Ref: Ro J Pharm Pract. 2022;15(2)
DOI: 10.37897/RJPhP.2022.2.2

Nonirritating concentrations for skin testing in immediate antibiotic allergy

Florin-Dan POPESCU[1,2], Maria CARAGHIULEA[2], Carmen Saviana GANEA[2], Mariana PREDA[1,2], Ana Maria Alexandra STANESCU[1]

[1] *"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*
[2] *"Nicolae Malaxa" Clinical Hospital, Bucharest, Romania*

Abstract

Accurate identification of a culprit drug involved in an immediate hypersensitivity adverse reaction has critical consequences on antibiotic stewardship and patient quality of life, therefore simple reliable diagnostic skin tests are of great importance.

Succinct information on the use of positive and negative controls and nonirritating concentrations of antibiotics, according to recent international guidelines, used for skin prick tests and intradermal tests for the allergy workup is required to avoid false negative or false positive results. The method used, the appropriate drug concentrations, and the criteria for positive skin testing influence the specificity and sensitivity of allergy skin tests.

Keywords: antibiotics, skin prick test, intradermal test, drug concentrations

INTRODUCTION

Proper identification of the offender drug associated with an immediate hypersensitivity adverse reaction can have significant health and financial consequences, highlighting the need for reliable and simple diagnostic tests. An appropriate assessment must always include a comprehensive clinical history and physical examination, including a careful reading of the patient's medical records and analyzing photos of cutaneous lesions, if available, followed by one or more of the subsequent procedures: skin prick tests (SPT) and intradermal tests (IDT), *in vitro* tests, and, when suitable, drug provocation tests (DPT).

SPT and IDT with immediate reading are useful in the diagnostic process of immediate drug hypersensitivity. It is imperative to emphasize that a detailed clinical history is needed before performing skin tests. Consequently, skin tests should only be done in patients with a suggestive anamnesis of adverse reactions that are consistent with drug hypersensitivity, not as general screening in patients without previous exposure to the drug or cross-reactive substances. The aim of skin testing is to detect allergen-specific IgE molecules bound to mast cells. The drug allergen exposure by skin testing cross-links the specific IgE molecules bound on mast cell. Skin prick and intradermal tests assess the reaction of the mast cell degranulation as a consequence of the exposure to drug molecules [1,2].

SPT to antibiotics that induce direct mast cell activation, such as fluoroquinolones or vancomycin, may lead to false-positive results, sometimes

Corresponding author:
Florin-Dan Popescu
E-mail: florindanpopescu@gmail.com

Article History:
Received: 21 June 2022
Accepted: 24 June 2022

complicating without reason the treatment decision-making process for a patient. Typically, SPT is performed first because it is more specific and safer, followed by IDT which is more sensitive, if necessary. Both are performed usually on the volar aspect of the forearms. The amount of drug solution applied epicutaneously during the SPT corresponds to 3 x 10-3 µL (3 nL) with the puncture being made using a 1-mm tip metal lancet. For IDT with drugs, a fixed volume of 20 µL (0.02 mL) is injected intradermally, obtaining a papule of 3-5 mm. Immediate reading of the skin tests is recorded at 15-20 min. Needles of 25-30G do not affect the IDT, but a small 1-mL syringe is needed to enable an accurate measurement of small solution volumes. SPT or IDT results are defined as positive if the papule is ≥ 3 mm than the negative control and accompanied by erythema ≥ 5 mm. Adding a second SPT reading 40 min after the first one increases the sensitivity in the diagnostic workup for immediate hypersensitivity to beta-lactams. IDT with delayed reading at 24 h and/or patch testing may be used for delayed hypersensitivity adverse drug reactions [3-5].

If a SPT is negative, an IDT can be done, also on the volar forearm. Other areas, such as the upper back, can also be used for testing. Application in duplicate should be considered.

Skin tests should be done by medical personnel trained to treat systemic reactions if needed even if these events rarely happen, therefore precautions are needed regarding the availability of emergency treatment and the additional risk related to patient comorbid conditions and/or its treatment. In order to limit false-negative outcomes, they should be performed at least 4-6 weeks after the reaction. An appointment must be made, and written consent should be obtained before testing. Skin tests may also be useful for subjects who are anxious or uncomfortable with drug challenges.

A positive SPT suggests that a subject has an IgE-mediated sensitization to the drug but a positive skin test should at all times be correlated with the adverse reaction history. The negative predictive value of a skin test is only useful with penicillin since the specificity of this test has been appropriately assessed. For most antibiotics, commercial skin test formulations do not exist. Specific standardized skin test reagents are available only for beta-lactams in some countries. For all other non-beta-lactam antibiotics, the tested material is obtained from the drugs commercially available on the market, prior to expiry date. In order to reach adequate standardization, skin testing should be done with injectable products, such as those used for the parenteral route. According to guidelines, they should be diluted in 0.9% sodium chloride solution or not, depending of the type of the skin test. Some intravenous preparations necessitate dilution in sterile water for stability, but this can lead to irritant reactions. The antibiotics are diluted in 0.9% NaCl not more than two hours before use. Drugs that are not available in a soluble form may be tested by SPT after the tablets have been smashed in a capsule or mortar, the content being diluted with sterile saline. Many oral drugs contain inactive fillers having the potential to induce irritating reactions [2,6-8].

Normal saline is used as negative skin test control, because dermographism may pose a real problem in interpreting the results if not used. Histamine dihydrochloride 10 mg/mL (equivalent with histamine base 6 mg/mL) must be used as positive skin test control, to ensure that there are no influences of drugs with H1 antihistamine effects. The antibiotic concentration used for skin tests should at all times not be higher than the highest concentration which does not produce a direct skin irritation. IDTs with many undiluted drugs, including antibiotics, produce irritative responses in both controls and patients, whereas this phenomenon is infrequently in SPT. The optimal concentration of a particular drug for skin testing, especially IDT, is the highest concentration that does not induce any irritative skin reactions in patients who have never been exposed to the drug, as well as in patients who have been exposed to the drug and have tolerated it, but may produce positive results in those with drug hypersensitivity. For example, initially, the SPT is performed at a low concentration (usually 1/100 of the parenteral formulation) and, if no reaction occurs, the concentration is increased tenfold each time until a positive reaction occurs. If no such reaction is elicited by the SPT, the IDT starts normally with a dilution of 1/100 of the SPT concentration and the concentration is increased until the final nonirritant one is reached. When nonirritant concentrations are used in drug allergy, skin tests are generally characterized by a high specificity and a relatively low sensitivity [3-8].

