Asia Pac Allergy. 2020 Oct;10(4):e44
https://doi.org/10.5415/apallergy.2020.10.e44
pISSN 2233-8276·eISSN 2233-8268

Asia Pacific allergy

Current Review

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Clinical value of *in vitro* tests for the management of severe drug hypersensitivity reactions

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OPEN ACCESS

Received: May 1, 2020
Accepted: Oct 29, 2020

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Conflict of Interest
The authors have no financial conflicts of interest.

ABSTRACT

Drug hypersensitivity reactions (DHRs) occasionally present with severe cutaneous adverse reactions (SCARs) which result in a high risk of morbidity and mortality. Although SCARs are rare, the occurrence could lead to a significant increase in healthcare and economic burden, especially when more than one possible culprit drug is implicated. Therefore, the accurate identification of the culprit drug(s) is important for correct labeling and subsequent patient education and avoidance. To date, clinical evaluation using causality assessment has limitations because the assessment may be inaccurate due to the overlapping timelines when multiple drugs are initiated/continued. Moreover, drug provocation tests (DPTs) which is the gold standard in diagnosis, are contraindicated, and *in vivo* skin tests may also be associated with risks of triggering SCAR. The European Network for Drug Allergy recommended that *in vitro* tests, if available, should be performed before any *in vivo* tests. Basophil activation tests and lymphocyte transformation tests, could serve as reliable *in vitro* tests for both immediate and delayed-type DHR. Many academic medical centers with affiliated laboratory services offer these tests in the diagnostic evaluation of SCARs in clinical practice. This not only complements identification of the culprit drug(s), but may also be used to test for potentially non cross-reactive alternatives, hence avoiding DPTs. In this review, we summarize the roles of *in vitro* tests in identifying the culprit drug(s) in SCARs, issues with utilization and interpretation of test results, and our experience in clinical practice.

Keywords: Basophil activation tests; Drug provocation tests; Lymphocyte transformation tests; Skin tests; Severe cutaneous adverse reactions

INTRODUCTION

Drug hypersensitivity reactions (DHRs) are an important public health problem. DHRs manifest with mild to life-threatening symptoms in susceptible individuals [1]. Life-threatening manifestations of DHR in the form of severe cutaneous adverse reactions (SCARs), are associated with high risks of morbidity and mortality, albeit its low prevalence [1]. Risk factors for SCARs include host genetic factors, threshold/eliciting dose of the drug, renal impairment, and inappropriate immune responses. Therefore, diagnosis of

Author Contributions

Conceptualization: Yuttana Srinoulprasert, Mongkhon Sompornrattanaphan. Data curation: Yuttana Srinoulprasert. Formal analysis: Yuttana Srinoulprasert, Ticha Rerkpattanapipat, Mongkhon Sompornrattanaphan, Chamard Wongsa. Funding acquisition: Yuttana Srinoulprasert. Methodology: Yuttana Srinoulprasert. Project administration: Yuttana Srinoulprasert. Visualization: Yuttana Srinoulprasert, Chamard Wongsa. Writing - original draft: Yuttana Srinoulprasert, Ticha Rerkpattanapipat, Mongkhon Sompornrattanaphan, Chamard Wongsa, Duangjit Kanistanon. Writing - review & editing: Yuttana Srinoulprasert, Duangjit Kanistanon.

SCARs involves the correlation between timeline of symptoms, and corroborative clinical and laboratory findings in relation to the history of drug exposure [2, 3]. Finally, a definitive diagnosis with causative drug identification is needed. To start to identify culprit drug causing SCARs, one could gather a history of drug exposure in detail as well as a timeline of SCAR onset and progression; to include the possible culprit drugs and exclude temporally irrelevant drugs [2]. However, this causality assessment has limitations because the outcome is usually inaccurate due to the overlapping timelines [4].

