, in the commercially available assay, total tryptase is quantified as the sum of continuously secreted baseline tryptase and β-tryptase released from degranulating mast cells (ImmunoCAP Thermofisher, Uppsala, Sweden). Since relevant increases have been observed way below the traditional decision threshold of 11.4 µg/L, it has been suggested to abandon this cut-off [6-8]. For example, an incremental threshold of 20% was shown to identify potential mast cell mediator release in an additional 14% of cases with peak tryptase between 5 and 14 µg/L and a further 15% with peak tryptase below 5 µg/L. Others have proposed that an increase of tryptase over baseline (24h after the acute event) levels is clinically relevant when it exceeds 2 + 1.2 x baseline [9, 10]. Although in the study by Sprung *et al.*, quantification of peak tryptase was performed between 30 minutes and 4 hours from the event, it is recommend to take the peak sample as close to 60 minutes after the reaction as possible, and if not possible later samples should still be taken and compared with a baseline taken at a later date [11]. Alternatively, by comparing the two measurements, anaphylaxis could be ruled out even for acute tryptase values > 11.4 µg/L in cases of baseline hypertryptasemia [9]. Quantifying baseline tryptase has another additional purpose, as elevated baseline levels might be indicative for underlying (clonal) mast cell disorders [4] that might underlie severe IHR, particularly in men who do not demonstrate urticaria/angioedema [12]. Levels of β-tryptase > 1 µg/L indicate mast cell degranulation. However, this test is not commercially available. Quantification of plasma histamine, although highly sensitive, was inferior to quantification of serum tryptase for

discrimination between IgE-dependent and IgE-independent anaesthesia-related IHR [13]. Resuscitation manoeuvres by themselves appear not to modify mediator concentrations [14]. Alternatively, it is important to stress that an elevated peak tryptase measurement does not necessarily indicate mast cell activation [9, 15]. In chronic renal failure elevated "peak" serum tryptase [15] might result from mast cell hyperplasia due to slow elimination of stem cell factor [16]. Note that tryptase is not cleared by the kidneys [17]. Tryptase can also be elevated in critically ill patients without anaphylaxis [18] and victims of trauma [19]. False negative results mainly result from incorrect sampling time (ideally 60-90 minutes after onset of symptoms).

## Principles of quantification of drug specific IgE (sIgE) antibodies and BAT

Like tissue resident mast cells, basophils can be triggered in IgE-dependent and various IgE-independent ways. Cross-linking of the surface-bound high-affinity IgE receptor (FcεRI) generally occurs through (glyco)proteins, chemical allergens or auto-antibodies directed against the FcεRI receptor or membrane-bound IgE antibodies. Quantification of sIgE antibodies predominantly relies upon quantification of a drug-(hapten)-carrier antibody complex in which the secondary antihuman IgE is conjugated to an enzyme with colorimetric reading in the enzyme linked immunosorbent test (ELISA) or with a fluorescence reading in the fluorescent enzyme immunoassay (FEIA) [20]. However, only a limited number of drug-specific sIgE immunoassays are available and most of these assays have not been thoroughly validated, mainly as a result of the unavailability of sufficient numbers of patients and exposed or challenged control individuals.

An IgE-independent, activation will mainly result from coupling of surface receptors with endogenous (*e.g.* cytokines, anaphylatoxins, chemokines, IgG, neuropeptides) or exogenous (*e.g.* pathogen associated molecular patterns) elements. Amongst these receptors is the Mas-

related G protein receptor MRGPRX2 that can lead to a quick but rather transient mast cell degranulation [21] and appears to be involved in different mast cell-associated conditions including non-immune immediate drug hypersensitivity reactions [22, 23]. Recently, McNeil *et al.* [2] described the potential of MRGPRX2-related mast cell activation by various drugs containing a tetrahydroisoquinoline (THIQ) motif such as some fluoroquinolones and various NMBA. The MRGPRX2 receptor has subsequently also been incriminated in reaction towards opioids [24] and vancomycin [25]. Alternatively, other largely unknown pathways might also induce degranulation.

