Performance characteristics of basophil activations tests for diagnosing penicillin allergy: a meta-analysis

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Abstract

Background: Approximately 10% of the global population identify themselves as penicillin allergic, yet 90% are not truly allergic and could safely tolerate penicillin. There is no simple way to identify these people. Current *in vitro* diagnostics include specific immunoglobulin E, sIgE (with a sensitivity of 19% and specificity of 97%) and basophil activation testing (BAT) with undefined sensitivity and specificity.

Objective: To define the sensitivity and specificity of BAT in the diagnosis of penicillin allergy

Methods: PubMed and EMBASE searched from inception to 04/02/2023 for original studies evaluating the performance characteristics of basophil activation test for penicillin allergy in adults. Study selection, data extraction, risk of bias, assessment with QUADAS-2 tool, certainty assessment with GRADE methodology were performed independently, in duplicate. Meta-analysis was performed using Reitsma methodology.

Results: Twenty-two studies fulfilled the inclusion criteria. Twelve used the same positive threshold giving a summary point sensitivity 51% (95% CI, 46%-56%) and specificity 89% (95% CI, 85%-93%). Significant risk of bias was identified due to patient selection. GRADE certainty of evidence rated sensitivity "very low" due to imprecision and specificity as "low". There was great heterogeneity in methods used. Use of 1000 basophils per test did not improve performance above 500 basophils.

Conclusion: BAT sensitivity is highly variable across studies and remains too low to be considered as a routine element of clinical practice. BAT specificity is not as good as sIgE in penicillin allergy diagnosis. Significant further work is required in this field before clinical application of BAT in routine practice.

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Introduction

Between 6 – 10% of the general population in high-income countries carry a label of penicillin allergy (1, 2). It is estimated that around 90-95% of those with a label of penicillin allergy are misdiagnosed and could safely use penicillin antibiotics (3). Patients with a label of penicillin allergy who require antibiotic treatment are often prescribed second line antimicrobial regimens, resulting in sub-optimal medical management (4). There is also a risk to population health through the unnecessary use of broad spectrum antibiotics in place of penicillin, which adds to rising antimicrobial resistance (5).

The current process for assessing IgE-mediated penicillin allergy varies between healthcare systems across the globe. For example, in the United States (US) there are many non-specialists undertaking skin tests (ST) and oral drug provocation tests (DPT). *In vitro* testing may not be included in this work up. However, in Europe, assessment involves referral to a tertiary allergy centre for specialist review, which may include detailed clinical history, ST, specific immunoglobulin E (sIgE) testing, and an oral/intravenous DPT. To streamline the process of de-labelling, there has been increased use of direct DPT, without prior ST in low-risk patients (6, 7). One British study estimated 65% of people with a label of penicillin allergy could be deemed "low-risk", and hence suitable for a direct DPT (7). Comprehensive specialist allergy assessment is still required for patients that do not meet low-risk selection criteria or have unclear results.

A recent meta-analysis showed ST alone has a sensitivity of around 30% and a specificity of 97% (8). Both the ST and DPT come with a small but significant risk of a systemic reaction, with rates reported between 0.12% – 11 % (9, 10). The risk of a systemic reaction in DPT was 0.06%, but if the index reaction was anaphylaxis, this goes up to 6% (11). A negative DPT is considered the gold standard to exclude true penicillin hypersensitivity (12).

Unlike the *in vivo* tests, sIgE carries no risk of a reaction, as this is a serum test. In a meta-analysis of mostly European studies, sIgE in penicillin allergy, has a specificity similar to ST (˜ 97%) (8). Of note, this value may differ in other regions with different healthcare structures and prescribing practices. However, the sensitivity of sIgE testing is very low (˜ 19% for amoxicillin) (8). Also, sIgE testing is only available for a limited number of penicillins (penicillin V, benzylpenicillin, ampicillin, amoxicillin). Penicillin determinants, such as penicilloyl polylysine (PPL) and minor determinant mixture (MDM), have been developed to mimic the epitopes presented when penicillin antibiotics bind to proteins when in the circulation.

The 2020 European Academy of Allergy and Clinical Immunology (EAACI) position paper on improving diagnosis of beta-lactam hypersensitivity (12) recommends *in vitro* testing such as basophil activation test (BAT) or sIgE, prior to *in vivo* testing in high risk patients. Laboratory methods used for BAT are heterogeneous. Most commonly, BAT involves immediate processing (immediate to 48 hours) of whole blood samples in a flow cytometer. Blood cells are labelled with antibody markers for cell surface proteins to identify basophils (e.g. CD193+, CD123+, HLA-DR-), and to quantify basophil activation (CD63, CD203c) (13). Samples are then exposed to a minimum of two different concentrations of penicillin- based allergen. The penicillin used can be the specific culprit drug or another commonly used penicillin, and a penicillin determinant. Spontaneous activation of basophils without any exposure to an allergen is known to occur. To account for this, the stimulation index (SI) is calculated as the ratio of the percentage of activated basophils after exposure to drug, and the percentage of basophil activation when left untreated. For a positive result, treated basophils must demonstrate at least 5% activation, and an SI above a set threshold, commonly [?]2, for at least one of the concentrations of penicillin. There are variations in practice at almost every level of this process, with significant efforts being made to unify practice across Europe (14-16).