Skin tests (STs) are usually performed by well-trained allergists or dermatologists. However, the diagnostic utility of these tests varies with the agents used, concentration, lack of standardization of ST preparations, each with an unknown predictive value. Although STs have been evaluated as having acceptable sensitivity to identify some common culprit drugs, such as beta-lactam, perioperative drugs, radiocontrast media (RCM), and platinum-based chemotherapeutic drug, they still have low diagnostic potency for most drugs. In addition, validation of ST protocols are lacking, and their sensivity is suboptimal [5]. To identify culprit drug causing immediate drug hypersensitivity, skin prick test (SPT) and intradermal test (IDT) have been widely used as both tests could provide high sensitivity and very high negative predictive value, especially in the case of beta-lactam allergy [6, 7]. However, SPT is relatively contraindicated in pregnant people; and IDT is contraindicated in SCAR as it has a high risk of causing a systemic reaction [6, 8]. Additionally, it is difficult to perform ST in patients with severe dermatitis, dermographism or taking immunomodulating or antihistamine drugs [6]. For delayed-type drug hypersensitivity, delayed-reading IDT, and patch test (PT) have been employed to identify culprit drugs. Both IDT and PT provided high negative predictive value, however, IDT has higher sensitivity than PT. As IDT is contraindicated in severe drug reactions, hence PT has been safer and more widely performed with any form of drugs [8]. Positive rates of PT depend on type of SCARs, drug, and vehicle [7].

DPT has been considered to be a gold standard for DHR diagnosis if the observed reactions are compatible with drug allergy. Nevertheless, DPT is contraindicated in cases with SCAR. The position paper from the European Academy of Allergy and Clinical Immunology/ European Network of Drug Allergy recommended that if the *in vitro* tests are available, they could be the first choice to help the clinician to arrive at the correct diagnosis [9]. These tests do not only identify culprit drugs with excellent specificity, but they also do not put vulnerable patients under a high-risk procedure. Although the *in vitro* tests often provide poor sensitivity, they could complement identification of the culprit drug, and assist physicians to seek safer alternative drugs.

The *in vitro* tests may be considered before *in vivo* procedures. Ideally, the diagnostic tests with high accuracy (high sensitivity and specificity) should be useful for high-risk patients with severe index reactions. A positive test result could provide a firm diagnosis of the culprit drug, therefore, decreasing the need for *in vivo* or unnecessary DPTs. A negative test result could decrease the likelihood of certain drugs being the putative drug, the physicians could move on to a further *in vivo* test or sometimes DPTs [10]. Hence, the *in vitro* tests, if available, could be considered as the investigation of choice in high-risk patients. However, there are many concerning issues involving the management of *in vitro* tests that will be discussed in this review.

The objective of this article is to review clinical applications of the *in vitro* tests for immediate-type DHR (basophil activation test, BAT) and delayed-type DHR (lymphocyte transformation

test, LTT) for which we recently published our experience from Thailand [11-14]. Some concerning issues for the *in vitro* test interpretation and laboratory limitations are also included. We summarize the utility of *in vitro* tests, laboratory interpretation, and clinical interpretation to physicians, not only to improve the understanding of these tests, but also to increase awareness of the limitations of the *in vitro* tests.

IMPLICATION OF *IN VITRO* TESTS IN CLINICAL PRACTICE

The *in vitro* tests have been developed according to the types of DHRs. So far, there have been many *in vitro* tests developed for culprit drug identification [9]. Basophil histamine release (BHR) assay and BAT have been established for immediate-type DHRs. However, the latter has been used more widely because BAT provides significantly higher sensitivity as compared to BHR. BAT can be performed with low number of basophils, while BHR cannot [15, 16]. For delayed-type DHR, the *in vitro* tests have been developed, such as cytokine detection by ELISA or ELISpot and LTT [17]. Although ELISA and ELISpot are moderately complicated tests and require a few days to complete the whole assay, standardization of cutoff values, selection of suitable cytokines for various types of delayed-type DHR remain issues. Even LTT is a laborious assay and needs an incubation time for cell proliferaton, it has been reliably utilized as a tool to identify culprit drug for delayed-type DHR for T-cell proliferation[18]. Therefore, the scope of this review will focus on BAT and LTT.