The foundations of current flow-assisted BAT were laid 25 years ago [26] and in the meantime the technique has largely supplanted older mediator release assays that rely upon difficult quantification of mediators released in the supernatant. Actually, the last reviews on mediator release tests date back to 2003 [27, 28]. To our knowledge, since then no large case-control studies including more than 15 patients and significant numbers of (exposed) control individuals on the application of the various mediator release tests have been published, except one report including patients who suffered from peri-operative hypersensitivity reactions resulting from various causes [29].

Traditional BAT relies upon a flow cytometric analysis of various activation and degranulation markers on the surface membrane. These changes can be detected and quantified on a single-cell level using specific monoclonal antibodies conjugated with different LASER-excitable fluorochromes. The technical principles and requirements of BAT have been detailed elsewhere [30]. Basophils are traditionally identified by markers such as CCR3 (CD193)/CD3, CD123/HLA-DR or IgE/CD203c. Of these markers, only CD203c, is lineage specific. After activation, the appearance and/or up-regulation of surface activation and/or degranulation markers, such as CD203c and/or CD63 is quantified. For a review on the applications and

limitations of the BAT in drug IHR see Mangodt et al [31]. Histamine release can also be quantified by flow cytometry [32] and the technique is applicable in IHR to drugs [33].

## β-lactams

The most studied sIgE assays are those for β-lactams, especially amoxicillin and benzyl penicilloyl. Although, several cases of positive sIgE results in IHR with negative skin tests have been described [34-38], sIgE assays for β-lactams, as shown in table 1, generally exhibit a poor sensitivity that decreases over time [39]. Besides these disappointing sensitivity data, there is increasing evidence supporting low specificity of the tests [35, 37, 40-43]. In some studies false positivity could have resulted from nonspecific binding in the solid phase assay as a result of elevated total IgE titres [41-44]. An alternative explanation for false-positive sIgE penicillin seems to be sIgE antibodies to phenylethylamine that test negative in a BAT [44]. In summary, sIgE antibodies to β-lactams seem of restricted value and should ideally not be used in isolation to exclude or confirm IHR to these antibiotics. In order to avoid misdiagnosis, these assays should be complemented with BAT, skin testing and, where appropriate a DPT [45, 46]. Table 2 summarizes the data of BAT in IHR to β-lactams. Up to now, 10 studies have investigated the BAT as a diagnostic in IHR to β-lactams, mainly to amoxicillin. Compared with the quantification of sIgE antibodies, BAT shows a higher sensitivity (about 50%) and specificity (approximately 90%). As for specific IgE, sensitivity of BAT to β-lactams is rather low and decreases over time but both tests can remain positive for years [39, 47]. Therefore, we cannot adhere to the recommendation by ENDA/EAACI drug allergy interest group not to perform drug-sIgE tests after three years [48], particularly as also skin test responsiveness decreases over time [39, 49]. Finally, it should be kept in mind that sensitisation to amoxicillin/clavulanate can also result from sensitization to clavulanic acid [50], which needs specific testing [51]. For a diagnostic algorithm for IHR to β-lactam antibiotic the reader is referred elsewhere [52].