However, BAT is limited in its clinical application by the need for immediate flow cytometric analysis of whole blood samples and access to laboratories and trained staff who can deliver this. Basophil activation has been shown to be stable in samples stored for up to 24 hours if samples are stored at 4°C (17) . Access to such facilities and expertise within 24 hours is operationally challenging in the real-life setting. Especially compared to SIgE, which can instead be collected and stored for convenient future analysis.

To support the use of BAT in the diagnosis of penicillin allergy, there is a need for clarity on the sensitivity and specificity of the test is, and how this might alter with different BAT methods. This work brings together all published studies with data on sensitivity and specificity of BAT used in penicillin allergy diagnosis. Through sub-group analysis, it aims to explore how this sensitivity and specificity is affected by variations between methods, to guide decision making for allergists considering the use of BAT in penicillin allergy diagnosis.

Methods

The review was registered with PROSPERO number CRD42021223880, 25/05/2021. Methodology was in accordance with PRISMA-DTA (18) and grading of recommendations, assessment, development and evaluation (GRADE) guidelines (19).

A search of PubMed and EMBASE databases was carried out from inception to 04/02/2023 using the terms “penicillin” AND “basophil” AND “allergy” with no limits. Duplicated results were automatically removed by EndnoteX9 reference manager and remaining titles and abstracts were blindly and independently reviewed by two authors using rayyan.ai software. Inclusion criteria were predefined as original, retrospective or prospective studies evaluating the performance characteristics of basophil activation test for identifying penicillin allergy in adults (age >18). Exclusion criteria included case reports and studies with insufficient

key information. Manuscript authors were contacted through private communication to avoid duplication of results where multiple papers used similar cohorts and also where information was missing for key findings (true positive, true negative, false positive, false negative, SI threshold, minimum number of basophils used). This raw data was used to calculate sensitivity and specificity as our primary outcomes for this work.

Bivariate diagnostic random-effects meta-analysis and heterogeneity analysis was undertaken using RStudio (R version 4.2.0) using mada (meta-analysis of diagnostic accuracy) package (version 0.5.11). This allows the bivariate model of Reitsma et al (20, 21) to be fitted and generates sensitivity and specificity values with 95% confidence intervals (CI) and heterogeneity value. Restricted maximum likelihood (REML) was used for calculating the variance components. Figures were generated using the package meta, mada, metafor; summary receiver operator characteristic (SROC) curves to summarise studies which had multiple different positive thresholds, and forest plots demonstrating summary points for sensitivity and specificity were generated for studies which used the same positive thresholds (22). No covariates or predictors were used as we did not have access to individual participant data for all included studies.

Publication bias analysis was undertaken using methods outlined by Deeks et al (23) as the recommended method for meta-analysis of diagnostic test accuracy in The Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy (24).

Results

Database searches found 288 results in total (Figure 1). Citation manager removed 27 duplicates, leaving 261 titles and abstracts which were independently reviewed by two authors. This resulted in assessment of 58 full texts. Of six authors who were contacted to clarify key information, three responded. Final analysis included 22 publications with sufficient detail for risk of bias (RoB) assessment, Figure 2 (25-46). No amendments were made to the registered protocol.

Characteristics of all included publications are summarised in Online Repository (OLR) Table E1. This included results for a total of 935 penicillin allergy cases (median cases per study 28, range 2 – 158). The majority of cohorts were from Europe (n=20, 91%) and two (9%) from the USA. Nearly all studies, 95% (20 of 21 that included this information) were based in dedicated specialist Allergy Centres/Units. Time interval from most recent reaction to time of BAT was reported in 19 (83%) studies, with the maximum time for any one study up to 540 months. Time from sample collection to sample processing was only reported in nine (41%) studies. Of these, one (11%) reported "immediate" analysis, one (11%) reported "<2 hours", and six (67%) reported <24 hours. Penicillin allergy definition was based on European allergy diagnostic criteria as outlined by the EAACI/ European Network for Drug Allergy (ENDA) (12, 47) in eight (36%) of studies. Clinical history and at least one of skin test results or sIgE or drug provocation tests was used in a further 11 studies (50%). History alone was used in three (14%).

