

Controversies in drug allergy: *In vitro* testing



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Despite their low frequency, drug hypersensitivity reactions (DHRs) can be serious and result in lifelong sequelae. The diagnosis is critical to avert future reactions and should identify the culprit drug or drugs and safe alternatives. However, making the diagnosis can be complex and challenging. Reliable *in vitro* tests can offer the potential to improve 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. There are several *in vitro* approaches for the different endotypes of DHRs. However, only few are available for routine diagnosis, and many are restricted to research laboratories. *In vitro* tests exhibit varying sensitivity and specificity depending on the drug involved and the clinical phenotype. *In vitro* tests can complement skin tests, especially in patients with negative or equivocal skin test responses

inconsistent with the clinical presentation and in severe reactions in which drug provocation tests are contraindicated. The main unmet need for many *in vitro* tests for the diagnosis of DHRs is validation in larger studies with standardized controls that could harmonize diagnostic management between the United States, European Union, and other regions of the world. (J Allergy Clin Immunol 2019;143:56-65.)

Key words: Drug hypersensitivity, *in vitro* tests, diagnostic, IgE, T-cells, *p-i* concept, anaphylaxis, severe cutaneous reactions, proliferation, cytokine

Drug hypersensitivity reactions (DHRs) can mechanistically be classified as immune (allergic), either antibody mediated (IgE/IgG) or T cell mediated, and nonimmune (nonallergic) when other mechanisms are involved. Despite its relatively low frequency, DHRs can be serious (anaphylaxis and severe cutaneous allergic reactions [SCARs]) and can result in death or lifelong sequelae with decreased quality of life.

Establishing that the adverse drug reaction is causal (rather than coincidental) and associated with an increased risk for reoccurrence during re-exposure is a critical aspect of management. Moreover, correct diagnosis not only implies identification of the culprit drug but also investigation of all cross-reactive structures and a search for safe alternatives. In patients with DHRs, both underdiagnosis and overdiagnosis are potential problems.¹ Thus it is critical on one hand to avoid false-positive results that incorrectly classify subjects as allergic and, on the other hand, to reduce the numbers of false-negative results, which can severely impair patient safety, especially in those with severe reactions.

An accurate diagnostic test might also help identify patients who could benefit from desensitization and monitor the effect of this procedure. Moreover, the screening of some HLA alleles might be able to predict patients at higher risk for DHRs for specific drugs.

The diagnosis of DHRs starts with a detailed clinical history and thorough review of the patient's records complemented with skin tests (STs) when indicated. However, ST responses might not always be predictive of the clinical outcome of subsequent

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Abbreviations used

BAT:	Basophil activation test
BL:	β -Lactam
DHR:	Drug hypersensitivity reaction
DPT:	Drug provocation test
DRESS:	Drug rash with eosinophilia and systemic symptoms
IDHR:	Immediate drug hypersensitivity reaction
LTT:	Lymphocyte transformation test
MRGPRX2:	Mas-related G-protein receptor
NIDHR:	Nonimmediate drug hypersensitivity reaction
NMBA:	Neuromuscular blocking agent
NSAID:	Nonsteroidal anti-inflammatory drug
SCAR:	Severe cutaneous allergic reaction
sIgE:	Specific IgE
SJS:	Stevens-Johnson syndrome
ST:	Skin test
TEN:	Toxic epidermal necrolysis

exposure.^{2,3} Consequently, the definitive diagnosis of DHR might require additional investigations, such as controlled drug provocation tests (DPTs). However, DPTs should not be performed in high-risk patients (eg, those with Stevens-Johnson syndrome [SJS] or toxic epidermal necrolysis [TEN] and drug rash with eosinophilia and systemic symptoms [DRESS]). In patients with life-threatening anaphylaxis, DPTs after negative ST responses should only be performed after balancing the potential for benefit with the potential for harm in each case.⁴ Moreover, even DPTs might not exhibit absolute predictive values,^{1,5} and their mainstream use can be hampered by ethical and practical considerations.