BAT for immediate drug hypersensitivity

The BAT is a cell-based assay using flow cytometry to evaluate the status of basophils by measuring changes in activation markers (commonly CD63 and/or CD203c) expressed on the cell membrane of basophils [15]. The general principle of BAT is that CD63, which is a membrane-bound molecule and intragranularly expressed, would be exposed on the cell surface during degranulation where granular membrane fuses with the cellular membrane. Additionally, CD203c, which is constitutively expressed on the basophil surface membrane, is significantly upregulated when basophils are activated. Even though the principles behind BATs are straightforward, the use of multiple negative and positive controls as well as consistent cytometer settings are required in order to limit the risk of incorrect reporting and interpretation of results.

BAT has been shown to be a reliable tool in culprit drug identification even though wide sensitivity (28%–100%) and specificity (80%–100%) have been reported. Examples of drugs commonly identified with BAT are RCM, beta-lactams, quinolones, nonsteroidal anti-inflammatory drugs (NSAIDs), and neuromuscular blocking agents (NMBAs) as summarized in **Table 1** [19-29]. This table also shows the current literature published on the value of BAT in the diagnosis of immediate DHR to beta-lactams, quinolones, NMBAs, RCM, and NSAIDs. Additionally, our in-house BAT studies (unpublished data) on these drugs and antituberculosis drugs were included. It appears that CD63 was consistently used as a primary marker for basophil activation and CD203c was employed as a complementary marker. It seemed that cutoff point was quite consistent criteria when percentage of basophil positive for activation markers was ≥5% and stimulation index (SI as calculated by a ratio of percentage of basophil positive between drug-stimulated condition and unstimulated condition) was ≥2 (according to manufacturer's recommendation). In our studies using in-house BAT, both CD63, and CD203c were evaluated and BAT was interpreted as positive when either CD63 or CD203c reached cutoff criteria (≥5% and SI≥2). We included both activation markers as different drugs

Table 1. Utility of BAT for culprit drug identification in immediate DHR

Drugs	Markers	Cutoff	Sensitivity (%)	Specificity (%)	Reference
Beta-lactams					
AMX, AMP, PEN, CEFU	CD63	≥5%, SI≥2	49–55	91–100	[19, 20]
AMX, AMP, CEF-3, CEFT, CEFU	CD63, CD203c	≥5%, SI≥2	33	100	*
Quinolones					
MOX, LEV, CIP, OLF	CD63, CD203c	≥5%, SI≥2	71	100	[21, 22]
NMBAs					
PAN, ROC, ATA, SUX	CD63, CD203c	>4%, >10%	80–100	96–100	[23, 24]
RCM					
IOB, IOX, IOP, IOH, IOPA, IOM	CD63	≥5%, SI≥2	46–63	89–100	[25, 26]
IOB, IOH, IOP, IOX	CD63, CD203c	≥5%, SI≥2	25	ND	*
NSAIDs					
ASA, DIC, KET, CEL, ACT	CD63	≥5%, SI≥ (1.71–2.18)	37–61	90–91	[27–29]
DIC, IBU, ACT	CD63, CD203c	≥5%, SI≥2	33	100	*

BAT, basophil activation tests; DHR, drug hypersensitivity reactions NMBAs, neuromuscular blocking agents; RCM, radiocontrast media; NSAIDs, nonsteroidal anti-inflammatory drugs containing ibuprofen, diclofenac, tramadol; ND, not determined; AMX, amoxicillin; AMP, ampicillin; PEN, penicillin; CEFU, cefuroxime; CEF-3, ceftriaxone; CEFT, ceftazidime; MOX, moxifloxacin; LEV, levofloxacin; CIP, ciprofloxacin; OLF, ofloxacin; PAN, pancuronium; ROC, rocuronium; ATA, atacurium; SUX, sumamethonium; IOB, iobitridol; IOH, iohexol; IOM, iomeprol; IOP, iopromide; IOPA, iopamidol; IOX, ioxithalamate; ASA, aspirin; DIC, diclofenac; KET, ketoprofen; CEL, celecoxib; ACT, acetaminophen; SI, stimulation index.
*Data of our studies, unpublished data.