Sensitivity and specificity values, their risk of bias and applicability concerns are presented for all 22 studies in Figure 2. The SI threshold for positivity varied across the publications (2, 2.5 and 3 were all used). An estimation of a summary receiver operator characteristic (SROC) curve was generated using results from all 22 studies (Figure 3). The Higgins' I^2 of heterogeneity was 55.3% with a 95% CI 27.9% - 72.4%, indicating moderate between-study heterogeneity, and tau^2 equal to 0.2522 with a p-value <0.0001 of the Cochrane Q statistic suggests the result is statistically significant. Twelve of the studies which undertook flow cytometric analysis of whole blood and used an SI of 2 as positive threshold for the diagnostic test, (Figure 4). As a summary point should only be completed using methods with the same positive threshold, this allowed calculation of a summary point sensitivity of 51% (95% CI, 46% – 56%), and specificity of 89% (95% CI, 85% – 93%), AUC 0.666, I^2 14.4% (95% CI, 0% - 54%), tau^2 0, p =0.30 (Figure 4).

From the twenty-two manuscripts reporting both on sensitivity and specificity, six reported results for two different BAT assay types. 18 (64%) measured flow cytometric analysis of activation of basophils collected directly from the patient. Four (14%) measured sulfidoleukotriene production. Two (7%) measured histamine

release, two (7%) undertook indirect observation, and two (7%) underwent a direct observation of where basophils morphology was examined under a microscope to determine activation. The different methods had similar sensitivity and specificity profiles as can be seen in comparison of SROC curves (Figure 6), and as seen by an even spread across the SROC curve of all 22 studies (Figure 3).

The minimum number of basophils required for a sample to be analysed was reported in 16 studies (73%) with a median value of 500 basophils required per sample, with a range from 200 -1000. Eleven studies that used an SI threshold of 2, and had details of the minimum number of basophils used in their assay, allowed an estimated summary points for sensitivity and specificity to be generated (Figure 4). The use of a minimum of 1000 basophils (sensitivity 0.58 (95% CI, 0.48-0.68) and specificity 0.91 (95% CI, 0.82 - 0.96)) per test did not confer any significant improvement in sensitivity or specificity over a minimum of 500 (sensitivity 0.44 (95% CI, 0.36 – 0.51) and specificity 0.91(95% CI, 0.80 – 0.96)).

Two papers directly compared CD63 and CD203c as markers of basophil activation and suggested that CD203c was potentially a better marker (25, 46). Statistical comparison of summary points could not be undertaken as one study did not define its positive result threshold.

All studies were of high or at least unclear risk of bias (RoB). The most frequent source of potential bias was due to the patient selection process with 14 of 22 studies (64%) rated as high risk in this domain, Figure 2. This was largely due to the fact that most studies did not specify how patients were identified.

In keeping with GRADE guidance on grading the certainty of evidence in diagnostic test accuracy, we have considered the domains of imprecision and publication bias (19). There was considerable inconsistency in the reported sensitivity (ranging from 0.23 to 0.94) with minimal overlapping of the 95% CI (Figure 2). This did however improve when we considered only those studies looking at flow cytometric analysis of whole blood with a positive SI threshold of 2 (Figure 4). Specificity was found to be fairly consistent (ranging from 0.67 to 0.99). The specificity also demonstrated extensive overlapping of 95% CI, Figure 2, suggesting good consistency. Although there was variation in CI width for the reported sensitivity, the majority of studies (16 of 22, 73%) showed a 95% CI that was entirely above the sensitivity of 0.19 seen with sIgE, which is the relevant clinical comparison which we hope to improve upon with BAT. The 95% CI for specificity were much narrower than for sensitivity, demonstrating no need to lower the grading of the certainty of the evidence based on imprecision.

Publication bias was assessed for all 22 studies using a funnel plot (OLR Figure E1). The asymmetry suggests that there may be evidence of publication bias. However funnel plots may overestimate publication bias in meta-analyses of diagnostic test accuracy (23). Although one study showed BAT was more likely to be positive in those with a severe reaction (27), this work did not show any sensitivity-specificity relationship, and we have therefore not upgraded the certainty of evidence. Overall GRADE certainty of the evidence for sensitivity is “very low”, and for specificity is “low”, suggesting “the true effect might be markedly different from the estimated effect”.

Discussion

This work primarily highlights the significant variation in BAT sensitivity across all studies. Our primary finding from this work is that using flow cytometric analysis with an SI threshold of 2, BAT in penicillin allergy has an estimated summary point sensitivity of 51% (46% – 56%) and specificity of 89% (85% – 93%). For comparison, sIgE, (another *in vitro* diagnostic recognized for use in penicillin allergy diagnosis), showed poorer sensitivity (19.3% (95%CI, 12 – 29)) but higher specificity (sIgE specificity of 97.4% (95% CI, 95.2%-98.6%)) than BAT (8).