Reliable *in vitro* tests offer the potential to improve the ability to accurately diagnose DHRs, particularly because these tests do not pose a risk to the patient. However, many of them require further technical and clinical validation and harmonization, as well as standardization of the protocols. The performance of *in vitro* tests in the DHR diagnostic algorithm would generally be placed before or after the STs but always before DPTs. Ideally, the performance of these tests should contribute to identifying both offending compounds and safe alternatives and enable investigation of the underlying pathomechanisms and possibly high risk markers.

Based on the development of new technologies, there is an expanding array of diagnostic tests that are becoming available for clinical use. 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, should have been validated in studies with a blind comparison to a reference standard in a representative study population,⁶ and will add information that will influence medical decision making. A positive response will lead to an increase in the probability that a diagnosis of DHR is present and to subsequent drug avoidance. A negative test response should lead to a reduction in the probability of the association between the investigated drug and the DHR, such that the potential morbidity associated with treatment, unnecessary avoidance of the suspected drug, and further diagnostic testing can be avoided.

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, differentiating immediate drug hypersensitivity reactions (IDHRs) from nonimmediate drug hypersensitivity reactions (NIDHRs). However, some controversies on the classification persist (eg, because some reactions are overlapping⁷ and because the appearance of similar symptoms might be due to quite different immune and even nonimmune mechanisms).^{7,8}

For testing, it is important to consider that IDHRs and NIDHRs usually correspond to different immunopathologic mechanisms (ie, endotypes). In patients with IDHRs, mast cell degranulation through IgE or other mechanisms occurs, whereas NIDHRs generally involve IgG and mainly T cells with specificity to the responsible drug. This needs to be taken into account when selecting the *in vitro* diagnostic approach to be applied.⁹ Moreover, nonimmune/nonallergic DHRs resulting in IDHRs can be based on excessive inhibition of specific enzymes or off-target occupation of (nonimmune) receptors. For example, hypersensitivity to nonsteroidal anti-inflammatory drugs (NSAIDs) has been related to the inhibition of COX-1.^{10,11} IDHRs can also result from an off-target occupation of the Mas-related G-protein receptor (MRGPRX2) by drugs, such as some fluorquinolones, neuromuscular blocking agent (NMBAs), and opiates.¹²⁻¹⁴ In patients with NIDHRs, stimulation of drug-reactive T cells can occur not only through the covalent binding of haptenic drugs to carrier proteins (allergic-immune stimulations) but also if the drug binds through noncovalent means to immune receptors (HLA: pharmacologic interaction [p-i] with immune receptors).^{8,15}

There are several *in vitro* approaches for identifying the pathologic process involved and evaluating the different DHRs.⁹ One might differentiate between assays that measure a general activation, tests that try to identify the eliciting drug, and genetic testing that can identify high-risk markers in the host, such as HLA haplotypes.

The potential diagnostic utility and limitations of *in vitro* diagnostics in DHRs have been reviewed elsewhere^{9,16-18} and are summarized in Table I. Although *in vitro* tests can contribute to correct diagnosis of DHRs, mainly IgE-dependent DHRs, it appears that there is significant room for improvement before these assays can enter mainstream use. Actually, from these reviews, it appears that one of the most important hurdles hampering their application in daily clinical practice is the absence of methodologically sound studies executed in significant numbers of well-characterized patients and (exposed) control subjects that included comparison with a reference (ie, “gold”) standard: the DPT. However, because DPTs are frequently not performed for ethical reasons, for the time being, in many cases a convincing clinical history and use of STs are considered the “reference test.” For evaluation of the role of different tests for this review, we have included only studies that selected patients receiving diagnoses based on STs, DPTs, or both and enrolled more than 5 patients and control subjects.

IDHRs

IDHRs can be evaluated at the acute phase of the reaction or at the resolution phase.