could possibly stimulate different mechanisms leading to expression of different activation markers. Additionally, we also successfully developed BAT to identify chlorhexidine and ortho-phthalaldehyde as culprit drugs in anaphylactic cases [13, 30]. In conclusion, although sensitivity of BAT varied among these studies, its specificity was excellent.

Discussion points for BAT setting

Interestingly, basophils from 5%–20% of patients do not respond to BAT positive controls, therefore, it is hard to interpret results for diagnosis. To increase the chance of positive control responsiveness, our in-house setting for BAT included various positive controls for basophil activation, IgE-dependent (anti-IgE and anti-FceRI), and IgE-independent (N-formyl-L-methionyl-L-leucyl-phenylalanine), as a positive control panel. In addition, a wide range of drug concentrations (at least 3 concentrations) were set in our assay. Additionally, a negative control consisting of stimulation buffer alone should also be included to assess the level of background or spontaneous activation of basophils. In case of interleukin (IL)-3 priming, an additional negative control for IL-3 in the stimulation buffer should be included. It is important to set standardization, quality assurance of the laboratory procedures, flow cytometry acquisition, and data analyses in order for laboratory BAT to be applied correctly to clinical practice. In addition, clinical validation with BAT studies could be performed to assure the accuracy of test results. As mentioned earlier, a wide range of BAT sensitivity was reported, which could be due to (1) nonestablished standardization of BAT (various methods, techniques and instrument setting), (2) type and optimal concentration of drug, (3) cutoff criteria, (4) quality of fresh blood sample, and (5) recruitment of appropriate cases with well-defined clinical histories [31, 32].

BAT is not useful for evaluation of nonallergic hypersensitivity to any stimulants if the stimulants can activate via alternative IgE-independent effector cell activation mechanisms, such as through off-target occupation of the MRGPRX2 receptor [33]. This event can occur in patients who have positive ST but negative BAT because skin mast cells express MRGPRX2, unlike basophils which barely express such receptor. Therefore, BAT may probably give low sensitivity in case of MRGPRX2 activation leading to DHR in the case of NSAIDs and quinolone hypersensitivity [31, 34].

LTT for delayed drug hypersensitivity

LTT is a cellular testing approach to reveal the existence of drug-specific memory lymphocytes in circulating blood and its proliferative response to identify culprit drug and confirm DHR. These drug-specific lymphocyte responses rely on ^{3}H-thymidine incorporation during lymphocyte proliferation. Its specificity was 63%–100% (mostly >90%), whereas its sensitivity varied depending on the drug and types of DHR as summarized in **Table 2** [17, 18, 35-43].

LTT has been used as a tool to identify culprit drug for diagnosis of delayed DHR. As summarized in **Table 2**, the most common culprit drugs reported as culprit drugs identified by LTT were beta-lactams and antiepileptic drugs. Additionally, LTT could identify causative drugs for delayed DHR in other drug groups such as anti-TB drugs, cotrimoxazole, quinolones, vancomycin, and NSAIDs. LTT studies for identification of anti-TB drugs as culprit drugs have been reported from Asian countries. Our LTT setting has also been used to identify culprit drugs from various drugs as aforementioned. In addition, other drugs, such as allopurinol, antiviral drug, and heavy metal (nickel) have also been identified by our LTT setting. In our experience, positive results of LTT correlated with well-defined cases (consistent history, timeline, and clinical manifestation) and high score of Naranjo's assessment [11]. According to many reports, LTT yielded higher sensitivity than STs, making it a promising *in vitro* diagnostic tool for delayed type DHR [18, 35, 44].