Flow cytometric immediate analysis of whole blood was the most commonly described assay type (seventeen of twenty-two papers). All used CD63 as a marker of basophil activation. Only two studies looked at the use of CD203c as a marker of basophil activation, both suggested slightly improved performance over CD63 (25,

46). However only one of these defined the positive threshold used, and so comparative sub group analysis of could not be undertaken.

Subgroup analysis of the minimum number of identified basophils required for any single BAT test demonstrated very similar sensitivity and specificity, suggesting no statistically significant difference between the use of 500 or 1000 basophils. This is clinically pertinent as it suggested a smaller collection volume may suffice, thereby increasing the likelihood of collecting an usable sample from the patient.

One study looked at the use of a novel dendrimeric antigens (DeAns) as carrier molecules for benzylpenicilloyl and amoxicilloyl in dense and stable hapten-carrier conjugates (35). This did not provide any diagnostic benefit above the use of benzylpenicilloyl, amoxicilloyl or free penicillin in BAT in this small sample.

Two US studies were considered in the overall analysis and generation of an SROC curve (Figure 3). However, once we looked at studies using flow cytometric analysis of whole blood that also used the same positive threshold of an SI of 2 to generate out primary results of the summary point sensitivity and specificity (Figure 4), all twelve of these studies were in fact from European centres. Due to differences in prescribing practices, healthcare system structures and population genetics, these results may differ in different countries. Further work with greater geographic diversity would be valuable.

The current order in which BAT can be used in penicillin allergy testing, according to European guidelines, is before ST for patients with a high-risk history, and after ST for low-risk patients (14). However, although the sensitivity of BAT was better than skin prick testing (51% vs 30%), sensitivity still remains extremely low. As the specificity of BAT is lower than skin testing (89% vs 97%), this paper does not provide strong evidence for the use of BAT to improve accuracy of investigations in penicillin allergy.

A questionnaire from 2007 with responses from 82 allergists across the world suggested 54% of responders used BAT in the work up of drug allergy hypersensitivity (48). A 2018 worldwide survey of the cost of allergy assessment, which included responses from 51 allergists, found the median cost for BAT was $129 (all values here given in US$ and adjusted for inflation to allow direct comparison), with only DPT costing more ($273) (49). Even with the cost of DPT, economic evaluations in both European and American healthcare systems have repeatedly concluded that widespread penicillin allergy testing with ST and DPT would be cost saving due to fewer courses of antibiotics, fewer outpatient visits and a need for fewer in hospital days (50, 51). Savings ranged from $50 to $7133 depending on the model used. One potential use of BAT might be to further decrease the costs of testing by decreasing the number of costly, and inevitably risky, DPT that need to be performed.

The 2020 EAACI position paper on beta-lactam allergy diagnosis suggests that "it is advisable to perform *in vitro* tests in addition to ST in high-risk patients in order to improve the sensitivity of the allergy workup and thus reduce the need for DPT", but does not clarifying if one or both tests should be done, or which test is preferred (12). BAT shows clearly improved sensitivity above sIgE (51% vs 19%), (8). However, including BAT and sIgE with their respective specificity of 89% and 97%, would still mean a small proportion of patients may erroneously be considered positive for penicillin allergy after optimal assessment, despite being able to tolerate penicillin. For BAT to become a routine part of the diagnostic work up for penicillin, it must either have a sensitivity that is high enough for it to be used as a screening test, or a specificity higher than skin test or sIgE (>97%).

One recurring theme across all twenty-two papers included was that there was a significant RoB through patient selection (Figure 2). The majority of papers only included final results on patients with definite immediate allergy compared to control groups with no history of allergy and able to tolerate oral penicillin. This aids clarity in understanding what a diagnostic test is showing, but it is not applicable to clinical practice, where indeterminate results and alternate diagnosis, such as delayed drug hypersensitivity and chronic spontaneous urticaria, complicate the clinical picture. Future work to overcome this issue should be undertaken, with prospectively collected consecutive samples from participants with suspected penicillin allergy who undergo the gold standard specialist work up.

It should also be noted that as we did not have access to individual data sets, we were unable to adjust for covariates or predictors which may have influenced results. Due to the significant heterogeneity in reporting of potential explanatory variable that may have influenced study outcomes across the manuscripts, we did not perform a meta-regression analysis. This may have identified further bias.