## Neuromuscular blocking agents (NMBA)

In many countries, curarizing neuromuscular blocking agents (NMBA) represent a significant cause of anaesthesia-related anaphylaxis [53-57]. As indicated in our diagnostic algorithm for NMBA (figure 1), skin tests are the primary instrument to confirm IHR to NMBA and cannot be substituted by BAT or quantification of sIgE [58]. However, the predictive value of skin testing is not absolute thereby leaving room for additional *in vitro* tests. In the absence of readily available assays, for about 2 decades, several groups have tried to define the accuracy of various home-made NMBA-sIgE assays (table 3) [59-62]. At present, IHR to NMBA are serologically assessed indirectly through assays measuring IgE reactivity to tertiary and quaternary substituted ammonium structures that have been shown to be the major epitopes of NMBA [63, 64]. Most frequently applied methods are a choline chloride [59, 60, 65-70], a p-aminophenyl phosphoryl choline (PAPPC) [59, 65, 66, 71] and/or morphine-based assays [59-61, 71-76]. With respect to the ImmunoCAP FEIA for suxamethonium, rocuronium, atracurium and morphine the sensitivity and specificity for the individual NMBA-specific varies between 38.5-92% and 85.7-100%, respectively. Furthermore, it has been demonstrated that a morphine-based immuno assay is a valuable test to detect suxamethonium and rocuronium-reactive antibodies but not to depict atracurium-reactive antibodies [72, 74]. Quantifying IgE reactivity to tertiary and quaternary substituted ammonium structures to identify patients at risk or to document NMBA hypersensitivity [77, 78] might cause a large number of false positive results as they are prevalent in the general population [73, 74, 76]. Therefore, these assays cannot be recommended as a screening tool to identify patients at risk or to document NMBA hypersensitivity [77, 78]. For example, Leysen *et al.* [78], showed that a positive sIgE to morphine is not a contraindication for administration of atracurium and cisatracurium in patients with negative skin tests to these benzylisoquinolines. Explanations for these false-positive sIgE results are an elevated total IgE

[74] and intake of the opiate antitussive pholcodine [79]. Specific IgE antibodies to tertiary and quaternary substituted ammonium epitopes can remain positive for several years after the acute reaction [77, 80]. Alternatively, as recently stressed by Spoerl *et al.* [3], IHR to NMBA such as rocuronium might occur independently from IgE/FcεRI cross-linking and relate to MRGPRX2-mediated activation of mast cells [2] and, therefore, not be detected by sIgE assays.

Table 4 displays the data about BAT in IHR to NMBA. In general, sensitivity of the assay varies between 36 and 92%, whereas specificity reaches 95%. Importantly, BAT not only enables identification of the culprit drug but also provides the opportunity to study cross-reactivity and identify safe alternatives for future anaesthesia [81, 82]. Alternatively, as resting basophils barely express MRGPRX2, it is unlikely that traditional BAT using cells in steady state be of value in reactions occurring from off-target occupation of this receptor [83]. Our current policy is to offer BAT and sIgE to patients with negative or equivocal skin tests who suffered from severe anaphylaxis and in whom no alternative cause was demonstrable.

## Opiates and (semi-)synthetic opioids

Genuine IgE-mediated allergies to opiates (morphine, codeine) and semi-synthetic opioid pholcodine remain rare notwithstanding their frequent and universal use. Additionally, correct diagnosis is not straightforward, mainly because of uncertainties associated with measurement of drug-specific IgE antibodies and skin testing [84]. Recently, it has been suggested that the two commercially available sIgE assays for a *Papaver somniferum* (poppy seed) extract and morphine can add to the diagnosis of IgE-mediated opiate allergy [85, 86]. However, using DPT we were unable to confirm these data [87], mainly because of the high prevalence of sIgE antibodies to these compounds in an allergic population. This observation is highly relevant when facing patients for whom correct identification of the causative compound(s) is impeded because of simultaneous intake or administration of different agents,

*e.g.*, during general anaesthesia. Erroneous opiate allergy diagnosis might not only entail unnecessary avoidance measures but also, most importantly, ultimately put patients at risk by overlooking alternative diagnoses such as an allergy to rocuronium or suxamethonium. For the time being the sole *in vitro* method to document opiate allergy is the basophil activation test, as basophils from opiate tolerant individuals, unlike their cutaneous mast cells, are unresponsive to opiates [87, 88]. Moreover, negative BAT, along with negative skin testing for different NMBA and negative provocation tests for the structurally almost similar opiates suggest that these drugs are probably safe in pholcodine hypersensitivity [88]. Therefore, for opiates and semi-synthetic opioids we recommend to start with BAT, and, if negative, a DPT. In contrast, for synthetic opioids such as fentanyl, sufentanyl and remifentanil diagnosis can start with skin testing eventually complemented with BAT and/or DPT for difficult cases [84, 89].

