Controversies in drug allergy : in vitro testing

CONTROVERSIES IN DRUG ALLERGY. IN VITRO TESTING

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Abbreviations:

DHRs: Drug hypersensitivity reactions

SCARs: Severe cutaneous allergic reactions

STs: Skin tests

DPT: Drug provocation tests

SJS: Steven-Johnson syndrome

TEN: Toxic epidermal necrolysis

DRESS: Drug rash with eosinophilia and systemic symptoms

IR: immediate reactions

NIR: Non-immediate reactions

NSAIDs: Non-steroidal anti-inflammatory drugs

COX-1: Cyclooxygenase-1 enzyme

MRGPRX2: Mas-related G-protein receptor

NMBAs: Neuromuscular blocking agents

TCR: T cell receptor

HLA: Human leukocyte antigen

sIgE: Specific IgE

BAT: Basophil activation tests

LTT: Lymphocyte transformation tests

ELISA: Enzyme-linked immunosorbent assay

ELISpot: Enzyme-linked immunosorbent spot assay

RCM: Radio contrast media

FcERI: High affinity IgE receptor

Cyto-LTT: Cyto-lymphocyte transformation test

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ABSTRACT

Drug hypersensitivity reactions (DHRs), despite their low frequency, can be serious and result in life-long sequelae. The diagnosis is critical to avert future reactions and should not only entail the identification of the culprit drug(s) but also cross-reactive structures and safe alternatives. However, making the diagnosis can be complex. Reliable *in vitro* tests can offer the potential to improve our ability to accurately establish a diagnosis of DHR and influence medical decision making. Importantly, *in vitro* testing is frequently not performed as a test in isolation, but rather as a component of a diagnostic algorithm, along with additional tests, for evaluating whether a DHR is present or not.

There are several *in vitro* approaches depending on the pathomechanism involved for different endotypes of DHRs. However, only a restricted number are in the area of routine diagnosis, and many require an experienced laboratory and critical interpretation. Data from different studies indicate that *in vitro* tests, although generally highly specific, exhibit varying sensitivity highly dependent on the drug involved and, particularly for non-immediate reactions, on the clinical phenotype. Nevertheless, *in vitro* tests can complement traditional *in vivo* testing such as skin tests, especially in patients in whom the *in vivo* test shows negative or equivocal results and in severe cases in which drug provocation tests are contra-indicated or impossible. The main unmet need for *in vitro* tests for diagnosis of DHRs is the absence of validation in larger studies with a blinded comparison to a reference standard, in representative study populations.

INTRODUCTION

Drug hypersensitivity reactions (DHRs) can mechanistically be classified as allergic, either antibody mediated or T-cell mediated, and non-allergic when other mechanisms, including direct mast-cell degranulation are involved. Despite it's relatively low frequency DHRs can be serious (anaphylaxis, severe cutaneous allergic reactions (SCARs)) and might result in life-long sequelae.

Establishing that the adverse reaction is causal (rather than coincidental) and is associated with increased risk for adverse reaction with re-exposure is a critical aspect of management. Correct diagnosis is mandatory to avert future reactions and implies not only the identification of the culprit(s) but also of all cross-reactive structures and safe alternatives. In DHRs both underdiagnosis and overdiagnosis are potential problems[1]. Thus an accurate diagnosis is important: on one hand to avoid false positive results that incorrectly classify subjects as allergic. And false negative results can impair patient safety, especially in those with severe reactions.

In addition, correct diagnostic tests might help to identify those patients who might need desensitization and provide benefit in monitoring the effect of this process.

Diagnosis of DHRs starts with a detailed clinical history and thorough revision of the patient's record that is generally complemented with skin tests (STs). However, the predictive value of STs remains frequently unknown and their responses might not always be predictive for the clinical outcome of subsequent exposure[2]. Consequently, the definitive diagnosis of DHR might require additional investigations, mainly drug provocation tests (DPT). However, this procedure should not be performed in high risk patients (e.g. severe life-threatening anaphylaxis, Steven-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN) and DRESS (drug rash with eosinophilia and systemic

symptoms)) and should only be performed after meticulously balancing the potential for benefit with the potential for harm in each individual case[3]. Moreover, even DPT might not exhibit absolute predictive values[1, 4] and their entrance in mainstream use can be hampered for practical reasons.