Another limitation is that, while many of the studies confirmed that patients were classified according to the EAACI or ENDA guidelines, not all participants will have had exactly the same assessment. DPT is felt to be the closest to a "gold standard test". However, given that the risk of anaphylaxis increases to 6% for patients with a history of anaphylaxis to penicillin (12), it is not appropriate to undertake DPT in most high risk cases. Furthermore, it is now well documented that skin testing can also lead to false positives with a recent meta-analysis reporting a summary sensitivity of 31% (95% CI, 19%-46%) and a specificity of 97% (95% CI, 94%-98%), (8). There was also significant heterogeneity in the definition of an "immediate reaction", with definitions ranging from less than 30 minutes (31, 36), to those occurring up to 24 hours (52), after drug administration.

The majority (91%) of these participants were recruited from Allergy Centres, when they have had an outpatient referral for assessment. While there has been work looking at de-labelling inpatients with DPT, no studies reported BAT results from an inpatient setting. Future work is required to explore if BAT can be used in different clinical settings, such as an emergency department, or in other outpatient facilities other than a highly specialised allergy clinic.

The time since the last reaction and BAT assessment also varied widely both between studies, and within studies. It was therefore not possible to undertake any sub-group analysis and comment on how this may have influenced the BAT outcome. As one potential use for BAT might be to investigate penicillin allergy in a person with a distant history of reaction, it would be important to know if a BAT result is reliable many years after the last penicillin exposure. A study published by Fernandez et al. showed that BAT reactivity decreased significantly even over a four-year study period (53). Only 1 of 41 patients was BAT positive at the four-year mark. When we consider this, along with the low sensitivity of the test from this meta-analysis, the clinical utility of BAT as a "rule-out" test may be limited. However, with its high specificity, BAT may be a good "rule-in" test and, if positive, could save patients from having a potentially harmful positive DPT. Future studies looking at the use of BAT as a diagnostic test should be clear about the time from reaction for the samples analysed, as this may have a significant effect on the BAT outcome.

There is still an unmet clinical need for improved *in vitro* diagnostics in penicillin allergy. BAT represents one potential avenue for investigation, however the evidence shown here has low certainty and is not yet ready for clinical application. Any future work looking at the application of BAT in the diagnosis of penicillin allergy can use this result as a benchmark of current practice, and should use an SI threshold of 2 and aim to improve upon the sensitivity of 51% and specificity of 89%. The use of a minimum number of 500 basophils per test is supported by this work.

Data and template data collection forms can be made available or request with corresponding author.