This article does not intend to present any risk stratification in antibiotic allergy according to index reaction(s) or algorithms for the diagnosis of immediate hypersensitivity adverse reactions to

antibiotics. It only presents information on nonirritating concentrations of antibiotics used for skin testing according to new European and American publications, because presenting appropriate drug concentrations, the type of method, and the criteria for positivity influence the specificity and sensitivity of allergy skin testing.

ALLERGY SKIN TESTING WITH BETA-LACTAM ANTIBIOTICS

Beta-lactam antibiotics are currently a first choice for the treatment of many bacterial infections and at the same time, are considered the most frequently immunological trigger for hypersensitivity adverse drug reactions. The beta-lactam ring is present to all beta-lactam antibiotics.

In **penicillins**, the beta-lactam ring is fused to a five-member thiazolidine ring (high-tensioned structure) and possesses one side chain (R1). Beta-lactams are haptens that become immunogenic only after binding to a protein structure. A main carrier protein is represented by the human serum albumin. Binding to the amino acid, lysine, is possible by the opening of the beta-lactam ring. This results in the development of primarily benzylpenicilloyl from benzylpenicillin.

For skin testing, the enclosure of major and minor determinants of benzylpenicillin is highly indorsed (Table 1). Major antigenic determinants consist of multivalent conjugates of benzylpenicilloyl coupled through an amide bond to the carrier polymer, such as penicilloyl poly-lysine (PPL) or benzylpenicilloyl-octa-lysine (BP-OL), with chemical stability. Minor determinants are formed by other bonds, but for stability reasons, the currently available test preparation contains only one minor determinant, the sodium benzylpenilloate.

Skin tests using reagents with antigenic proteins must start by assessing cutaneous reactivity to the major determinant available in Europe as benzylpenicilloyl octa-L-lysine 0.04 mg/mL and, if negative, to the minor determinant benzylpenilloate sodium 0.5 mg/mL, benzylpenicillin 10,000 IU/mL (6 mg/mL as sodium salt), amoxicillin 20 mg/mL and clavulanic acid 20 mg/mL (Table 1). Skin testing must always start with SPT, followed by IDT only when SPT results are negative. As a precautionary measure, it is prudent to use a set of dilutions, at a ratio of 1:100 and 1:10, prior to starting IDT. In subjects with a personal history of severe reactions, or at a high risk, the dilutions may even begin at 1:1,000 [9-11].

Skin testing with benzylpenicillin only, without the use of the major antigenic determinant PPL, is not endorsed, because up to 70% of subjects who have a positive skin test result react only to PPL, and these can still have a severe allergic reaction. Moreover, it has been assessed that skin testing with PPL and benzylpenicillin, without the use of minor determinants, may miss 10% to 20% of penicillin-sensitized subjects [11].

Besides benzylpenicillin (Penicillin G), there are other commercial available parenteral penicillins used for

TABLE 1. Nonirritating concentrations for SPT and IDT with penicillins

Determinant/Drug/Class/Group	Abbreviation	Maximum nonirritating concentrations
Major penicillin determinants:		
benzylpenicilloyl poly-L-lysine	PPL	6 x 10^{-5} mol/L benzylpenicilloyl (BP)
benzylpenicilloyl octa-L-lysine	BP-OL	0.04 mg/mL (8.64 x 10^{-5} mol/L BP)
Minor penicillin determinants:		
benzylpenilloate sodium	PO	0.5 mg/mL (1.5 x 10^{-3} mol/L PO)
benzylpenicillin	BP/Penicillin G	10,000 IU/mL (6 mg/mL BP as sodium salt)
Aminopenicillins:		
ampicillin	AMP	20-25 mg/mL
amoxicillin	AX	20 mg/mL
Beta-lactam beta-lactamase inhibitor [BLI]:		
clavulanic acid	CLV	20 mg/mL
Combination aminopenicillinBLI:		
amoxicillin-clavulanic acid	AX-CVL	20 mg/mL AX - 4 mg/mL CLV
Other semisynthetic penicillins		20 mg/mL

Note: **In vivo* reagents for penicillin testing with antigenic determinants and other commercial products.

skin testing like ampicillin injectable solution and amoxicillin with clavulanic acid. Their maximum nonirritating concentrations are 20-25 mg/mL. Ampicillin and amoxicillin are aminopenicillins, and clavulanic acid is a beta-lactam beta-lactamase inhibitor.

Amoxicillin injectable solution 20 mg/mL with clavulanic acid 4 mg/mL is nonirritating for SPT and IDT. By adding 19.1 mL saline solution in a vial containing 1000 mg amoxicillin (as the sodium salt) and 200 mg clavulanic acid (as the potassium salt) a concentration of 50 mg/mL amoxicillin is obtained, then extract 0.4 mL from this solution in a 1 mL syringe and add 0.6 mL saline to obtain the solution with 20 mg/mL amoxicillin and 4 mg/mL clavulanic acid which may be applied for SPT and IDT as the maximum nonirritating solution. Further dilutions 1:1,000, 1:100, 1:10 may be used in clinical practice for individuals at high risk [3,9,11-16].