Reliable *in vitro* tests offer the potential to improve our ability to accurately diagnosis DHR, particularly since these tests are readily available and can be easy to harmonize/standardize. The performance of these *in vitro* tests in the DHR diagnostic algorithm would appropriately be placed before or after the STs but always before DPT. Ideally, these tests should contribute to identify both offending compound(s) and safe alternatives, and enable investigation of the underlying pathomechanism(s).

Based on the emergence of new technologies, there is an expanding array of diagnostic tests available for diagnosis of a DHR. It is important for the clinician to understand the potential promise of in vitro tests for confirming or ruling out a diagnosis of DHR, and to recognize the limitations of currently available diagnostic tests. An ideal screening test is associated with optimal sensitivity and specificity, is safe to perform, and would have been validated in studies with a blinded comparison to a reference standard, in a representative study population [5]. An ideal diagnostic test will add additional information that will influence medical decision making: a positive test will lead to an increase in the probability that a diagnosis of DHR is present, such that appropriate treatment will be prescribed, drug avoidance will be recommended, and further diagnostic testing will be avoided; a negative test will lead to a reduction in the probability an DHR is present, such that the potential morbidity associated with treatment, suspension of the suspected drug, and further diagnostic testing can be avoided. Furthermore, the clinician should understand that in vitro testing is

frequently not performed as a test in isolation, but rather as a component of a diagnostic strategy, along with additional tests, for evaluating whether a DHR is present.

DHRs have been classified by different means: In practical terms, the time elapsed between intake/administration and onset of symptoms is still the most widely used basis for a subclassification. It differentiates immediate, accelerated, or delayed forms, and more recently as immediate reactions (IR) and non-immediate reactions (NIR). However, some controversies on the classification persist, e.g. as some reactions are overlapping[6] and as the appearance of similar symptoms may be due to quite different immune-mechanism[6].Thus, Pichler & Hausmann proposed a subclassification based on the mode-of-action of drugs: drugs forming an new antigen lead to allergic-immune reactions, while pharmacological interaction with immune receptors cause “p-i” and binding of drugs to proteins of effector cells result in “pseudoallergic” reactions[7].

For testing, it is important to consider that IR and NIR usually correspond to different immunopathological mechanisms, i.e. endotypes. In IR, mast cell degranulation via IgE or other mechanism occur; whereas in NIR, IgG and mainly T cells with specificity to the responsible drug play a role. This needs to be taken into account when selecting the *in vitro* diagnostic approaches to be applied[8]. Moreover, non-immune/non-allergic DHRs resulting in IR may be based on excessive inhibition of specific enzymes or off-target occupation of (immune) receptors. For example, hypersensitivity to non-steroidal anti-inflammatory drugs (NSAIDs) has been related to the inhibition of cyclooxygenase-1 enzyme (COX-1)[9, 10]. IR may also result from an off-target occupation of the Mas-related G-protein receptor (MRGPRX2) by drugs such as some

fluoroquinolones, neuromuscular blocking agent (NMBAs), and opiates[11-13]. In NIR, stimulation of drug-reactive T cells may occur not only by the covalent binding of the haptenic drugs to carrier proteins (allergic-immune stimulations), but also if the drug binds by non-covalent means to immune receptors (TCR, HLA: pharmacological interaction (p-i) with immune receptors). This may result in strong and restricted T cell stimulations, which can be measurable *in vitro* or with skin tests (patch, delayed intradermal testing)[7, 14].

There are several *in vitro* approaches for identifying the pathomechanism involved and evaluating the different DHRs[8]. One might differentiate between parameters, which measure a general activation, or assays, which try to identify the eliciting drug.