Acute phase

The main goal of the *in vitro* test performed during the acute phase of the reaction is assessment of mast cell and/or basophil

TABLE I. *In vitro* tests for evaluating different types of DHRs

	Advantages	Limitations
IDHRs at the acute phase: Markers for severity and type of reaction		
Tryptase determination	● Assessment of mast cell involvement	● There are difficulties in performing the test in the right time kinetic of peak tryptase caused by 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 test is available.
IDHRs at the resolution phase: Identifying the relevant drug or drugs		
sIgE by using an 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.
BAT	● Identification of the culprit drug ● Available for a wide panel of drugs	● Blood samples cannot be stored. ● BAT is not useful for nonallergic DHRs. ● Time interval from reaction is critical for sensitivity.
NIDHRs at the resolution phase: Identifying the relevant drug or drugs		
LTT	● Identification of the culprit drug based on proliferation	● Highly dependent on clinical entities ● Low sensitivity in severe bullous skin reactions
Cytokine determination by means of ELISA, ELISpot, and bead assay	● Identification of the culprit drug based on cytokine secretion	● Highly dependent on 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 and cytotoxicity	● Increased sensitivity and still excellent specificity; might even help in diagnosis of subset of patients with SJS/TEN

involvement and activation by means of quantification of inflammatory mediators, such as tryptase, histamine, and prostaglandins or leukotrienes.¹⁹ The determination of peak serum tryptase levels during the acute phase with subsequent quantification of baseline tryptase disclosed mast cell activation in a variable percentage of patient (31% to 67%).²⁰⁻²² In the case of histamine, it is also quite variable in different studies (61% to 92%).²⁰⁻²² This variability could be related to the severity of the reaction.^{23,24} Moreover, different definitions for mast cell activation have been used, and results depend on the half-life of these molecules. In fact, determination of histamine levels in blood is limited because of the circadian variations, and efforts are ongoing to determine an optimal time for collection before full validation. Regarding tryptase results, it has been suggested that the threshold of 11.4 µg/L should be revised, and as an alternative, an international consensus equation (ie, $1.2 \times [\text{Basal tryptase value}] + 2 \mu\text{g/L}$) should be applied,^{23,25} which has shown high specificity and positive predictive value but moderate sensitivity and negative predictive value in patients with perioperative anaphylaxis.

The determination of other inflammatory mediators can also have clinical utility. Urinary leukotriene E₄ has been associated with a high negative predictive value for identifying asthmatic patients with aspirin-exacerbated respiratory disease, such that compared with clinical parameters alone, the determination of urinary leukotriene E₄ levels combined with clinical parameters enhances the accuracy of the aspirin-exacerbated respiratory disease diagnosis.²⁶

Resolution phase

Confirmatory testing for identification of the responsible drug or drugs performed in the resolution phase can involve quantification of serum drug-specific IgE (sIgE) measurements and direct/indirect basophil activation tests (BATs).

Immunoassays

sIgE detection in serum is traditionally performed by using a solid-phase immunoassay; however, only a restricted number of drug sIgE assays are currently available. DHRs to β-lactams (BLs; using the major determinant, penicilloyl), NMBAs, quinolones, and biological agents have been evaluated in different studies.^{18,27} Performance varies depending on the drug involved, with a range of positivity of 38% to 85% for BLs,²⁸⁻³² 44% to 92% for NMBAs,³³⁻³⁸ 26% to 68% for biological agents,^{39,40} and 59% to 75% for platins.⁴¹ For BL hypersensitivity evaluation, the inclusion of all suspected drugs has been associated with an increase in sensitivity, as has been observed for patients with IDHRs to the combination of amoxicillin-clavulanic acid.⁴²⁻⁴⁴ For NMBAs, it has been shown that the diagnosis should not rely on quantification of sIgE in isolation⁴⁵ and that sIgE to the opiate morphine used as a marker for sensitization to tertiary and quaternary substituted ammonium structures can add to the diagnosis of anaphylaxis from rocuronium³⁸ and suxamethonium but not atracurium.⁴⁶ In the case of platins, it has been found that carboplatin sIgE measurement is very specific but less sensitive. In contrast, oxaliplatin sIgE had greater

sensitivity but lower specificity; moreover, patients with sIgE to oxaliplatin have a high degree of cross-reactivity to cisplatin and carboplatin.⁴¹