In **cephalosporins**, the beta-lactam ring is associated to a six-member dihydrothiazine ring; they also have two side chains (R1 and R2). The cephalosporin R2 side chain acts as a "leaving group" being usually lost after the opening of the beta-lactam ring, therefore it is less likely to cause IgE mediated hypersensitivity. The molecular recognition is primarily directed to the R1 side chain and the fragment of beta-lactam ring that binds to a carrier protein. Immediate allergic reactions to cephalosporins seem to be correlated to antigenic responses to the R1 group/side chain rather than the core beta-lactam part of the molecule. Aminopenicillins cross-react with aminocephalosporins such as cefaclor, cefadroxil, and cefalexin in some individuals. Cefazoline as well as cephalosporins with methoxyiimino group in the R1 side chain do not have similar or identical chains to penicillins. Methoxyimino cephalosporins reveal cross-reactivity with penicillins only in individual cases, but cross-reactivity between them is possible. The most important cephalosporins used for skin testing are presented below [3,9,11-14].

Cefazolin has a unique R1 side chain and seems to have very low cross-reactivity with penicillins despite being a first generation cephalosporin. The nonirritating concentration of cefazolin according to the EAACI Position Paper is 20 mg/mL for SPT and IDT. According to the AAAAI Drug allergy 2022 practice parameter the nonirritating concentration of cefazolin is 330 mg/mL for SPT, and 33 mg/mL for IDT. Optional for subjects with a history of severe and/or recurrent reactions the concentration of cefazolin 3.3 mg/mL may be additionally first used for IDT. In order to obtain these concentrations, for example, if a cefazolin 1 gram vial is available, add 3 mL sodium chloride 0.9% to create the solution containing 330 mg/mL cefazolin. Then further dilute by 1:10 to obtain 33 mg/mL cefazolin. Alternatively, by diluting 1:100, a concentration of 3.3 mg/mL, is obtained. These concentrations are nonirritating for SPT and IDT as mentioned.

Cefuroxime is a second-generation cephalosporin antibiotic with a methoxyimino group in the R1 side chain. The nonirritating concentration of cefuroxime according to the recent EAACI Position Paper is 20 mg/mL for SPT and IDT. According to the latest Drug allergy practice parameter the concentrations are 90 mg/mL for SPT and 10 mg/mL for IDT. Optional for patients with a history of severe and/or recurrent adverse reactions the concentration of 1 mg/mL may be initially used for IDT. For example, to obtain such dilutions, if a cefuroxime 1.5 gram vial is available, add 16 mL sodium chloride 0.9% to create a solution containing the concentration of 90 mg/mL cefuroxime. Then further dilute by 1:2 to obtain 45 mg/mL cefuroxime and extract 0.22 mL in a 1 mL syringe and add 0.78 mL saline to obtain 10 mg/mL cefuroxime used for IDT.

Ceftriaxone is a third-generation cephalosporin antibiotic with a methoxyimino group in the R1 side chain. The nonirritating concentration of ceftriaxone according to the EAACI Position Paper is 20 mg/mL for SPT and IDT. According to the AAAAI Drug allergy 2022 practice parameter is 100 mg/mL for SPT, and 10 mg/mL for IDT. Optional for subjects with a history of severe and/or recurrent adverse reactions 1 mg/mL may be initially used for IDT. For example, if a ceftriaxone 1 gram vial is available, add 9.6 mL sodium chloride 0.9% to obtain a solution containing 100 mg/mL ceftriaxone. Then further dilute by 1:10 to obtain 10 mg/mL ceftriaxone. Alternatively, diluting by 1:5 the solution containing 100 mg/mL a concentration of 20 mg/mL is obtained, or diluting by 1:100 the concentration 1 mg/mL is created. These concentrations are nonirritating for SPT and IDT as mentioned.

Ceftazidime is a third-generation cephalosporin antibiotic with an alkoxyimino group in the R1 side chain. Although ceftazidime and aztreonam (the only monobactam) have identical R1 side chain, this is of

only partial relevance in clinical practice. The nonirritating concentration of ceftazidime according to the recent EAACI Position Paper is 20 mg/mL for SPT and IDT. According to the latest AAAAI Drug allergy practice parameter the concentrations are 100 mg/mL for SPT, and 10 mg/mL for IDT. Optional, for patients with a history of severe and/or recurrent reactions 1 mg/mL may be used initially for IDT. In practice, for example, if a ceftazidime 1 gram vial is available, add 9.4 mL sodium chloride 0.9% to obtain a solution containing 100 mg/mL ceftazidime. Then further dilute by 1:10 to create 10 mg/mL ceftazidime. Alternatively, by diluting 1:5 the solution with 100 mg/mL ceftazidime a concentration of 20 mg/mL is obtained or by diluting 1:100 a concentration of 1 mg/mL is created. These concentrations are nonirritating for SPT and IDT as mentioned. Ceftazidime may also be used in clinical practice in a combination with avibactam, a non-beta-lactam beta-lactamase inhibitor.

Cefepime is a fourth-generation cephalosporin antibiotic with a methoxyimino structure in the R1 side chain. Patients with cefepime allergy should avoid ceftriaxone and cefotaxime (identical R1 methoxyimino groups). The nonirritating concentration of cefepime according to the latest AAAAI Drug allergy practice parameter and EAACI Position Paper is 2 mg/mL for SPT, the same as for IDT. For example, if a cefepime 1 gram vial is available, add 8.7 mL sodium chloride 0.9% to obtain the resulting solution containing 90 mg/mL cefepime. Then further dilute by 1:10 to obtain the concentration of 9 mg/mL cefepime, and extract from it 0.22 mL in a 1 mL syringe and add 0.78 saline solution to obtain 2 mg/mL cefepime used for SPT and IDT.