Discussion points for LTT setting

To set LTT standardization, quality assurance of the cell culture procedures, such as capacity for cell growth of each preparation batch of culture medium and supplements (especially pooled AB plasma) and capacity to induce cell proliferation of each lot of positive control (phytohemagglutinin [PHA] and tetanus toxoid), have to be regularly assessed [45]. In order to interpret LTT results, sufficient amount and percentage of viable peripheral blood mononuclear cells (PBMCs) were routinely evaluated in our LTT setting. Positive controls in our LTT included nonspecific mitogen (PHA) and recall antigens (tetanus toxoid and purified protein derivative (in the case of tuberculosis). Every concentration of drugs or reagents was

Table 2. Utility of LTT for culprit drug identification in delayed-type DHR

Drugs	Clinical manifestation	Sensitivity (%)	Specificity (%)	Reference
Beta-lactams				
AMX, AMP, PEN, BEN	MPE, EXT, AGEP, TEN	68–83	85–100	[18, 35–38]
AMX, AMP, PEN, CEF-3	MPE, DRESS, AGEP, SJS/TEN	21	100	*
Anti-TB drugs				
IRZE	BUL, EXT, DILI	29–87	90–100	[39–41]
IRZEL	MPE, DRESS, SJS/TEN	52	89	*
Antibiotics				
VAN, COT, CIP, LEV, OLF, MOX, MER, CLO, PIP	LABD, DRESS, AGEP, SJS/TEN	77	100	*
Antiepileptic drugs				
CBZ, LTG	MPE, EXT, SJS/TEN	26–66	63–100	[17, 37, 42, 43]
CBZ, PHE, VAL, LTG	DRESS, SJS/TEN	55	91	[11], *
NSAIDs				
FEN, FLU	TEN	44	63	[43]
IBU, TRA, ACT, DIC, MEF	DRESS, SJS/TEN	50	98	*

LTT, lymphocyte transformation tests; DHR, drug hypersensitivity reaction; AMX, amoxicillin; AMP, ampicillin; PEN, penicillin; BEN, benzylpenicillin; CEF-3, ceftriaxone; MPE, maculopapular eruption; EXT, exanthem; DRESS, drug reaction with eosinophilia and systemic symptoms; SJS/TEN, Stevens-Johnson syndrome/toxic epidermal necrolysis; AGEP, acute generalised exanthematous pustulosis; TB, tuberculosis; IRZEL, isoniazid, rifampicin, pyrazinamide, ethambutol, levofloxacin; BUL, bullous; DILI, drug-induced liver injury; NSAIDs, nonsteroidal anti-inflammatory drugs containing ibuprofen, diclofenac, mefenamic acid, acetaminophen-tramadol; VAN, vancomycin; COT, cotrimoxazole; LEV, levofloxacin; CIP, ciprofloxacin; OLF, ofloxacin; MOX, moxifloxacin; MER, meropenem; CLO, cloxacillin; PIP, piperacillin; LABD, linear immunoglobulin A bullous dermatosis; CBZ, carbamazepine; PHE, phenytoin; VAL, valproic acid; LTG, lamotrigine; FEN, fenbrufen; FLU, flurbiprofen; IBU, ibuprofen; TRA, tramadol; DIC, diclofenac; ACT, acetaminophen; MEF, mefenamic acid.
*Data of our studies, unpublished data.