## Chlorhexidine

Chlorhexidine, a cationic bisguanide antiseptic and disinfectant, is used as the (di)acetate or (di)glucuronide salt. These chlorhexidine salts can trigger irritant dermatitis, allergic contact dermatitis [90], IDHR (including life-threatening anaphylaxis) [91-94] and even a combination of both, contact dermatitis and IDHR [95]. For a traditional arbitrarily chosen decision threshold of 0.35 kUA/L, the sensitivity of sIgE chlorhexidine varied between 84.2-91.6% and specificity between 93.7-100%. For a ROC-generated threshold of 0.20 kUA/L sensitivity was 94.1% and specificity 90.7% [93, 94]. As for β-lactam [42-44] and NMBA [74], raised total IgE levels were shown to have an impact on chlorhexidine sIgE measurement at levels higher than 500 kU/L and more particularly at levels higher than 2,000 kU/L [94]. Recently, it was demonstrated the optimal sampling time for sIgE chlorhexidine was between 1 and 4 months [96], but sIgE might persist for years [55].

## Latex

Another significant cause of anaesthesia-related anaphylaxis is *Hevea* latex (*Hevea brasiliensis; Hev b*). Diagnosis of latex allergy mainly relies upon skin testing and/or various in vitro tests. However, correct diagnosis of IgE-mediated allergy to natural latex is not always straightforward, mainly because of the false-positive sIgE results [97, 98], especially in patients suffering from grass and weed pollen allergy who are sensitized to cross-reactive carbohydrate determinants and/or profilin Hev b 8 [99-103]. Therefore, in a significant number of patients additional tests such as skin tests, component resolved diagnosis [100-103] and eventually BAT [98, 104-107] might be required to establish correct diagnosis. For a review on component resolved diagnosis the reader is referred elsewhere [108].

## Other agents

Bovine gelatine constitutes the active component in certain plasma substitutes, haemostatic sponges and can be present in various other drugs such as vaccines. Since the first descriptions of the allergenicity of gelatine [109], IgE-mediated IHR to this compound, including fatal anaphylaxis, have been increasingly reported. Today, 2 distinct types of IgE-mediated bovine gelatine allergy are recognized. First, genuine gelatine allergy that results from sensitization to the protein part of the molecule. Second, gelatine allergy resulting from a sensitization to a glycan moiety of the molecule, *i.e.* galactose-α(1,3)-galactose (α-gal) [110-112], as first described by Chung *et al.* [113] and Commins *et al.* [114]. To our knowledge, there are no studies that have determined the diagnostic accuracy of sIgE gelatine. However, it is of note that patients with life-threatening anaphylaxis to gelatine as a result of α-gal sensitization are generally overlooked by traditional gelatine-sIgE assay and need additional testing including quantification of α-gal specific IgE antibodies and gelatine skin testing [110-112].

## Discussion

Correct management of anaphylaxis during anaesthesia requires a multidisciplinary approach with prompt recognition and treatment of the attending anaesthesiologist, quantification of peak tryptase, and subsequent determination of the responsible agent(s) with strict avoidance of all incriminated and cross-reactive compounds. From this review it emerges that diagnosis of anaphylaxis during anaesthesia is not always straight forward. It can be hindered as a broad spectrum of different drugs can elicit heterogeneous immune and non-immune hypersensitivity reactions. Problems are certainly compounded as multiple drugs are administered simultaneously and non-anaesthesia related drugs or compounds (e.g. premedication, disinfection, antibiotic rinsing by surgeon, lymph node mapping) can also be the cause of an IHR. Nevertheless, diagnostic work-up should be offered to all patients with a clinical suspicion of anaesthesia-related IHR, irrespective of the grade of severity. Evaluation should comprise all agents the patient was exposed to and it should be kept in mind that a patient might demonstrate multiple sensitizations. In an own survey of over 650 patients double sensitization occurs in approximately 8% of the cases (unpublished data).