BATs

In the case of a BAT, most studies have been performed in patients with IDHRs to BLs, NMBA, quinolones, dipyrone, and iodinated radiocontrast media. Although BATs generally display specificity (>90%), sensitivity rates have been highly variable, depending on the drug involved. Positivity rates ranged from 44% to 63% for BLs,^{42,43,47-52} 36% to 92% for NMBAs,^{35,36,53-58} 42% to 65% for dipyrone,⁵⁹⁻⁶¹ 57% to 77% for quinolones,⁶²⁻⁶⁵ and 46% to 63% for radiocontrast media.^{66,67} Regarding platin BATs, a study has provided insight into the risk of breakthrough reactions during desensitization.⁶⁸

As noted above, critical appraisal of these studies reveals that assessment of the BAT might to some extent have been hampered by several considerations, including the magnitude of the study, heterogeneous inclusions, use of drugs that might demonstrate specific optimal stimulation concentrations but cytotoxic effects, and application of arbitrary thresholds.⁶⁹ Moreover, BAT results can be affected by the use of a particular basophil activation marker, CD63 or CD203c, that specifically upregulates after drug stimulation, as has been demonstrated for BLs, quinolones, platins, and recently for omeprazole,^{52,64,68,70} and on clinical presentation.^{64,71} Nevertheless, we believe that the BAT can be an important asset and merits further evaluation.

Alternatively, BATs offer the potential to study involvement of basophils, irrespective of the activation pathway. IgE-mediated pathogenesis can be confirmed by evaluating the reduction of basophil activation after blocking with phosphoinositide 3-kinase inhibitors, such as wortmannin.^{65,72-74} Moreover, preliminary comparative studies between BATs and STs might help to discriminate between genuine IgE-mediated reactions⁷⁵ and reactions resulting from alternative IgE-independent effector cell activation, such as through off-target occupation of the MRGPRX2 receptor. Because basophils, unlike cutaneous mast cells, barely express MRGPRX2, these cells will not respond in steady-state conditions of traditional BATs. Therefore, unlike IgE-dependent reactions, DHR from MRGPRX2 occupancy will probably yield negative BAT results.⁷⁶

BATs are not useful for evaluation of nonallergic hypersensitivity to NSAIDs.^{9,73} BATs show a low sensitivity when including one NSAID in the test,⁷⁷⁻⁸⁰ and although the sensitivity could increase when including several NSAIDs, the specificity decreases dramatically.⁷⁹⁻⁸²

When using BATs, it should be taken into account that in about 10% to 20% of patients, basophils cannot be activated after positive control stimuli or specific drugs and are called “nonresponders.” In these cases BAT results should be considered not interpretable. Nonresponsiveness is attributed to differences in the intracellular signaling pathway of the high-affinity IgE receptor (FcεRI), particularly expression of Syk, and in some patients basophils can revert to an activation state, showing cyclical activity.⁸³ These patients usually have positive ST responses, indicating mast cell but not basophil responsiveness.⁸⁴ A negative BAT response and positive ST responses can also result from lower sensitivity of BATs and/or an alternative MRGPRX2-dependent activation in skin mast cells.

NIDHRs

Regarding *in vitro* tests for evaluating NIDHRs, additional limitations exist because they include very heterogeneous clinical presentations (phenotypes) with different degrees of severity and variable underlying immunologic mechanisms, which impair the identification of a unique biomarker to be determined in routine *in vitro* tests for early diagnosis of the disease or identification of the culprit drug. *In vitro* approaches are cellular tests including determination of the cellular proliferative response in lymphocyte transformation tests (LTTs) and several modifications all relying on stimulation of drug-specific T cells and measurement of T-cell reactions like upregulation of surface markers, production of cytokines measured by using an ELISA or ELISpot, or measurement of cytotoxicity.