For IR, during the acute phase of the reaction, mediators such as tryptase, histamine or leukotrienes, should be quantified. Although these assays do not provide information about the culprit drug, they can point to involvement of mast cells and or basophils in DHRs. For confirmatory diagnosis and identification of the responsible drug, quantification of drug-specific IgE(sIgE) antibodies or direct/indirect basophil activation tests (BAT) can be performed. However, only a restricted number of drug-sIgE assays are currently available and the execution of BAT is still an area between research and routine diagnosis, and requires an experienced laboratory and critical interpretation.

For NIR or cell-mediated reactions, the *in vitro* approaches are lymphocyte transformation tests (LTT) which evaluates the drug specific proliferative response of T-lymphocytes. Moreover, several modifications of the LTT have been evaluated – all relying on a drug-specific stimulation of T cells and measurement of T cell reactions like upregulation of surface markers (CD25, CD69, HLA-DR), production of cytokines,

177 measured by enzyme-linked immunosorbent assay (ELISA) and enzyme-linked
178 immunosorbent spot assay (ELISpot),or measurement of cytotoxicity (51-Cr release,
179 granzyme B and/or granulysin measurement).

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181 **I. WHAT WE KNOW (ALREADY) AND WHAT WE HAVE (MOST RECENTLY) LEARNED**

182 The potential diagnostic utility and limitations of *in vitro* diagnostics in DHRs have been
183 reviewed elsewhere[8, 15-17]. Although *in vitro* tests can contribute to correct diagnosis of
184 DHRs, mainly IgE-dependent, it appears that there is significant room for improvement
185 before these assays can enter mainstream use. Actually, from these reviews it appears
186 that one of the most important hurdles hampering their application in daily clinical
187 practice is the absence of methodologically sound studies executed in significant
188 numbers of well-characterized patients and (exposed) control individuals that included
189 comparison with a reference (i.e., "gold") standard: DPT. However, as DPT is frequently
190 not performed due to obvious ethical reasons, in many cases convincing clinical history
191 and STs are reference tests. For the evaluation of the role of different tests for this
192 review we have included only studies that selected patients diagnosed using STs
193 and/or DPT and enrolled more than 5 patients and controls.

194 **I.a. In vitro tests for evaluating IR**

195 IR can be evaluated at the acute phase of the reaction or at the resolution phase. The
196 main goal of the *in vitro* test performed at the acute phase of the reaction is the
197 assessment of mast cell and/or basophil involvement and activation by quantification
198 of inflammatory mediators such as tryptase and histamine[18]. The determination of
199 peak tryptase during the acute phase with (or without in older studies) subsequent

quantification of baseline tryptase disclosed mast cell activation in a variable percentage of patients, 31-66.6%[19-21]. In the case of histamine, it is also quite variable in the different studies, 61-92% [19, 20]. However, different criteria were adopted for determination of mast cell activation and results could be restricted due to the short half-life of these molecules. In fact, the determination of histamine in blood is limited due to the circadian variations. Histamine and its metabolite N-Methylhistamine could be collected in 24 hours urine and some efforts are doing to their determination in an optimal time of collection. Regarding the values to consider positive results, today, it has been suggested that the arbitrary tryptase threshold of 11.4 µg/L should be revised and as an alternative, an international consensus equation (i.e., 1.2 x [basal value] + 2 µg/L) should be applied [22, 23],that has shown a high specificity, and positive predictive value although a moderate sensitivity and negative predictive value in perioperative anaphylaxis.