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Tables

	Data Source	Total positive cases	Country (Setting)	Reaction types included	Penicillin Allergy definition	Control group	Definition of immediate penicillin allergy; time to reaction	Time since last reaction median(range)	Time from samples collection to processing, hours	All BAT methods used	Penicillin used in assay	Marker of basophil activation	SI threshold for positive result	Minimum number of basophils per test
Abauf 2008	Clinical and Experimental Allergy	27	France (Allergy Centre)	20/27 anaphylaxis, 7/27 urticaria and angioedema	13 positive on SPT, 14 positive on IDT to Amoxicillin/Ampicillin/Cefuroxime	14 NSAID allergy, 6 dealyed skin reaction to amoxicillin		12		Flow cytometry	Amoxicillin, Ampicillin	CD63, CD203c	2	200
Arribas 2017	Journal of Investigational Allergology and Clinical Immunology	19	Spain (Allergy Units)	9/19 anaphylaxis, 7/19 urticaria,2/19 erythema, 1 unknown	sIgE to amoxicillin >0.35kU/L	10 previously received amoxicillin with no adverse effect, negative ST and sIgE to amoxicillin	<90 minutes from amoxicillin exposure to reaction	3(2-8)		Passive histamine release test	API,(amoxicillinyl-octa-L-lysine), amoxicillin			
Banni 2017	Italian Journal of Pediatrics	16*	Italy (Allergy Unit)	10 erythema, 9 LUC, 7 itching, 6 urticaria, 3 angioedema, 3 dyspnoea, 2 confusion, 1 throat tightness	According to ENDA criteria; 2 skin prick positive , 11 intradermal testing positive, 1 positive sIgE, 1 positive drug provocation test	5 negative on DPT	<1 hour after antibiotic; in the first 24–36 h of treatment	4(2-6)	<24	Flow cytometry (FAST)	Amoxicillin, amoxicillin/clavulanate potassium	CD63	2	500
Cecere 2017	Journal of Gerontology and Geriatrics	37	Italy (Immuno-Allergy Unit)	35/37 urticaria/agioedema, 237 anaphylaxis	Clinical history and skin tests according to EAACI criteria	21 Non allergic exposed subjects	Within 2 hours after taking the drug	(1-24)		Flow cytometry (Flow Cast kit)	Penicillin V, penicillin G, amoxicillin, ampicillin, PPL, MDM, cefuroxime	CD63	2	
De Weck 2009	Journal of Investigational Allergology and Clinical Immunology	158	European (Allergology Centres)	118/178 anaphylaxis, 28/178 angioedema, 58/178 urticaria, 4/178 morbilliform exanthema	ENDA criteria; 132 skin tests, 19 DPT or history of reaction documented by a physician of multiple clinical events, 40 sIgE	81 control patients, 76/81 no history of allergic reaction, 5/81 allergic to other drugs		(1-300)	<24	Flow cytometry (Flow-CAST) and sulfidoleukotriene	BPL, PPL, MDM, amoxicillin, ampicillin	CD63	2	500
Eberlein 2010	Clinical and Experimental Allergy	22*	Germany (Allergy Service)	4 anaphylaxis, 15 urticaria, 6 angioedema	History of type-I allergic reaction and positive skin test to beta-lactams (except one patient). 14 positive skin prick test, 17 positive IDT, 4 sIgE	16 individuals with negative history for antibiotic allergy, negative skin tests, negative sIgE		56(1-204)	<24	Flow cytometry (Flow-CAST and Flow2 CAST)	Penicillin G, penicillin V, PPL, MDM, Ampicillin, Amoxicillin, Cefuroxime	CD63	2	300
Gamboa 2004	Journal of Investigational Allergology and Clinical Immunology	23	Spain (Allergy Service)	10 anaphylaxis, 13 urticaria	History of two or more episodes of urticia/angioedema after beta-lactam administration or positive sIgE or positive drug provocation test.	30 non beta lactam allergic adults with negative skin test and DPT.	Within 30 minutes after administration	15(1-120)	<24	Flow cytometry (FAST), antigen specific sulfidoleukotriene	Penicillin G, PPL, MDM, Ampicillin, Amoxicillin	CD63	2	500
García-Avilés 2005	Journal of Investigational Allergology and Clinical Immunology	61	Spain (Allergy Service)	44 anaphylaxis, 17 urticaria	History of anphylaxis, urticaria or angioedema and positive skin test to at least one beta-lactam	30 adult patients negative skin test and DPT.	<1 hour after administration	2 (1-11)		Antigen specific sulfidoleukotriene	Penicillin G , ampicillin, amoxicillin, MDM, PPL, cefuroxime, cefazolin		3	
Heremans 2022	Journal of Allergy and Clinical Immunology: In Practice	66	Belgium (Allergy Service)		EAAO/ENDA Guidelines. History and skin prick or intradermal test positive, or DPT positive	70 in whom diagnosis of AX allergy was excluded after complete negative workup		54(0-540)		Flow cytometry		CD63, CD203c		
Joerg 2021	World Allergy Organization Journal	2	Switzerland (Allergy Centre)	2 anaphylaxis	History of anaphylaxis according to EAACI/ENDA standard and and skin test positive	3 IDT skin test negative		4(2-60)	<24 hours	Flow cytometry	Amoxicillin, Amoxicillin/Clavulanic acid	CD63	2.5	