Carbapenems, in discrepancy to penicillin, have a carbon atom instead of sulfur in the thiazolidine ring, which is associated to the beta-lactam ring, as well as side chains at the R1 and R2 position. Studies revealed an absence or very low (1%) rate of cross-reactivity between penicillins and carbapenems, therefore, the new Drug allergy 2022 practice parameter proposes that in patients with a personal history of penicillin or cephalosporin allergy, a carbapenem may be administered without skin testing or extra precautions irrespective of whether the hypersensitivity adverse reaction was anaphylaxis or not. The nonirritating concentrations for skin tests to usual carbapenems are: ertapenem 1 mg/mL, imipenem-cilastatin 0.5-0.5 mg/mL and meropenem 1 mg/mL. For example, if a meropenem 1 gram vial is available, add 19.1 mL diluent and shake gently to dissolve (total volume of 20 mL), the resulting solution containing 50 mg/mL meropenem. Then further dilute 0.2 mL of the 50 mg/mL meropenem solution with 0.8 mL sodium chloride 0.9% (total volume of 1 mL), and the resulting solution contains 10 mg/mL meropenem, which will be further diluted by 1:10 to the concentration of 1 mg/mL meropenem which can be applied for SPT and IDT [3,9,11-12].

ALLERGY SKIN TESTING WITH NON-BETA-LACTAM ANTIBIOTICS

Fluoroquinolones are commonly prescribed antibiotics. For diagnosing immediate hypersensitivity reactions to fluoroquinolones there is controversy regarding the utility of skin testing because of low sensitivity and high rate of false-positive results, likely due to their capacity to trigger mast cell degranulation straight by activating a mast cell-specific receptor named Mas-related G protein-coupled receptor X2 (MRGPRX2) and induce direct histamine release, due to a tetrahydroisoquinoline motif, similar to neuromuscular blocking agents [11, 20-22]. Recommended concentrations of injectable solutions of floroquinolones for SPT are 2 mg/mL for ciprofloxacine, 5 mg/mL for levofloxacine, 1.6 mg/mL for moxifloxacin, 2 mg/mL or 5 mg/mL for ofloxacin. Although these concentrations are currently suggested for skin tests, there is disagreement about nonirritating concentrations [11]. Because the non-irritating IDT concentrations are very difficult to accurately assess. It is difficult to define an appropriate drug concentration to be used for IDT in order to detect IgE-mediated mast cell degranulation (good sensitivity), but not to trigger direct mast cell degranulation (good specificity) [23]. Some authors attempted to define IDT using sophisticated criteria by assessing the weal and flare diameters versus saline, histamine and specific fluoroquinolone at specific concentrations of 0.025 mg/mL and 0.005 mg/mL, but this needs to be further validated. Given that fluoroquinolones generate many false-positive results ascribed to nonspecific histamine release, mostly the case of IDT, a recent Spanish guidelines recommended including only SPTs, and not IDT, in the diagnostic approach for immediate reactions to fluoroquinolones, as negative SPT results may be useful for assessing the introduction of an alternative quinolones [24-25].

Macrolides are macrocyclic lactone ring antibiotics

classified as 14- (erythromycin, clarithromycin, roxithromycin), 15- (azithromycin), or 16- (spiramycin) membered ring molecules. Although there are fears about false-positive and false-negative results associated with macrolide skin tests, The highest nonirritating concentrations mentioned for SPT and IDT are 100 mg/mL and 0.01 mg/mL, respectively, for azithromycin, and 50 mg/mL and 0.5 mg/mL, respectively, for clarithromycin. The clarithromycin concentration 0.05 mg/mL is also specified by some authors for IDT. By injecting 10 mL solvent into a vial containing 500 mg clarithromycin (as lactobionate), after shaking until the contents have dissolved, 1 ml of the vial solution contains 50 mg clarithromycin lactobionate. The highest published nonirritant concentrations for SPT with other individual macrolide antibiotics are: erythromycin 50 mg/mL, roxithromycin SPT 30 mg/mL, spiramycin 10 mg/mL. In general, skin tests to macrolides seems to have inadequate diagnostic potential. A very recent American Drug allergy practice parameter consensus-based statement suggests using a 1- or 2-step drug challenge without preceding skin tests to confirm tolerance in subjects with a history of non-anaphylactic reactions to fluoroquinolones or macrolides [3,11,26-29].

Metronidazole is a 5-nitroimidazole drug with structural similarity to tinidazole. Few reports mentioned SPT with metronidazole 125 mg/mL, but these skin tests are considered with low sensitivity. Other authors specified SPT with metronidazole at 5 mg/mL and IDT with 0.05 mg/mL (1:100 dilution of metronidazole 5 mg/mL solution for infusion [3,11, 30-33].

Aminoglycosides are a broad-spectrum group of antibiotics structurally constituted of hydrophilic sugars containing amine and hydroxyl functional groups. Skin testing for immediate reactions should be approached with caution to confirm IgE-mediated allergy. The highest nonirritating concentration for gentamycin is 40 mg/mL in case of SPT and 0.4 mg/mL (1:100 dilution of the usual commercially available 40 mg/mL intravenous solution) for IDT. A concentration of 4 mg/mL may be used for IDT, if the gentamycin formulation is preservative-free. An additional dilution step is advised for patients with a history of anaphylaxis to gentamycin in case of IDT (0.04 mg/mL). For tobramycin, the concentration used for SPT is 40 mg/mL while for IDT are 0.4 mg/mL and 4 mg/mL (1:100 and 1:10 dilutions of the commercially available 40 mg/mL solution). Similar, an optional dilution step is suggested for patients with a history of severe immediate hypersensitivity reaction to tobramycin in case of IDT (0.04 mg/mL). Initially, for SPTs and IDT with streptomycin, concentrations of 0.1-1 mg/mL are used, increasing then step by step the concentrations (as high as 20 mg/mL) if the result is negative, with the mention that the irritant properties of higher concentrations have not been properly assessed [1,34,35].

Sulfonamide antimicrobials hypersensitivity have no validated diagnostic testing currently available. Sulfamethoxazole-trimethoprim (co-trimoxazole) is the most commonly used arylamine sulfonamide. A nonirritating concentration of 1:100 dilution of 80 mg/mL, or 0.8 mg/mL, is mentioned for skin testing in case of highly suspected IgE-mediated adverse drug reaction [11,22]. If used, skin tests have not revealed an acceptable level of sensitivity. Considering the diversity of sulfonamide hypersensitivity along with its complex underlying mechanisms, it is difficult to suppose that skin testing alone has a significant decisional impact. A 2022 American Drug allergy practice parameter consensus-based statement suggests that for individuals with a history of benign cutaneous reactions to sulfonamide antibiotics that happened more than five years ago, a 1-step drug challenge with trimethoprim-sulfamethoxazole should be done when there needing to delabel a sulfonamide antibiotic allergy [11,36].