Confirmatory testing for identification of the responsible drug(s) should be performed in the resolution phase, and might imply quantification of the serum drug-sIgE and BAT. Specific IgE detection in serum is traditionally performed by a solid-phase immunoassay. By this approach, DHRs mainly to beta-lactam (BLs) (using the major determinant, penicilloyl), NMBAs, quinolones and biological agents have been evaluated in different studies, with a sensitivity of 62.9% and specificity of 89.2%[24]. These data vary depending on the drug involved with a range of positivity of 37.8-85% for BLs[25-30], 44-92% for NMBAs[31-36]and 26-68% for biological agents[26, 37].For BLs hypersensitivity evaluation, the inclusion of all suspected drugs has been associated with an increase in sensitivity as has been observed for patients with IR to the combination amoxicillin-clavulanic acid[38-40]. For NMBA, it has been shown that

diagnosis should not rely upon quantification of sIgE in isolation[41]and that sIgE to morphine can add to the diagnosis of anaphylaxis from rocuronium and suxamethonium but not atracurium[42].In the case of BAT, most studies have been performed in patients with immediate DHRs to BLs, NMBA, quinolones, dipyrone and radio contrast media (RCM).Although BAT generally display excellent specificity (exceeding 90%), sensitivity rates have been found to be highly variable and very dependent on the drug involved. The positivity rates ranged from 44.4-63% for BLs[40, 43-48], 36.1-91.7% for NMBAs[33, 35, 49-54], 42.3-65% for dipyrone[55-57], 57.1-76.5% for quinolones [58-61]and 46.2-62.5% for RCM[62, 63]. As noted above, critical appraisal of these studies reveals that assessment of the BAT might have been hampered by several considerations including magnitude of the study, non-proper and heterogeneous selection of patients and control individuals, pooling of drugs that might demonstrate specific optimal stimulation concentrations and cytotoxic effects, and application of arbitrary decision thresholds[64]. Moreover, BAT results can be affected by the use of a particular basophil activation marker, CD63 or CD203c, that specifically upregulates after the drug stimulation, as has been demonstrated for BLs, quinolones and recently for omeprazole[43, 61, 65], and also on the clinical manifestation of the patients[61, 66].

Alternatively, BAT offers the potential to study involvement of basophils, irrespective the activation pathway. IgE-mediated pathogenesis can be confirmed by evaluating the reduction of basophil activation after blocking with PI3Kinase inhibitors such as wortmannin[58, 67-69]. Moreover, preliminary, comparative studies between BAT and STs might help to discriminate between genuine IgE-mediated reactions[70]and reactions resulting from alternative IgE-independent effector cell activation, e.g. via off-target occupation of the MRGPRX2 receptor. As basophils, unlike cutaneous mast cell barely

express MRGPRX2, these cells will not respond in steady state conditions of traditional BAT. Therefore, unlike IgE-dependent reactions, DHR from MRGPRX2 occupancy will probably yield negative BAT[71].

In non-allergic hypersensitivity to NSAIDs, BAT show a low sensitivity when including one NSAIDs in the test[72-75] and although the sensitivity could increase when including several NSAIDs, the specificity decreases dramatically[72, 73, 76, 77]implying that BAT is not useful for evaluating these reactions[24, 69].

When using BAT, it should be taken into account that some patient's basophils cannot be activated after a positive control stimuli or specific drugs and are called "non-responders". These non-responders account for 10-20 % of the population and should be considered as false negative. It is attributed to differences in the intracellular signaling pathway of high affinity IgE receptor (FcERI). These patients usually have positive skin tests, indicating basophil, and not mast cell compromise[78]. A negative BAT result and positive STs can be due beside to non-responder basophils, to the genuine lower sensitivity of BAT of a MRGPRX2 off-target occupation. Recently Spoerl et al have proposed to reclassify reactions to NMBA as off-target reactions [79]however, this cannot be entirely correct since a single drug (class) might actually have different endotypes (with similar phenotype)[13, 80-82].