Katz 1964	Journal of the American Medical Association	100	US (Naval Hospital)		History (questionnaire)	56 no history of penicillin allergy			1-150 days	Indirect technequé	Penicillin G, (PPL and 6-aminopenicillenicacid)			20
Koller 1992	Allergy	7	Austria (Allergy Service)	7 immediate systemic, 6 pruritus, 2 dyspnoea, 2 lip swelling, 1 rash	History of immediate allergy with positive sIgE or skin test positive	4 patients previously taken penicillin G or V and shown no hypersensitivity and no sIgE		2		Histamine release	PPL			
Marraccini 2018	International Archives of Allergy and Immunology	5	Italy (AllergyCentre)	58% angioedema and urticaria, 10%anaphylaxis, 32% no documentation*	History, positive sIgE or BAT, positive skin prick or intradermal test, drug provocation challenge.	24 negative sIgE, sin test and penicillin DPT		<2 years	Immediate	Flow cytometry	Penicilloyl G, penicilloyl V, amoxicillin, ampicillin, cefaclor	CD63	2	400
Molina 2018	Molecules	3	Spain	3 anaphylaxis	European Academy of Allergy and Clinical Immunology (EAACI)/European Network for Drug Allergy (ENDA) guidelines. History and skin test positive or sIgE positive	2 penicillin tolerant, cephalosporin allergic		5(5-6)		Flow cytometry	BPO-De-An-G2, BPO-De-AN-G4, AXO-De-An-G2, AXO-DeAn-G4	GP53	2	1000
Sainte-Laudy 2007	Inflammation Research	12	France (Respiratory Pathologies, Allergology and Anaesthesia)	4 anaphylaxis, 4 angio-oedem, 4 urticaria	History alone or history and skin prick or intradermal tests.	16 tolerated beta-lactam in the last 6 months; 3 dealyed beta-lactam reaction, with negative skin tests, 2 negative DPT, 7 NSAID urticaria, 2 muscle relaxant allergy	< 30 minutes	(NA-12)	<2	Flow cytometry	Amoxicillin, ceftriaxone, penicillin G	CD63	2	500
Soler 2018	Journal of Allergy and Clinical Immunology	57	Spain (Allergy Service)	13 anaphylaxic shock, 40 anaphylaxis, 4 urticaria/angioedema	EAAO/ENDA Guidelines. History and skin prick or intradermal test positive, or DPT positive	28 negative skin testing or DPT to penicillin but positive skin test or DPT to clavulanic acid		10(0-24)		Flow cytometry	Amoxicillin, amoxicillin/clavulanate potassium	CD63	2	500
Sanz 2002 ACI	Allergy and clinical immunology international	57	Spain (Allergy Service)	46 anaphylaxis, 11 urticaria-angioedema	History of immediate allergy with positive skin test	30 adults nonallergic adverse reactions to other drug. Negative skin tests, tolerates beta-lactam		96(1-360)	<24	Flow cytometry (FLOW-CAST), sulfidoleukotriene	Amoxicillin, benzylpenicillin	CD63	2	500
Sastre-Dominguez 1986	Allergologia et immunopathologia	16	Spain (Allergy Service)	12 urticaria, 1 anaphylactic shock, 8 angioedema, 2 pruritus	History of immediate reaction with either skin test or oral/parenteral drug provocation test	10 non atopic controls		22(2-96)		Flow cytometry	Benzyl-penicilyol ovalbumin, Penicillin G			20
Shelley 1963	Journal of the American Medical Association	138	USA (Allergy Service)		History of immediate allergy	70 adults recently tolerate penicillin* of these 66 TN, 4 FN on both direct and indirect fixation technique				Indirect technique and direct fixation	6 -amino-penicilloic acid, potassium penicillin G, Sodium penicillin O, potassium phenoymethyl penicillin, potassium penicillin V, potassium phenethicillin, methicillin, oxacillin			20
Thinnes 2018	Cutaneous and Ocular Toxicology	9	Germany (Allergy Centre)	6 urticaria, 1 angioedema , 2 other	History of type I hypersensitivity reaction and skin prick or patch test or sIgE positive	3 negative skin test, sIgE and penicillin drug provocation test		137(2-360)		Flow cytometry	Amoxicillin, penicillin, ampicillin	CD63	3	500
Torres 2004	Clinical and Experimental Allergy	61	Spain (Allergy Service)	15 urticaria, 46 anaphylaxis	European Network for Drug Allergy (ENDA). Hisotry of immediate reaction and sIgE or skin testing or DPT positive	40 skin test negative patients with toleranceto beta-lactams		8(1-24)		Flow cytometry (basotest)	PPL, MDM, penicillin G, amoxicillin, ampicillin, cefuroxime, cefacor	GP53	2	1000
Torres 2010 JACI	Journal of Allergy and Clinical Immunology	39	Spain (Allergy Centre)		History of immediate allergic reaction and positive skin test	30 good tolerance of amoxicillin/clavulanic acid				Flow cytometry (basotest)	Benzylpenicillin, amoxicillin, amoxicillin/clavulanate potassium	GP53	2	1000