Fosfomycin is an antibiotic structurally dissimilar to other antibiotics, belonging to the phosphonic acid family. There are no validated skin tests for the diagnosis of immediate hypersensitivity to fosfomycin. SPT with fosfomycin may be performed with a solution obtained from the granules at a concentration of 10 mg/mL. One sachet has 5.631 g of fosfomycin trometamol equivalent to 3 g fosfomycin, as granules for oral solution. IDT may also be performed at a concentration of 5 mg/mL, with saline solution as a negative control. Commercial preparations of fosfomycin used for *in vivo* tests at a concentration of 10 mg/mL were also reported diluted in phosphate buffered saline, pH 7.3. SPT may be better performed with 100 mg/mL fosfomycin in saline solution and IDT with 1 and 10 mg/mL fosfomycin in saline solution. One vial with 2.69 g of powder contains 2.64 g disodium fosfomycin, matching to 2 g fosfomycin and 0.64 g sodium, for solution in 50 mL of solvent, therefore one mL of solution for infusion has 40 mg fosfomycin [37,38].

Tetracyclines represent a broad-spectrum antibiotic class. There is currently no standardized skin testing for these antibiotics. SPT with doxycycline may be performed at 10 mg/mL after dissolving a 100 mg capsule content in 10 mL of saline. A better approach is to formulate a solution containing 10 mg/mL, the contents of a vial with doxycycline hyclate corresponding to 100 mg doxycycline for injection being reconstituted with 10 mL 0.9% sodium chloride injectable solution. For, **tigecycline**, a glycylcycline considered a new-generation tetracycline, the 50 mg of powder from a vial should be created with 5.3 mL of sodium chloride 9 mg/mL solution to achieve by gently swirling a concentration of 10 mg/mL of tigecycline for SPT. The IDT maximum concentration for tigecycline tested was 0.1 mg/mL (1:100 dilution of STP solution) [11,39,40].

Vancomycin, a tricyclic glycopeptide antibiotic, may induce a common adverse drug reaction, caused by rate-dependent infusion, non-IgE mediated, direct mast cell degranulation, known as vancomycin infusion reaction (previously called "red man syndrome") involving MRGPRX2 receptors. In addition, vancomycin-specific IgE detection has not been reported. Therefore, vancomycin skin testing produces high false positive rates [11,22,39]. In addition, no allergenic determinants of the vancomycin molecule have been detected [42]. Case reports of anaphylaxis/ anaphylactoid adverse reactions to vancomycin mentioned positive IDT with this antibiotic at concentrations of 0.1 to 5 µg/mL which may be interpreted as IgE-mediated hypersensitivity. However, such cutaneous reactivity, even at 0.1 mg/mL, may represent an increased propensity for direct mast cell degranulation. The general consensus is that 50 mg/ mL for SPT and 0.005 mg/mL for IDT are nonirritating dilutions.

At the time of use, adding 20 mL of 0.9% sodium chloride to a vial with 1000 mg vancomycin powder will obtain a solution of 50 mg/mL, and after that a dilution of at least 1:10,000 (10-4) may be needed for IDT. Some guidelines discuss IDT concentrations of 0.1 µg/mL (1:1,000,000 dilution of an initial concentration of 100 mg/mL). Nevertheless, immediate hypersensitivity skin testing has not been validated in terms of its negative or positive predictive value, for predicting vancomycin infusion reaction or its severity. Moreover, vancomycin skin testing may be of no benefit for assessing IgE-mediated hypersensitivity to vancomycin, since all patients present a positive reaction at concentrations equal to or greater than 10 µg/mL at IDT [11,3,42,43].

Teicoplanin, a semisynthetic glycopeptide antibiotic, does not activate the MRGPRX2 receptors, thus infusion reactions cross-reactivity between vancomycin and teicoplanin is unlikely. But immunological cross-reactivity between teicoplanin and vancomycin has been documented in some individuals, therefore teicoplanin may not be a perfect alternative option for patients with vancomycin immediate adverse reactions. Moreover, vancomycin should be used with caution in those with hypersensitivity reactions to teicoplanin, since cross-reactivity, including anaphylaxis, may occur [11,41, 44-46]. A possible immune cross-reactivity between the newer lipoglycopeptide telavancin, a synthetic derivative of vancomycin, and teicoplanin or vancomycin was recently discussed. Though cross-reactivity between vancomycin and its semi-synthetic derivatives of vancomycin, oritavancin or dalbavancin, is not well known, thus attention is needed when these drugs are administered in patients with a history of hypersensitivity to other glycopeptides and should only be used if the benefit is greater than risks [47, 48]. Skin testing with teicoplanin comprises SPT with or without IDT. The concentrations used for SPT and IDT vary substantially between experts and clinics, due to the absence of validated skin testing concentrations for teicoplanin. Reconstituting a 400 mg vial with the 3.14 mL diluent will give a 400 mg/3mL solution. SPT with teicoplanin may then be performed with prepared concentrations of 20 mg/mL and IDT with 2 mg/mL. SPT may also be performed with prepared concentrations of 75 mg/mL or 125 mg/mL, whereas IDT with 75 mg/mL or 62.5 mg/mL, respectively [49, 50].

Daptomycin is a cyclic lipopeptide for which a single case report mentioned IDT with solution 5 mg/mL representing 1:10 (10-1) dilution of the original vial concentration (50 mg/mL) obtained from a vial containing 500 mg daptomycin after reconstitution with 10 mL of sodium chloride 9 mg/mL [51].

Clindamycin is a semisynthetic derivative of lincomycin for which SPT at 150 mg/mL (ampoule with a solution containing clindamycin phosphate equivalent to 150 mg clindamycin in 1 mL) and IDT at 15 mg/mL (1:10 of prick solution concentration) have been recognized as nonirritating concentrations. But skin testing for clindamycin immediate hypersensitivity does not seem

to be useful in clinical practice due to many false negative tests [11,29, 52].