I.b. In vitro tests for evaluating NIR

Regarding *in vitro* tests for evaluating NIR, additional limitations exist, as they include very heterogeneous clinical presentations (phenotypes), with different degrees of severity and variable underlying immunological mechanisms which impair the identification of a unique biomarker to be determined in routine *in vitro* tests for the

early diagnosis of the disease or the identification of the culprit drug. Most data are mainly available from classical LTT with [3H] thymidine incorporation as a measure of T cell proliferation and show a global sensitivity of 56.1%, and specificity of 93.9%. Interestingly these data are strongly influenced by the type of reaction with mild and moderate reactions showing higher sensitivity (57.9-88.8%)[83-86]and specificity (92.8-100%)compared to data obtained in severe bullous reactions with sensitivity ranging from 25-75% and specificity from63-100%[24, 87-90].Moreover, also within each type of reaction the positivity will depend on the drug tested with higher results when evaluating anticonvulsant induced-DRESS[90-94]than SJS/TEN[88, 91].

In recent years, based on the need to focus on the effector response[95], other *in vitro* tests, which are modification of LTT, have been used. In these tests, cells are stimulated with the culprit drug and if activated they will release cytokines (IL-4, IL-5, IFN-γ) or cytotoxic markers (granzyme B, granulysin) which can be detected by ELISpot, beads assay/flow cytometry or ELISA. These tests have shown to be valuable in evaluating drug-induced-SCARs where ELISpot, measuring the number of cells producing IFN-γ or IL-4,has shown a higher level of positivity,82% compared to only 50% with LTT[87]. Moreover, analyzing different studies, it has been observed that ELISPOT measuring IFN-γ producing cells didnot increase sensitivity compared to LTT when evaluating patients with DRESS, but markedly increased sensitivity in patients with SJS/TEN from 35% to 71% with no changes in specificity (96%)[96].Furthermore, as demonstrated by Porebski et al in patients with drug induced SJS/TEN, the combination of results obtained by different *in vitro* approaches evaluating inflammatory mediators in effector cells increases the overall *in vitro* sensitivity to 80% maintaining the high specificity, 95%[88]. Based on the improved sensitivity by combining

the analysis of cytokines with cytotoxicity, the Bernese group recently developed the "Cyto-LTT", which combines measurements of IL-5, IL-13, IFNγ with cytotoxicity (granzymeB and granulysin): It seems to be far more sensitive (>80%) than the usual, proliferation based LTT in NIR like maculopapular exanthems, acute generalized exanthematous pustulosis and DRESS and is still affordable for routine diagnosis as it is based on a beads assay[97]it avoids the use of radioactivity, one of the main obstacles for using LTT. Thus, the new Cyto-LTT seems to open new possibilities for routine testing of T cell mediated NIR.

In last decades, different studies have indicated associations between some HLA alleles and increase risk of suffering SCARS particularly in those reactions induced by abacavir, carbamazepine, and allopurinol. In this sense, HLA-B*57:01 has been associated with a severe drug hypersensitivity reaction induced by abacavir with a sensitivity from 45.5-80% and specificity of 97.6-99%) demonstrating that this screening reduces the prevalence of abacavir-induced hypersensitivity[98-100]. In the case of carbamazepine-induced SJS/TEN, HLA-B*15:02 has been strongly associated in Asian populations[101-104]and its determination has been recommended by European Medicines Agency and U.S. Food and Drug Administration in at-risk populations[105]. The HLA-B*58:01 allele has been associated to allopurinol-induced DRESS and SJS/TEN[106, 107]although its usefulness is not currently clear[105, 108].

II. WHAT IS (STILL) CONTROVERSIAL AND/OR DONE DIFFERENTLY IN DIFFERENT INSTITUTIONS OR GEOGRAPHICAL AREAS.

Although there is a general consensus about the need for better *in vitro* tests for diagnostic evaluation of DHRs, currently there are in consistencies in their use, with recommendations for these assays varying depending on the geographical area and type of health system. Regarding the sIgE immunoassays, in some European countries they are recommended for evaluating IR to BLs, NMBAs, chlorhexidine, and biological agents[8]but only for penicillins in the United States[109]. BAT is only recommended in Europe for patients who presented a severe life-threatening reaction or when ST are not available or yield equivocal or negative results[69].