https://doi.org/10.5415/apallergy.2020.10.e44

also tested as nontoxic levels for cell culture. In our LTT setting, irrelevant drugs were also included to clarify specificity of the test. Nevertheless, false-positive results could occur when PBMCs were stimulated with some drugs, such as vancomycin, acetaminophen and RCMs [45] and valproic acid (our finding; unpublished data), or when LTT was performed at an inappropriate time (such as acute phase in DRESS [drug reaction, eosinophilia with systemic symptoms]) [46]. *In vitro* tests are usually performed after remission of disease, as PBMCs obtained during the acute drug allergy stage are strongly activated due to bystander activation of innate cells. High background proliferation might make it difficult to detect proliferation of drug-specific T-cell clones. The time interval between the acute stage and test performance has been recommended as 4–8 weeks after recovery of delayed-type hypersensitivity reaction and extended for up to 12 weeks after DRESS remission to wash out the incriminated drug and any other drugs [46, 47]. Even though LTT could provide very high specificity (85%–100%), wide range of sensitivity could occur according to (1) recruitment of cases with mixed-up Naranjo's score and not well-defined clinical history, (2) drugs, (3) threshold of cutoff point, and (4) clinical manifestations (maculopapular eruption, Stevens-Johnson syndrome/toxic epidermal necrolysis) [45]. We have previously demonstrated that SI well corresponded to Naranjo's score and SI ≥ 2.0 could be the optimal cutoff point after receiver operating characteristic analysis when compared with other SI values [11].

LABORATORY RESULTS CONTRIBUTE TO CLINICAL PRACTICE

Positive results

As these *in vitro* tests could provide excellent specificity, positive results could identify culprit drug and provide definite DHR diagnosis. The positive results could convince allergy practitioners that the suspected drug could be the cause of DHR and no further *in vivo* investigation is needed.

Negative results

Although negative results from these tests with varying sensitivity could not rule out a suspected drug, they maintain sufficient negative predictive value for allergists to work up with some clues represented by *in vitro* nonresponsiveness. With the nonresponsive clues, they may help the allergist manage and ensure availability of appropriate drugs for further *in vivo* test or drug provocation test. Some representative cases of our experience to perform *in vivo* test or challenge test according to clues from *in vitro* tests are demonstrated in **Table 3**.

According to our finding in **Table 3**, the majority of negative *in vitro* cases could pass a drug provocation/challenge test or be confirmed by *in vivo* tests. This suggests that patients may not really be allergic to the suspected drug or have a chance to use alternative drugs. Our management was consistent with other studies showing that DHR patients, who obtained negative *in vitro* test with unresponsiveness in challenge test or DPT, had a chance to successfully retake the suspected drugs [10].

CONCERNED INTERPRETATION

As *in vivo* and *in vitro* tests for DHR provide low-to-moderate sensitivity; technical improvement and test combination are required to enhance sensitivity of these tests [48].

https://doi.org/10.5415/apallergy.2020.10.e44

Table 3. Utilization of *in vitro* tests for severe DHRs in our clinical practice

Immediate DHR					
DHRs manifestation	Possible culprits	*In vitro*-tested drugs	BAT	STs	DPT
Anaphylaxis	Lidocaine	Lidocaine	Negative	Negative	ND
		Mepivacaine	Negative	Negative	Negative
	Final drug use: Mepivacaine				
Anaphylaxis	Iobitridol	Iobitridol	Negative	ND	ND
		Iopromide	Negative	ND	Negative
		Iohexol	Negative	ND	ND
		Ioxagate	Negative	ND	ND
	Final drug use: Iopromide (no premed, no ADR)				
Possible Kounis syndrome	Amoxicillin/clavulanic	Amoxicillin	Negative	Negative	ND
		Clavulanic	Negative	Negative	ND
	Continue to avoid amoxicillin/clavulanic				
Delayed-type DHR					
DHRs manifestation	Possible culprits	*In vitro*-tested drugs	LTT	STs	DPT
DRESS	IRZE	Isoniazid (I)	Negative	ND	Negative
		Rifampicin (R)	Positive	ND	ND
		Ethambutol (E)	Negative	ND	Negative
		Pyrazinamide (Z)	Positive	ND	ND
		Levofloxacin (L)	Negative	ND	Negative
	Final regimen: IEL				
DRESS	IRZE	Isoniazid (I)	Negative	ND	Negative
		Rifampicin (R)	Negative	ND	Negative
		Ethambutol (E)	Negative	ND	Negative
		Pyrazinamide (Z)	Positive	ND	ND
		Levofloxacin (L)	Positive	ND	ND
	Final regimen: IRE				

DHR, drug hypersensitivity reaction; BAT, basophil activation test; ST, skin test; DPT, drug provocation test; ND, not determined; ADR, adverse drug reaction; DRESS, drug reaction with eosinophilia and systemic symptoms; LTT, lymphocyte transformation test; IRZE, isoniazid, rifampicin, pyrazinamide, ethambutol.