Colistin is a cyclic polypeptide antibacterial drug belonging to the polymyxin group. SPT may be performed with a solution of colistimethate sodium with the concentration of 80 mg/mL obtained by reconstituting the contents of a vial with 1 million IU (80 mg colistimethate) with 1 mL isotonic sodium chloride 9 mg/mL with gentle shaking. IDT was not performed in several published case reports due to its possible irritant effect [53-56].

Finally, no standardized immediate skin testing protocols for the **oxazolidinone** antibiotics linezolid and tedizolid have been published yet [11,33].

CONCLUSION

This concise informative article is not intended to substitute in any way the referral to an allergy expert with experience in drug-allergic reactions when necessary. It is intended for information purposes only, to present nonirritative concentrations of antibiotics used for SPT and IDT according to the new EAACI position paper and the latest AAAAI practice parameter, to understand that antibiotics are not intended to be tested in any circumstances, in any patients as general screening and at any concentration, to avoid skin testing with antibiotics at irritant concentrations, without the use of negative and positive controls to allow assessing false-positive and false-negative results. Information presented here cannot be held liable or responsible for inappropriate health care associated with the inappropriate use of this paper. When assessing patients and making therapeutical decisions, healthcare professionals are strongly recommended to use their own professional judgment and to respect national and local regulations and guidelines. The medical setting is rapidly evolving, and not all recommendations will be suitable or valid to all patients and they may change over time [3,34].

Conflict of interest: none declared
Financial support: none declared

REFERENCES

1. Birch K, Pearson-Shaver AL. Allergy Testing. 2022 Jul 25. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan–. PMID: 30725705.
2. Greiwe J, Bernstein JA. *In Vitro* and *In Vivo* Tests for Drug Hypersensitivity Reactions. In: Khan DA, Banerji A, eds. Drug Allergy Testing. St. Louis: Elsevier; 2018: p. 98-108.
3. Khan DA, Banerji A, Blumenthal KG, Phillips EJ, Solensky R, White AA et al. Drug allergy: A 2022 practice parameter update. *J Allergy Clin Immunol.* 2022 Sep 17:S0091-6749(22)01186-1. doi: 10.1016/j.jaci.2022.08.028. PMID: 36122788.
4. Rosti B, Mahler V. Adding a second skin prick test reading and modifying the cut-off for beta-lactam-specific IgE enhances the sensitivity in the routine diagnostic workup for immediate beta-lactam hypersensitivity. *Contact Dermatitis*. 2020 Nov;83(5):361-71. doi: 10.1111/cod.13622. PMID: 32462721.
5. Barbaud A, Weinborn M, Garvey LH, Testi S, Kvedariene V, Bavbek S et al. Intradermal Tests With Drugs: An Approach to Standardization. *Front Med* (Lausanne). 2020 May 15;7:156. doi: 10.3389/fmed.2020.00156. PMID: 32500075; PMCID: PMC7243670.
6. Park MA, May SM. Basics of Skin Testing and Drug Challenges. In: Khan DA, Banerji A, eds. Drug Allergy Testing. St. Louis: Elsevier; 2018: p. 86-96.
7. Brockow K, Romano A. Skin tests in the diagnosis of drug hypersensitivity reactions. *Curr Pharm Des.* 2008;14(27):2778-91. doi: 10.2174/138161208786369821. PMID: 18991697.
8. Brockow K, Garvey LH, Aberer W, Atanaskovic-Markovic M, Barbaud A, Bilo MB et al. ENDA/EAACI Drug Allergy Interest Group. Skin test concentrations for systemically administered drugs - an ENDA/EAACI Drug Allergy Interest Group position paper. *Allergy*. 2013 Jun;68(6):702-12. doi: 10.1111/all.12142. PMID: 23617635.
9. Wurpts G, Aberer W, Dickel H, Brehler R, Jakob T, Kreft B et al. Guideline on diagnostic procedures for suspected hypersensitivity to beta-lactam antibiotics: Guideline of the German Society for Allergology and Clinical Immunology (DGAKI) in collaboration with the German Society of Allergology (AeDA), German Society for Pediatric Allergology and Environmental Medicine (GPA), the German Contact Dermatitis Research Group (DKG), the Austrian Society for Allergology and Immunology (ÖGAI), and the Paul-Ehrlich Society for Chemotherapy (PEG). *Allergol Select.* 2020 May 28;4:11-43. doi: 10.5414/ALX02104E. PMID: 32568254; PMCID: PMC7304290.
10. Mayorga C, Montañez MI, Najera F, Bogas G, Fernandez TD, Gil DR et al. The Role of Benzylpenicilloyl Epimers in Specific IgE Recognition. *Front Pharmacol.* 2021 Feb 26;12:585890. doi: 10.3389/fphar.2021.585890. PMID: 33716734; PMCID: PMC7952312.
11. Broyles AD, Banerji A, Barmettler S, Biggs CM, Blumenthal K, Brennan PJ et al. Practical Guidance for the Evaluation and Management of Drug Hypersensitivity: Specific Drugs. *J Allergy Clin Immunol Pract.* 2020 Oct;8(9S):S16-S116. doi: 10.1016/j.jaip.2020.08.006.
12. Romano A, Atanaskovic-Markovic M, Barbaud A, Bircher AJ, Brockow K, Caubet JC et al. Towards a more precise diagnosis of hypersensitivity to beta-lactams - an EAACI position paper.