LTTs

Most data are from LTTs with tritiated thymidine incorporation as a measure of T-cell proliferation and show an overall mean sensitivity and specificity of 56% and 94%, respectively.²⁷ Interestingly, these data are strongly influenced by the type of reaction, with mild and moderate reactions showing greater sensitivity (58% to 89%)⁸⁵⁻⁸⁸ and specificity (93% to 100%) compared with data obtained in patients with severe bullous reactions (sensitivity range, 25% to 75%; specificity range, 63% to 100%).^{27,89-92} Positivity will depend on the drug tested, with higher results when evaluating anticonvulsant-induced DRESS^{89,93-96} than SJS/TEN.^{90,93} Use of tritiated thymidine incorporation to measure cell proliferation can produce false-positive results when stimulating cells with some drugs, as demonstrated with methotrexate.^{97,98}

Other tests

In recent years, based on the need to focus on the effector response,⁹⁹ other *in vitro* tests that are modifications of LTTs have been used. In these tests cells are stimulated with the suspected culprit drug, and cytokine release (IL-4, IL-5, and IFN-γ) or cytotoxic markers (granzyme B and granulysin) can be detected by using an ELISpot, bead assay/flow cytometry, or ELISA. These tests have been shown to be valuable in evaluating drug-induced SCARs, whereas ELISpot, measuring the number of cells producing IFN-γ or IL-4, has shown a greater level of positivity (82% compared with only 50% with LTTs).⁹² ELISpots measuring numbers of IFN-γ-producing cells did not increase sensitivity compared with LTTs when evaluating patients with DRESS but markedly increased sensitivity in patients with SJS/TEN from 35% to 71%, with no changes in specificity (96%).¹⁰⁰ In patients with drug-induced SJS/TEN, the combination of results obtained by using different *in vitro* approaches evaluating inflammatory mediators in effector cells increases the overall *in vitro* sensitivity to 80% maintaining the high specificity (95%).¹⁰¹

Based on the improved sensitivity achieved by combining the analysis of cytokines with cytotoxicity, the Cyto-LTT was developed recently, which combines measurements of IL-5, IL-13, and IFN-γ with cytotoxicity (granzyme B and granulysin). It seems to be more sensitive (>80%) than LTTs in patients with maculopapular exanthems, acute generalized exanthematous pustulosis, and DRESS. The test can be used in routine diagnosis because it is based on a bead assay,¹⁰² avoiding use of radioactivity, one of the main obstacles for using LTTs. Thus the

Cyto-LTT opens new possibilities for routine testing of T cell-mediated NIDHRs.

HLA markers

Recently, studies have shown the association between HLA alleles and an increased risk for SCARs, particularly in reactions involving abacavir, carbamazepine, dapsone, and allopurinol. HLA-B*57:01 has been associated with severe DHR induced by abacavir with a sensitivity of 46% to 80% and a specificity of 98% to 99%, demonstrating that screening reduces the prevalence of abacavir-induced hypersensitivity.¹⁰³⁻¹⁰⁵ In the case of carbamazepine-induced SJS/TEN, HLA-B*15:02 has been strongly associated in Asian populations,¹⁰⁶⁻¹⁰⁹ and in patients with other SCARs, such as DRESS, HLA-A*31:01 has been shown to be a predisposing factor.¹¹⁰ Its determination has been recommended by the European Medicines Agency and US Food and Drug Administration in at-risk populations.¹¹¹ In patients with dapsone-induced DRESS, associations with HLA-B*1301 have been found, indicating the importance of genetic screening before dapsone therapy for patient safety.¹¹²⁻¹¹⁴

The HLA-B*58:01 allele has been associated with allopurinol-induced DRESS and SJS/TEN,^{115,116} and its predictive value is being evaluated in different populations.^{111,117}