From this review it appears that drug, chlorhexidine and latex-sIgE antibody testing can provide useful information but can rarely be applied as solitary diagnostic tests to exclude or document IHR, as they lack 100 percent predictive values. For β-lactam determinants the main issue is poor sensitivity, which could not be increased without significant loss of specificity [42]. For NMBA, drug-specific IgE tests seem to attain acceptable sensitivity and specificity, provided the application of drug-specific cut-offs [74, 94]. Although quantification of sIgE to morphine appears to be a reliable biomarker of sensitization to tertiary and quaternary ammonium structures, IgE reactivity to this compound in general and allergic population is as high a 5-10%. Therefore, the test should not be applied in isolation to diagnose IHR to NMBA or opiates. With respect to the unsatisfactory sensitivity of some tests it has been argued that

this observation relates to the time-interval elapsed between the acute reaction and testing. Although we agree that late testing can result in lower sensitivity we do not adhere to the recommendation of the ENDA/EAACI Drug Allergy Interest Group. Based upon a single publication about negativation of sIgE to β-lactams [39], further use of drug-sIgE is dissuaded when the time-interval exceeds 3 years in their Position Paper [48]. However, this is not our experience [77] and drug-sIgE may persist as long as 5-30 years [49, 80]. With respect to the low specificity of some tests it is reemphasized that correct interpretation of sIgE results requires taking into account total IgE values [42, 74, 94]. Whether the introduction of sIgE/total IgE ratio's increases specificity [42] remains to be confirmed. We currently do not apply these ratio's.

Since the earliest days of the BAT it was obvious that this technique would become an asset in the allergological diagnostic instrumentation to document IHR, particularly when diagnosis cannot be established by other means. Moreover, it is anticipated that BAT using "conditioned" basophils expressing the MRGPRX2 receptor might become an easy accessible instrument to study and diagnose IgE/FcεRI-independent IDHR that result from off-target occupation of this receptor. However, additional collaborative large-scale studies are needed to verify whether the BAT fulfils this promise, to optimize and harmonize the protocols, to avoid instigation of cynicism and scepticism, and to enable and justify its entrance in routine diagnostic application.

**Acknowledgement**

EDG is a Senior Clinical Researcher of the FWO (1800614N), SV is a Senior Clinical Researcher of the FWO (1804518N), VGAL is a fellow of the FWO (1113617N).

## Conflict of interest

The authors declare no conflict of interest related to this publication

| Table 1: Specific IgE to β-lactams | | | | | | |
|---|---|---|---|---|---|---|
| **Compound** | **Reference test** | **Assay** | **Sensitivity** | **Specificity** | **N** | **Reference** |
| Various β-lactams | H + ST | CAP-FEIA | BPO + AXO + peni G + AMP: 31.8% | BPO + AXO + peni G + AMP: 88.6% | 58 | [115] |
| Various β-lactams | H ± ST ± DPT | CAP-FEIA | BPO: 32%<br>AXO: 43%<br>BPO+AXO: 50% | BPO: 98%<br>AXO: 98%<br>BPO+AXO: 96% | 129 | [116] |
| Various β-lactams | H ± ST ± DPT | CAP-FEIA | BPO: 10-68%<br>AXO: 41-53% | BPO: 98%<br>AXO: 95% | 410 | [38] |
| Various β-lactams | H | CAP-FEIA | 37.9% | 86.7% | 58 | [117] |
| Various β-lactams[1] | H ± ST ± DPT | CAP-FEIA<br>RAST[2] | 0-25%[2]<br>42.9-75%[2] | 83.3-100%[2]<br>66.7-83.3%[2] | 45 | [34] |
| Various β-lactams | H ± ST | CAP-FEIA<br>CAP-FEIA | 85%[3]<br>44%[4] | 54%[3]<br>80%[4] | 176 | [42] |
| Various β-lactams | H ± ST | CAP-FEIA | 66% | 52% | 293 | [43] |
| Amoxicillin | H + ST + DPT | CAP-FEIA | 19% | NA | 57 | [51] |