As mentioned above, the BAT is not useful for evaluating non-allergic hypersensitivity to NSAIDs[72, 73, 110], although it is propagated by some commercial companies.

A potential role for BAT has recently been shown for monitoring response during desensitization to chemotherapeutics, with the possibility that BAT could serve as a biomarker for rapid drug desensitization[111, 112] and to predict breakthrough reactions during desensitization[112]. Additional studies are warranted to confirm these promising data.

With regards to NIR there is agreement about the urgent need for further studies since sensitivity of *in vivo* testing (patch testing or intradermal testing with delayed reading) is disappointingly low, particularly in SCARs where no DPT are possible because of obvious ethical reasons. However, as SCARs are rare, it is likely that validation and harmonization of these tests will require collaborative studies.

III. WHAT CONSENSUS RECOMMENDATIONS CAN BE MADE NOW?

Data from different studies indicate that *in vitro* tests, although generally highly specific, exhibit varying sensitivity. This sensitivity is highly dependent on the drug involved and, especially in NIR, on the clinical phenotype. Nevertheless, *in vitro* tests can complement traditional *in vivo* testing such as STs, especially in patients in whom the *in vivo* test shows negative or equivocal results and provided drug-specific thresholds are applied[31]. They can also be complementary to *in vivo* testing for the identification of cross-reactivity missed by STs[50].For IR, it has been proposed triple testing including STs, BAT or histamine release test and determination of drug-sIgE as a way to achieve a diagnosis when at least two out the three tests are positive [13, 64] although further studies are needed.

Finally, these assays can be valuable in severe cases in which DPT are contra-indicated. Therefore, their use is tailored to particular patients and there is not enough information to make a recommendation for all DHRs.

In vitro tests generally exhibit a favorable specificity. However, it is important to note that results of sIgE to morphine, a biomarker for sensitization to substituted ammonium structures, are frequently clinically irrelevant[31, 33, 41, 64]. The reason for these false-positive results to some extent relates to high titers of total IgE resulting in non-specific binding of IgE to morphine[31].Some concerns about specificity has also been found for sIgE to penicillins since some patients with suspected IgE-mediated hypersensitivity to penicillin and positive ImmunoCAP, can have sIgE to phenylethylamine, an allergenic structure related to penicillin, but different from the classical allergens[113]. These false-positive tests limit the ImmunoCAP value for the diagnosis of penicillin allergy[114].Alternatively, Vultaggio et al for BL [115] demonstrated

365 that high levels of total serum IgE can induce false-positive results to BL and that the
366 application of drug-sIgE/total IgE ratio's may correct the diagnosis[29].Whether the
367 principle of the drug-sIgE/total IgE ratio applies to other drugs or related compounds is
368 unknown.
369 BAT mirrors more closely the *in vivo* situation than traditional sIgE and might also
370 circumvent the issue of epitopes hidden in a solid phase assay (as shown for IgE to
371 quinolones[116].
372 Using drug metabolite(s) beside the native drugs or the right drug-carrier conjugates
373 could achieve an increase in sensitivity in some reactions, as has been demonstrated in
374 IR[68, 117] as well as in NIR[118]. Furthermore, and especially for NIR, it should be important
375 to focus on the right effector cells and biomarkers involved in each clinical entity[87, 88,
376 95, 119].
377 A major limitation of BAT to drugs is the need of fresh cells and the 10-20% no-
378 responders. Thus, it would be ideal to use patient's serum and passive sensitization of
379 donor basophils (or basophil/mast cell lines) as read out system. This approach has
380 been investigated in various labs: While the available cell lines were quite
381 disappointing, some success could be achieved with IgE stripped and resensitized
382 basophils of blood donors. e.g. in chlorhexidine allergy, 90% of direct BAT positive
383 patients reacted also in the passive BAT to chlorhexidine (Pichler WJ, personal
384 communication). Thus, while it is still an experimental procedure, it may have its role
385 under certain situations already now.
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IV. UNMET NEEDS

The main unmet needs for *in vitro* tests in the diagnosis of DHRs are that currently the tests are not robustly clinically validated and most of them are in the technical validation phase. Larger studies with well-characterized patients and controls are needed. The ideal situation to evaluate the role of *in vitro* tests to evaluate DHRs would be the inclusion of patients with a confirmed diagnosis of DHRs that in many cases would only be achieved after a DPT employed as a reference standard, with the understanding that for ethical reasons DPT cannot be performed in life-threatening reactions.