CONTROVERSIAL AREAS

There is general consensus about the need for better *in vitro* tests for diagnostic evaluation of DHRs. At the present time, recommendations for the current assays vary depending on the geographic area and type of health system. Regarding sIgE immunoassays, in some European countries they are recommended for evaluating IDHRs to BLs, NMBAs, chlorhexidine, and biological agents.⁹ In contrast, experts from the United States indicated that the current commercial immunoassay to determine sIgE levels to penicillin is not useful because of its suboptimal sensitivity and low concordance with STs and DPTs, probably because the assay only identifies IgE to the major determinant.^{118,119}

These *in vitro* tests generally exhibit a favorable specificity. However, there is some controversy about the importance of false-positive results of sIgE results, which will depend on the drug and therefore have a special influence in some geographic areas. Thus results of sIgE measurement to morphine, a biomarker for sensitization to substituted ammonium structures, are not consistent with the clinical presentation, with most sensitized patients presenting with no clinical symptoms.^{36,38,45,69} In some cases the reason for false-positive results relates to high titers of total IgE, resulting in nonspecific binding of IgE to morphine.³⁸ sIgE to penicillins in some patients with suspected IgE-mediated hypersensitivity to penicillin and a positive ImmunoCAP result can be directed to a cross-reactive epitope, phenylethylamine, an allergenic structure related to penicillin but different from the major and minor allergens.¹²⁰ These false-positive tests limit the ImmunoCAP value for the diagnosis of penicillin allergy.¹¹⁹ In addition, high levels of total serum IgE can induce false-positive results to BLs, and the application of drug sIgE/total IgE ratios is indicated.¹²¹ Whether the principle of the drug sIgE/total IgE ratio applies to other drugs or related compounds is unknown. Further studies to establish factors influencing false-positive results are needed.

Box 1. Expert consensus

- *In vitro* tests exhibit varying sensitivity depending on the drug involved and clinical phenotype.
- In the diagnostic strategy *in vitro* tests can complement *in vivo* testing, such as STs.
- These assays are of critical value in severe cases in which DPTs are contraindicated.
- BATs mirror more closely the *in vivo* presentation and clinical phenotype than traditional sIgE measurement.
- In desensitization to chemotherapeutics, BATs have a potential role for monitoring response and to predict breakthrough reactions.
- Inclusion of drug metabolites beside the native drugs or drug-carrier conjugates and determination of the effector response involved in each clinical entity could improve the sensitivity in some DHRs.

The BAT is recommended in European countries for patients who present with severe life-threatening reactions or when STs are not available or yielded equivocal or negative results.^{9,73} In the United States, however, no commercially available BAT assay has proved validity.^{5,122}

Another controversial issue is the role of BATs for evaluating nonallergic hypersensitivity to NSAIDs that has shown to be not useful when including patients with a confirmed non-IgE-mediated mechanism.^{79,80,123} There is actually a discussion about the basophil marker (ie, CD63 or CD203c) that better represents activation after culprit drug stimulation. In patients with quinolone-induced IDHRs the best sensitivity-specificity was obtained by using CD203c for moxifloxacin and CD63 for ciprofloxacin⁶⁴; in patients with omeprazole-induced IDHRs, CD63 was also the best marker.⁷⁰ These results should be taken into account given the possible differences in the stimulation mechanism induced by the drug that will lead to expression of different activation markers.⁷¹

With regard to NIDHRs, there is agreement about the urgent need for further studies because the sensitivity of *in vivo* testing (patch testing or intradermal testing with delayed reading) is disappointingly low, particularly in patients with SCARs, and DPTs are contraindicated for ethical reasons. However, because SCARs are rare, it is likely that validation and harmonization of these tests will require collaborative studies. From a technical point of view, there is a debate related to the right time at the acute or resolution phase to perform the *in vitro* test.¹⁰¹ It has been reported to depend on the clinical manifestation being more often positive at the acute phase when evaluating SJS or TEN, whereas better sensitivity is obtained in patients with DRESS at the resolution phase.⁹⁴

EXPERT CONSENSUS

Details based on expert consensus are provided in Box 1. 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 NIDHRs, on the clinical phenotype. *In vitro* testing is usually not performed as a test in isolation but rather as a component of a diagnostic strategy along with additional tests for evaluating the association between a given drug and an observed clinical