[1] home-made assay, [2] sensitivity and specificity vary according to clinical manifestations, [3] for a threshold of 0.10 kUA/L, [4] for a threshold of 0.35 kUA/L.
H: history, ST: skin test, DPT: drug provocation test, N: number
CAP-FEIA: fluorescence enzyme immunoassay available from Phadia Thermofisher. RAST: radio allergo sorbent test.
Peni G: penicillin G, AMP: ampicillin, BPO: benzyl penicilloyl, AXO: amoxicillin
Note that there is no IgE for clavulanic acid available

| Table 2: BAT in immediate β-lactam hypersensitivity | | | | | | |
|---|---|---|---|---|---|---|
| **Stimulus** | **Reference test** | **Activation marker** | **Sensitivity (%)** | **Specificity (%)** | **Number of patients and controls** | **Ref.** |
| β-lactam | H | CD63 | 50 | 93 | 88 | [117] |
| β-lactam | H + DPT[1] | CD63 | 39 | 93 | 53 | [118] |
| β-lactam | H ± ST ± IgE ± DPT | CD63 | 49 | 91 | 110 | [119] |
| Amoxicillin | H ± ST | CD203c<br>CD63 | 52<br>22 | 100<br>79 | 41 | [120] |
| β-lactam | H | CD63 | 50 | 89-97 | 262 | [121] |
| β-lactam | H ± ST ± IgE | CD63-CCR3<br>CD63-IgE | 55<br>53 | 100 | 39 | [122] |
| Amoxicillin | H | CD63 | 29 | / | 14 patients,<br>no controls | [123] |
| Amoxicillin | H ± ST ± DPT | CD63 | 50 | / | 61 patients, number of controls not mentioned | [124] |
| Amoxicillin | H ± ST | CD63 | 50 | / | 30 patients | [125] |
| Amoxicillin<br>Clavulanic acid | H ± ST ± DPT | CD63 | 47<br>62 | 93<br>89 | 57<br>58 | [51] |
| Cefazolin | H + ST | CD63<br>CD203c | 33<br>67 | 94<br>94 | 16 patients,<br>17 controls | [47] |
| For legend see table 1. | | | | | | |

**Table 3: Specific IgE to NMBA and substituted ammonium structures**

| Compound | Reference test | Assay | Sensitivity | Specificity | N | Reference |
|---|---|---|---|---|---|---|
| Various NMBA | H ± ST | RIA<br>RIA<br>RIA | PAPPC: 97%<br>MOR: 83%<br>QAS: 86% | PAPPC: 97%<br>NA<br>NA | 75 | [59] |
| Various NMBA | H + ST | RIA<br>RAST<br>RAST | QAS: 87.9%<br>SUC: 66.7%<br>Alcuronium: 40.7% | NA | 83 | [60] |
| Various NMBA | H + ST | RIA<br>RIA | MOR: 85%<br>NMBA-specific: 52% | 98% | 118 | [72] |
| Various NMBA | H + ST | CAP-FEIA<br>CAP-FEIA | SUXA: 38.5%<br>MOR: 67.7% | SUX: 96.3-99.6%<br>MOR: 90-95% | 866 | [73] |
| Rocuronium[1] | H + ST | CAP-FEIA | SUXA: 72%[2]<br>SUXA: 60%[3]<br>ROCU: 92%[2]<br>ROCU: 68%[3]<br>MOR: 88%<br>PHOL: 86% | SUXA: 100%[2]<br>SUXA: 100[3]<br>ROCU: 93%[2]<br>ROCU: 93%[3]<br>MOR: 100%<br>PHOL: 100% | 82 | [74] |
| Rocuronium | H + 2 tests* | CAP-FEIA | ROCU: 83% | ROCU: 72% | 66 | [77] |
| Various NMBA[1] | H + ST | CAP-FEIA | QAM[4]: 87.7% | QAM[4]: 90.7% | 168 | [76] |
| Atracurium[1] | H + ST | CAP-FEIA | SUXA: 28.6%<br>ATRA: 57.1%<br>MOR: 14.2% | SUXA: 85.7%<br>ATRA: 100%<br>MOR: 85.7% | 78 | [75] |