Allergy. 2020 Jun;75(6):1300-1315. doi: 10.1111/all.14122. PMID: 31749148.
13. Yuson CL, Katelaris CH, Smith WB. 'Cephalosporin allergy' label is misleading. *Aust Prescr.* 2018 Apr;41(2):37-41. doi: 10.18773/austprescr.2018.008. PMID: 29670309; PMCID: PMC5895476.
14. Chaudhry SB, Veve MP, Wagner JL. Cephalosporins: A Focus on Side Chains and β-Lactam Cross-Reactivity. *Pharmacy* (Basel). 2019 Jul 29;7(3):103. doi: 10.3390/pharmacy7030103. PMID: 31362351; PMCID: PMC6789778.
15. Torres MJ, Romano A, Celik G, Demoly P, Khan DA, Macy E et al. Approach to the diagnosis of drug hypersensitivity reactions: similarities and differences between Europe and North America. *Clin Transl Allergy.* 2017 Mar 13;7:7. doi: 10.1186/s13601-017-0144-0. PMID: 28293415; PMCID: PMC5347172.
16. Romano A, Gueant-Rodriguez R.M, Viola M et al. Diagnosing immediate reactions to cephalosporins. *Clin Exp Allergy.* 2005;35(9):1234–42.
17. www.sahealth.sa.gov.au
18. https://pch.health.wa.gov.au
19. www.accessdata.fda.gov
20. Azimi SF, Mainella V, Jeffres MN. Immediate Hypersensitivity to Fluoroquinolones: A Cohort Assessing Cross-Reactivity. *Open Forum Infect Dis.* 2022 Mar 2;9(4):ofac106. doi: 10.1093/ofid/ofac106. PMID: 35355888; PMCID: PMC8962755.
21. Porebski G, Kwiecien K, Pawica M, Kwitniewski M. Mas-Related G Protein-Coupled Receptor-X2 (MRGPRX2) in Drug Hypersensitivity Reactions. *Front Immunol.* 2018 Dec 20;9:3027. doi: 10.3389/fimmu.2018.03027. PMID: 30619367; PMCID: PMC6306423.
22. McNeil BD, Pundir P, Meeker S, Han L, Undem BJ, Kulka M, Dong X. Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions. *Nature*. 2015 Mar 12;519(7542):237-41. doi: 10.1038/nature14022. PMID: 25517090; PMCID: PMC4359082.
23. Kelso JM. MRGPRX2 signaling and skin test results. *J Allergy Clin Immunol Pract.* 2020 Jan;8(1):426. doi: 10.1016/j.jaip.2019.09.038. PMID: 31950910.
24. Krantz MS, Stone CA Jr, Yu R, Adams SN, Phillips EJ. Criteria for intradermal skin testing and oral challenge in patients labeled as fluoroquinolone allergic. *J Allergy Clin Immunol Pract.* 2021 Feb;9(2):1024-1028.e3. doi: 10.1016/j.jaip.2020.09.017. PMID: 32980582; PMCID: PMC8454706.
25. Doña I, Blanca-López N, Boteanu C, Cueva-Oliver B, Fernández-Sánchez FJ, Gajate P et al. Clinical Practice Guidelines for Diagnosis and Management of Hypersensitivity Reactions to Quinolones. *J Investig Allergol Clin Immunol.* 2021 Jul 26;31(4):292-307. doi: 10.18176/jiaci.0669. Epub 2021 Jan 19. PMID: 33461956.
26. Kuruvilla M. Macrolide Allergy. In: Khan DA, Banerji A, eds. Drug Allergy Testing. St. Louis: Elsevier; 2018: p. 141-49.
27. Sánchez-Morillas L, Laguna-Martínez JJ, Reaño-Martos M, Rojo-Andrés E, Gómez-Tembleque P, Santaolalla-Montoya M. Hypersensitivity to spiramycin with good tolerance of other macrolides. *J Investig Allergol Clin Immunol.* 2007;17(6):417-8. PMID: 18088029.
28. Mori F, Pecorari L, Pantano S, Rossi ME, Pucci N, De Martino M, Novembre E. Azithromycin anaphylaxis in children. *Int J Immunopathol Pharmacol.* 2014 Jan-Mar;27(1):121-6. doi: 10.1177/039463201402700116. PMID: 24674687.
29. Empedrad R, Darter AL, Earl HS, Gruchalla RS. Nonirritating intradermal skin test concentrations for commonly prescribed antibiotics. *J Allergy Clin Immunol.* 2003 Sep;112(3):629-30. doi: 10.1016/s0091-6749(03)01783-4. PMID: 13679828.
30. García-Rubio I, Martínez-Cócera C, Santos Magadán S, Rodríguez-Jiménez B, Vázquez-Cortés S. Hypersensitivity reactions to metronidazole. *Allergol Immunopathol* (Madr). 2006 Mar-Apr;34(2):70-2. doi: 10.1157/13086750. PMID: 16606549.
31. Asensio Sánchez T, Dávila I, Moreno E, Laffond E, Macías E, Ruiz A et al. Anaphylaxis due to metronidazole with positive skin prick test. *J Investig Allergol Clin Immunol.* 2008;18(2):138-9. PMID: 18447147.
32. Dilley M, Geng B. Immediate and Delayed Hypersensitivity Reactions to Antibiotics: Aminoglycosides, Clindamycin, Linezolid, and Metronidazole. *Clin Rev Allergy Immunol.* 2022 Jun;62(3):463-75. doi: 10.1007/s12016-021-08878-x. Epub 2021 Dec 15. PMID: 34910281; PMCID: PMC9156451.
33. Scolaro RJ, Crilly HM, Maycock EJ, McAleer PT, Nicholls KA, Rose MA, The R. Australian and New Zealand Anaesthetic Allergy Group Perioperative Anaphylaxis Investigation Guidelines. *Anaesth Intensive Care*. 2017 Sep;45(5):543-55. doi: 10.1177/0310057X1704500504. PMID: 28911283.
34. Solensky R. Hypersensitivity reactions to macrolides, aminoglycosides, tetracyclines, clindamycin, and metronidazole. *UpToDate.* Oct 2022. Accessed from www.uptodate.com
35. Romano A, Viola M, Di Fonso M, Rosaria Perrone M, Gaeta F, Andriolo M. Anaphylaxis to streptomycin. *Allergy*. 2002 Nov;57(11):1087-8. doi: 10.1034/j.1398-9995.2002.23836_9.x. PMID: 12359019.
36. Dorn JM, Volcheck GW. Sulfonamide Drug Allergy. In: Khan DA, Banerji A, eds. Drug Allergy Testing. St. Louis: Elsevier; 2018: p. 158-169.
37. Rosales MJ, Vega F. Anaphylactic shock due to fosfomycin. *Allergy*. 1998 Sep;53(9):905-7. doi: 10.1111/j.1398-9995.1998.tb04002.x. PMID: 9788697.
38. Gamboa PM, Antepara I, Jauregui I, Urrutia I, Sanz ML. Two patients with anaphylactic shock due to fosfomycin. *Ann Allergy Asthma Immunol.* 2011 Mar;106(3):260-1. doi: 10.1016/j.anai.2010.12.017. PMID: 21354031.
39. Zhu LJ, Liu AY, Wong PH, Arroyo AC. Road Less Traveled: Drug Hypersensitivity to Fluoroquinolones, Vancomycin, Tetracyclines, and Macrolides. *Clin Rev Allergy Immunol.* 2022 Jun;62(3):505-518. doi: 10.1007/s12016-021-08919-5. PMID: 35092578; PMCID: PMC9167562.
40. Maciag MC, Ward SL, O'Connell AE, Broyles AD. Hypersensitivity to tetracyclines: Skin testing, graded challenge, and desensitization regimens. *Ann Allergy Asthma Immunol.* 2020 Jun;124(6):589-593. doi: 10.1016/j.anai.2020.02.007. Epub 2020 Feb 20. PMID: 32087343; PMCID: PMC7250719.
41. Kayode OS, Rutkowski K. Vancomycin Hypersensitivity: It Is Not Always What It Seems. *J Allergy Clin Immunol Pract.* 2021 Feb;9(2):913-15. doi: 10.1016/j.jaip.2020.10.040. PMID: 33551043.
42. Fernandez JM, Fernandez AP, Lang DM. Other Antibiotic Allergy. In: Khan DA, Banerji A, eds. Drug Allergy Testing. St. Louis: Elsevier; 2018: p. 170-6.
43. Polk RE, Israel D, Wang J, Venitz J, Miller J, Stotka J. Vancomycin skin tests and prediction of "red man syndrome" in healthy volunteers. *Antimicrob Agents Chemother.* 1993 Oct;37(10):2139-43. doi: 10.1128/AAC.37.10.2139. PMID: 8257136; PMCID: PMC192241.
44. Hsiao SH, Chou CH, Lin WL, Lee EJ, Liao LH, Chang HJ et al. High risk of cross-reactivity between vancomycin and sequential teicoplanin therapy. *J Clin Pharm Ther*. 2012 Jun;37(3):296-300. doi: 10.1111/j.1365-2710.2011.01291.x. Epub 2011 Oct 23. PMID: 22017186.
45. Hung YP, Lee NY, Chang CM, Lee HC, Wu CJ, Chen PL et al. Tolerability of teicoplanin in 117 hospitalized adults with previous vancomycin-induced fever, rash, or neutropenia: a retrospective chart review. *Clin Ther.* 2009 Sep;31(9):1977-86. doi: 10.1016/j.clinthera.2009.09.010. PMID: 19843487.
46. Perrin-Lamarre A, Petitpain N, Trechot P, Cuny JF, Schmutz JL, Barbaud A. Glycopeptide-induced cutaneous adverse reaction: results of an immunoallergic investigation in eight patients. *Ann Dermatol Venereol.* 2010 Feb;137(2):101-5. doi: 10.1016/j.annder.2010.01.005. PMID: 20171430.