TABLE II. *In vitro* tests in the major categories of DHR

Type of reaction	<i>In vitro</i> test	Sensitivity	Comments/issues
IDHRs at the acute phase	Tryptase determination	31% to 67% ¹⁴⁻¹⁶	● This variability could be related to the type of reaction.^{17,18} ● Threshold should be revised or, alternatively, the international consensus equation should be applied.^{17,19}
	Histamine determination	61% to 92% ^{20,22}	● This variability could be related to the type of reaction.^{17,19}
IDHRs at the resolution phase identifying the relevant drug	sIgE by using immunoassay	38% to 85% for BLs ²¹⁻²⁵ 44% to 92% for NMBAs ²⁶⁻³² 26% to 68% for biological agents ^{39,40} 59% to 75% for platins ⁴¹	● sIgE to penicillins can produce false-positive results in patients with penicillin V allergy¹²⁰ and in those with high levels of total serum IgE.¹²¹
	BAT	44% to 63% for BLs ^{42,47-52} 36% to 92% for NMBAs ^{35,36,53-58} 42% to 65% for dipyron ⁵⁹⁻⁶¹ 57% to 77% for quinolones ⁶²⁻⁶⁵ 46% to 63% for RCM ^{66,67}	● Use of both markers CD203c and CD63 can improve BAT sensitivity. ● Some patients' basophils can be "nonresponders" in 10% to 20% of the population.⁷³
Non-IDHRs at the resolution phase identifying the relevant drug	LTT	56.1% 58% to 89% in moderate reactions ⁸⁵⁻⁸⁸ 25% to 75% in severe reactions ^{27,89-92}	● Strongly influenced by the type of reaction, with mild and moderate reactions showing greater sensitivity.
	Cytokine determination by means of ELISA, ELISpot, and bead assay	36.4% to 100% for IFN- γ , IL-5, IL-2, IL-4, or granzyme ^{27,92,99-102}	● Drug-induced SCARs ● There is low sensitivity in patients with DRESS but markedly increased sensitivity in patients with SJS/TEN compared with LTTs.
	Combined cytokine and cytotoxicity assays	>80% ^{92,101,102}	● MPE, AGEF, and DRESS
	HLA markers	45.5% to 80% for HLAB*57:01 in abacavir-induced DHR ¹⁰³⁻¹⁰⁵ 75% to 100% for HLA-B*15:02 in carbamazepine-induced DHR ^{107,108} 55% to 100% for HLA-B*58:01 in allopurinol-induced DHR ^{113,114}	● Screening for the presence of these alleles could be recommended for at-risk patients before receiving the drug.

AGEP, Acute generalized exanthematous pustulosis; MPE, maculopapular exanthema; NMBAs, neuromuscular blocking agents; RCM, radiocontrast media.

reaction. Thus *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 that drug-specific thresholds are standardized.³⁸ They can also be complementary to *in vivo* testing for the identification of cross-reactivity missed by STs.⁵⁷

When combining results from multiple *in vitro* and *in vivo* tests, for IDHRs, multiple testing has been proposed, including STs, BATs, and determination of drug sIgE levels, as a way to achieve a diagnosis and validate new tests. In fact, BATs can identify an additional percentage of patients (10% to 57%), as has been demonstrated for several drugs, such as BLs, quinolones, pyrazolones, NMBAs, and recently omeprazol.^{47,60,62,70} With this approach patients are considered to have a confirmed diagnosis when results of at least 2 of these 3 tests are congruently positive.^{36,124,125} Although this *post hoc* analysis might not be absolute, in our opinion it can add to diagnostic accuracy.

These assays are of critical value in severe cases in which DPTs are contraindicated. Therefore their use is tailored to specific patients, and there is currently not enough information to make a general recommendation for all DHRs.