* For validation purposes, diagnosis of rocuronium allergy was considered definite when at least 2 out of 3 tests (skin test, BAT, sIgE) were positive, as rocuronium challenges at full dose are not possible for obvious reasons.

[1] applying ROC-generated drug-specific thresholds, [2] for a ROC-generated threshold of 0.11 kUA/L for suxamethonium and 0.13 kUA/L for rocuronium, [3] for a traditional threshold of 0.35 kUA/L.

[4] "optimized" morphine-based assay

H: history, ST: skin tests, RIA: radio immunoassay, RAST: radio allergosorbent test, CAP-FEIA: fluorescence enzyme immunoassay available from Phadia Thermofisher, PAPPC: p-aminophenyl phosphoryl choline, MOR: morphine, QAS: quaternary ammonium structure, SUC: succinyl choline, SUX: suxamethonium, ROCU: rocuronium, QAM: quaternary ammonium morphine, ATRA: atracurium. N: number. NA: not available.

| Table 4: BAT in immediate neuromuscular blocking agent (NMBA) hypersensitivity | | | | | | |
|---|---|---|---|---|---|---|
| **Stimulus** | **Reference Test** | **Activation marker** | **Sensitivity (%)** | **Specificity (%)** | **N** | **Ref.** |
| Various NMBA | H | CD63<br>CD45 | 64<br>43 | 81<br>96 | 26 | [126] |
| Various NMBA | H ± ST | CD63 | 54 | 100 | 56 | [127] |
| Various NMBA | H | CD63<br>CD203c | 79<br>36 | 100<br>100 | 31 | [128] |
| Various NMBA | H ± ST | CD63 | 36-86[1] | 93 | 92 | [129] |
| Rocuronium | H ± ST | CD63 | 92[2] | 100 | 22 | [81] |
| Various NMBA | H ± ST ± IgE | CD63 | 60 | 100 | 49 | [130] |
| Rocuronium | H + 2 tests* | CD63 | 80 | 96 | 104 | [77] |
| Various NMBA | H+ST | CD63 | 68 | 100 | 56 | [131] |
| Atracurium | H ± ST | CD63 | 71[3] | 100 | 75 | [82] |

* For validation purposes, diagnosis of rocuronium allergy was considered definite when at least 2 out of 3 tests (skin test, BAT, sIgE) were positive, as rocuronium challenges at full dose are not possible for obvious reasons.

NMBA: neuromuscular blocking agent, H: history, ST: skin test, N: number of patients and control individuals
[1] Increasing sensitivity when only the reactions that occurred during the 3 years were taken into account,
[2] taking into account the non-responders sensitivity is 76%,
[3] taking into account the non-responders sensitivity is 63%.

Figure 1: diagnostic algorithm NMBA

**Legend of the figure:**

NMBA: neuromuscular blocking agent
BAT: basophil activation test, sIgE: specific IgE
PPV: positive predictive value, NPV: negative predictive. Values are provided for rocuronium [77] using a ROC-calcultaed drug-specific decision threshold of 0.13 kUA/L [74].
[1] drug specific IgE available for suxamethonium, rocuronium and atracurium and should be using drug-specific thresholds. Specific IgE morphine is applied as a biomarker for sensitization to aminosteroids and suxamethonium (not useful for benzylisoquinolines).
[2] challenging at maximum 1/10$^{th}$ of therapeutic dose.

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