Positive results from tests with excellent specificity and negative predictive value could be useful to provide definitive diagnosis of DHR with culprit drug identified. However, negative results from low-to-moderate sensitivity and positive predictive value tests cannot be used to rule a suspected drug out, and further investigation or avoidance of the suspected drug is required. Likewise, false-positive and false-negative results have to be considered and analyzed during results interpretation. False-negative results may lead to serious outcomes, which can contribute to patient safety with re-exposure of culprit drug. Meanwhile, false-positive results do not affect patient safety, however, they make patients lose a chance to receive the first-line drugs with high potency. Nevertheless, investigation for culprit drug identification is necessary to give a definite diagnosis of DHR as well as no or very low risk from investigation has to be concerned. With such a situation, therefore, *in vitro* tests could be the most appropriate investigation to provide evidence for physicians to plan further treatment. Many factors contributing to either false-negative or false-positive results were summarized in **Table 4**.

Of note, antihistamine has no effect on BAT (both CD63 and CD203c), which make BAT more superior than *in vivo* test in patients who could not omit antihistamine [32, 49, 50]. LTT could be suitable in some decent conditions which may be performed with blood samples drawn from patients who take <0.2 mg/kg/day of prednisolone or who take methotrexate/azathioprine without lymphopenia [45]. Even though there was no consensus on standardization of ELISpot to identify culprit drug in delayed-type DHR, in case culprit drug identification is urgently needed, IFN-γ ELISpot might be an alternative assay as reported [12].

Table 4. Factors affecting results of BAT and LTT

Outcomes	BAT	LTT
False-negative results may be obtained if [31, 32, 45]	Performed within 1–2 weeks after recently reaction (refractory period)	-
	Systemic corticosteroid, immunosuppressant drugs (cyclosporin A)	Systemic corticosteroid, immunosuppressant drugs, chemotherapeutic drugs
	Last reaction > 1 yr	Last reaction > 2–3 yr
	Prolonged blood handling > 24 hr	Prolonged blood handling > 24 hr
	Nonresponder to controls	Nonresponder to controls
	-	High/toxic doses lead to cell death
False-positive results may be obtained if [32, 45]	-	AGEP, SJS/TEN: within 1 month after recovery
		DRESS: within 3 months after recovery
	-	Some drugs (vancomycin, acetaminophen, RCM, NSAIDs)
	High/irritating doses leading to nonspecific degranulation	-

BAT, basophil activation test; LTT, lymphocyte transformation test; AGEP, acute generalised exanthematous pustulosis; DRESS, drug reaction with eosinophilia and systemic symptoms; SJS/TEN, Stevens-Johnson syndrome/toxic epidermal necrolysis; RCM, radiocontrast media; NSAIDs, nonsteroidal anti-inflammatory drugs.

CONCLUSION AND PERSPECTIVE

In vitro tests are promising tools that can help allergists to identify culprit drugs for the definitive diagnosis of DHR. *In vitro* tests have been shown to be useful when *in vivo* diagnosis are lacking or not possible. To evaluate the role of *in vitro* tests in clinical practice for DHR diagnosis, they require DPT outcomes in many cases for validation analysis. However, with ethical reasons, DPTs cannot be performed in many patients with life-threatening reactions to serve as test validation. With this situation, clinical data with thorough assessment and STs, if available, are employed.

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.

ACKNOWLEDGEMENTS

This study was granted by Siriraj Foundation and research fund of Research Division, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. Y.S., M.S., C.W., and D.K. were supported by Chalermprakiat Fund.

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