BAT mirrors more closely the *in vivo* presentation and clinical phenotype than traditional sIgE measurement and might also circumvent the issue of epitopes hidden in a solid-phase assay (as shown for IgE to quinolones).¹²⁶

Recently, a potential role for BATs has been shown for monitoring the response during desensitization to chemotherapeutics, with the possibility that BATs could serve as a biomarker for rapid drug desensitization^{68,127} and to predict breakthrough reactions during desensitization.⁶⁸ Additional studies are warranted to confirm these promising data.

Moreover, using drug metabolites other than the native drugs or drug-carrier conjugates could achieve an increase in sensitivity in some reactions, as has been demonstrated in patients with IDHRs,^{74,128} as well as in those with NIDHRs.¹²⁹ Furthermore, especially for NIDHRs, it should be important to focus on the effector cells and biomarkers involved in each clinical entity.^{92,99,101,130}

UNMET NEEDS

Nowadays, there are *in vitro* tests that can be used in evaluation of the major categories of DHRs with variable sensitivity and limitations (Table II).*

In many regions of the world, there is a lack of widely available *in vitro* commercial assays that have demonstrated validity. Currently, most of the tests are not clinically validated, and

*References 14-32, 35, 36, 39-42, 47-67, 73, 85-92, 99-105, 107, 108, 113, 114, 120, and 121.

many of them are in the technical validation phase. Larger studies with well-characterized patients and control subjects are needed. The ideal situation to evaluate the role of *in vitro* tests in DHR evaluation would be inclusion of patients with a confirmed diagnosis of DHRs that in many cases would only be achieved after a DPT used as a reference standard, with the understanding that for ethical reasons, DPTs cannot be performed in patients with life-threatening reactions. For this reason, in real life patient inclusion could also be based on clinical history and STs and sometimes only on clinical history when STs are not available or produce equivocal results.

As a result of the prevalence of DHRs, another limitation of many studies is the small sample size. Therefore it is likely that multicenter studies will be needed because these can facilitate the harmonization of techniques and inclusion of sufficient numbers of patients/control subjects with more careful selection of phenotypes/endotypes.

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

- We need to complete our knowledge about mechanisms and effector cells involved in each clinical manifestation.
- We should determine the optimal drug concentration of the test from a dose-response analysis and check noncytotoxic concentrations, particularly when using cellular tests.
- The right thresholds for considering results positive by using receiver operating characteristic analysis should be determined.
- Techniques need to be developed to identify nonresponder cases in BATs.
- A major limitation of BATs to drugs is the need for fresh cells. Thus it would be ideal to use the patient's serum and passive sensitization of donor basophils (or basophil/mast cell lines) as read-out systems. This approach has been investigated in various laboratories: although the available cell lines were quite disappointing, some success could be achieved with IgE-stripped and resensitized basophils of blood donors. For example, among patients with chlorhexidine allergy, 90% of those with positive direct BAT results reacted in the passive BAT to chlorhexidine (Pichler W. J., personal communication). Thus although it is still an experimental procedure, it might have its role under certain situations.
- The optimal time to obtain the sample to perform the test to avoid false-negative results must be determined. For IDHRs (and depending on the drug), this is close to the acute phase of the reaction; for NIDHRs, this is at the acute phase or resolution phase depending on the clinical entity.
- Drug metabolites involved in DHR induction should be characterized.
- We need to increase our knowledge about the interaction of drug and the immunologic system to amplify the *in vitro* immunologic response. In this sense approaches using dendritic cells or Toll-like receptor ligands have been investigated and demonstrated an increase in sensitivity of *in vitro* tests in NIDHRs.^{86,131-133}
- The value of multiple *in vitro* and *in vivo* tests for IDHRs and NIDHRs induced by different drugs should be assessed.

- Different techniques for each *in vitro* testing and for the most common drugs eliciting hypersensitivity should be standardized.
- We should perform studies on hypersensitivity reactions to a single drug or drug group, avoiding miscellanea of drugs and, if possible, focusing on a specific clinical manifestation, mainly in patients with NIDHRs.

All of these approaches 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 in a personalized fashion.

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