47. Nakkam N, Trubiano J, Gibson A, Phillips EJ. Considerations for cross-reactivity between vancomycin and other glycopeptides. *J Allergy Clin Immunol Pract.* 2021 Aug;9(8):3233. doi: 10.1016/j.jaip.2021.04.013. PMID: 34366100; PMCID: PMC8496740.
48. Huang V, Clayton NA, Welker KH. Glycopeptide Hypersensitivity and Adverse Reactions. *Pharmacy* (Basel). 2020 Apr 21;8(2):70. doi: 10.3390/pharmacy8020070. PMID: 32326261; PMCID: PMC7357119.
49. Asero R. Teicoplanin-induced anaphylaxis. *Allergy*. 2006 Nov;61(11):1370. doi: 10.1111/j.1398-9995.2005.01021.x. PMID: 17002717.
50. Savic LC, Garcez T, Hopkins PM, Harper NJ, Savic S. Teicoplanin allergy - an emerging problem in the anaesthetic allergy clinic. *Br J Anaesth.* 2015 Oct;115(4):595-600. doi: 10.1093/bja/aev307. PMID: 26385667.
51. Gisler V, Müller S, Müller L, Jörg-Walther L, Sendi P. Acute Angioedema Triggered by Daptomycin. *Infect Dis Ther.* 2016 Jun;5(2):201-5. doi: 10.1007/s40121-016-0111-4. PMID: 27228997; PMCID: PMC4929090.
52. Notman MJ, Phillips EJ, Knowles SR, Weber EA, Shear NH. Clindamycin skin testing has limited diagnostic potential. *Contact Dermatitis.* 2005 Dec;53(6):335-8. doi: 10.1111/j.0105-1873.2005.00716.x. PMID: 16364122
53. Domínguez-Ortega J, Manteiga E, Abad-Schilling C, Juretzcke MA, Sánchez-Rubio J, Kindelan C. Induced tolerance to nebulized colistin after severe reaction to the drug. *J Investig Allergol Clin Immunol.* 2007;17(1):59-61. PMID: 17323867.
54. Sieber J, Renner S, Lakatos-Krepcik A, Szépfalusi Z. Case Report: Maintenance of Desensitization to Nebulized Colomycin Over 10 Years. *Front Pediatr.* 2021 Apr 1;9:663228. doi: 10.3389/fped.2021.663228. PMID: 33869120; PMCID: PMC8049140
55. www.anm.ro
56. www.medicines.org.uk