In real life, patient inclusion is based on clinical history and STs and sometimes only on clinical history when STs are not available or produce equivocal results.

Another limitation of many studies, as a result of the prevalence of DHRs, is the small sample size. Therefore, it is likely that multicenter studies will be needed, as these can benefit the harmonization of techniques and inclusion of sufficient numbers of patients/controls with more careful selection of phenotypes/endotypes.

Moreover, there is an urgent need for improvement *in vitro* testing since currently there is no method with optimal/sufficient sensitivity to diagnose DHRs. Future research should focus on:

- Determining the clinically relevant biomarkers by dissecting the effector immunological response (basophil, mast cells, lymphocytes,…).
- Using the optimal drug concentration of the test from a dose-response analysis, checking non-cytotoxic concentration particularly when using cellular tests.

- Determining the right thresholds for considering results as positive, by using ROC analysis.
- Determining the rate of non-responder cases in BAT.
- Determining the optimal time of performing the test in order to avoid false negative results. For IR and depending on the drug, close to the acute phase of the reaction; for NIR, at the acute phase or resolution phase depending on the clinical entity.
- Standardizing the different techniques for each in vitro testing and for the most common drugs eliciting hypersensitivity.
- Amplifying the *in vitro* immunological response (dendritic cells, TLR ligands,..)
- Combining results from multiple *in vitro* and *in vivo* tests.
- Performing studies on hypersensitivity reactions to a single drug or drug group, avoiding miscellanea of drugs and if possible focusing in a specific clinical manifestation mainly in NIR.
- Characterizing the drug metabolites involved in DHRs induction.

All this will encourage development of algorithms for identifying the best *in vitro* test approach for each patient according to the culprit drug, time elapsed since the reaction onset, and clinical manifestations.

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816

817 **Table 1. In vitro tests for evaluating different types of DHRs**
818

	Advantages	Limitations
Immediate DHRs at the acute phase: Markers for severity and type of reaction		
Tryptase determination	• Assessment of mast cell involvement	• Difficulties in performing the test in the right time kinetic of peak tryptase due to short half-life (30-120 min) • Comparison with basal levels is needed
Histamine determination	• Assessment of mast cell and/or basophil involvement	• Short half-life • Comparison with basal levels is needed • No commercial available
Immediate DHRs at the resolution phase: Identifying the relevant drug(s)		
sIgE by Immunoassay	• Identification of the culprit drug • Serum sample can be easily stored and transported	• Available for a limited number of drugs • Time interval from reaction is critical for sensitivity
Basophil activation test	• Identification of the culprit drug • Available for a wide panel of drugs	• Blood samples cannot be stored • Not useful for non-allergic DHRs • Time interval from reaction is critical for sensitivity
Non-Immediate DHRs at the resolution phase: Identifying the relevant drug(s)		
Lymphocyte transformation test	• Identification of the culprit drug Based on proliferation	• Highly dependent of the clinical entities • Low sensitivity in severe bullous skin reactions
Cytokine determination by ELISA, ELISPOT, bead assay	• Identification of the culprit drug Based on cytokine secretion;	• Highly dependent of the clinical entities • Low sensitivity in severe bullous skin reactions
Combined cytokine and cytotoxicity assays (cyto-LTT)	• Identification of the culprit drug based on cytokine production or cytotoxicity	• Increased sensitivity and still excellent specificity; may even help in diagnosis of part of SJS/TEN cases