Table Summary of characteristics of included studies, n=22

Figures

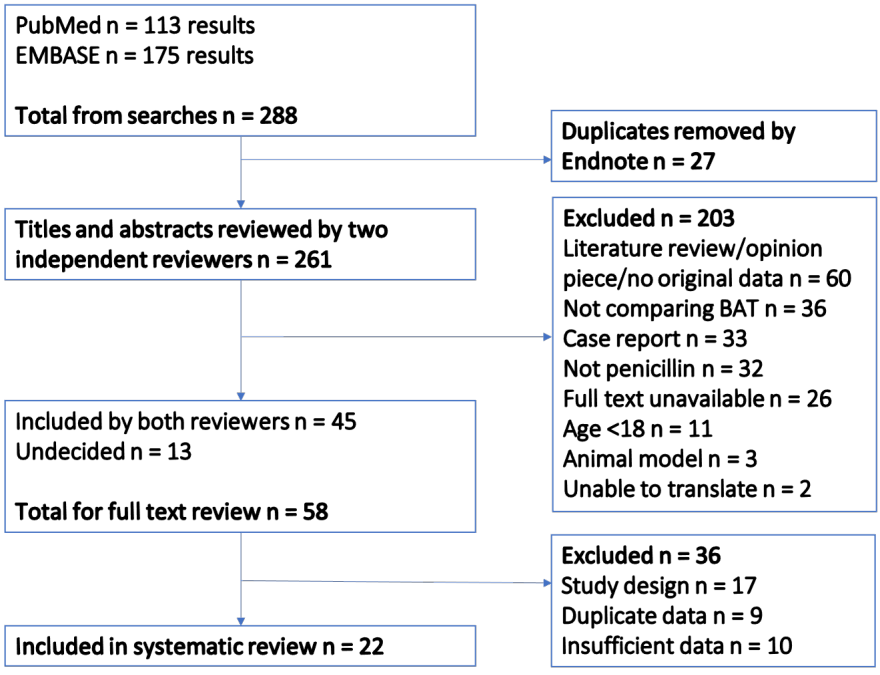


Figure Study Selection

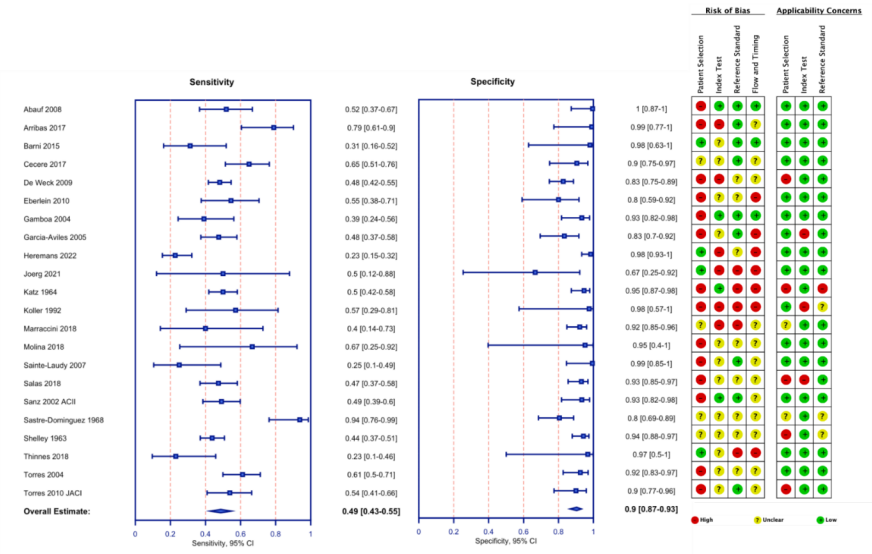


Figure Extracted sensitivity and specificity along-side risk of bias and applicability concerns summary: authors' judgement about each domain for each of the 22 included studies, using QUADAS-2.

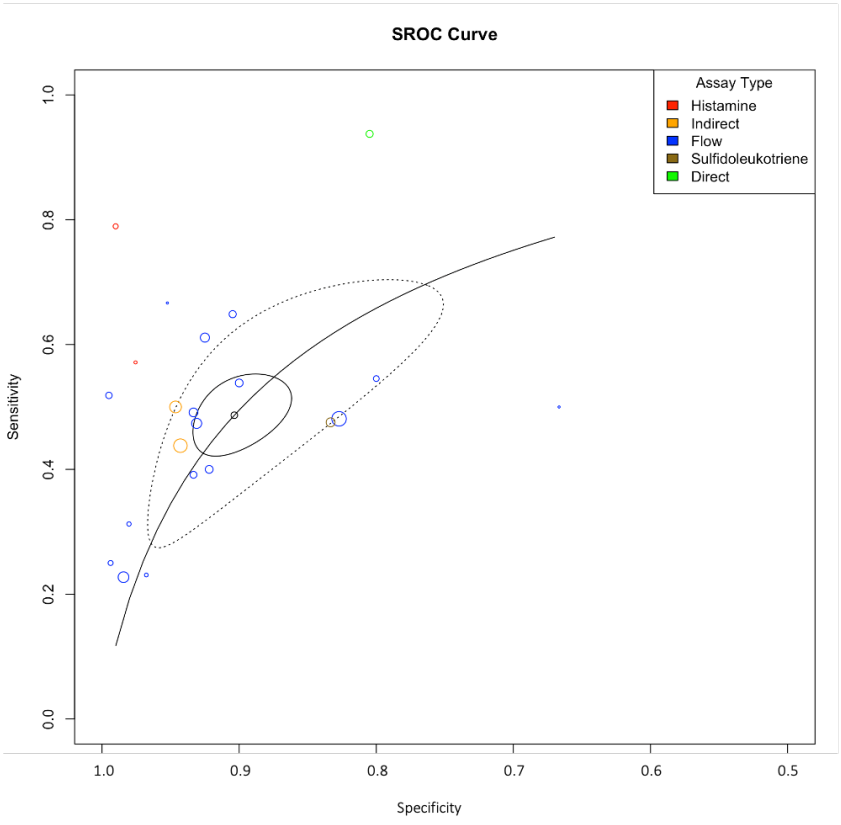


Figure Summary receiver operator characteristic curve (SROC) for the most sensitive assay type reported from each of the 22 studies with complete values for true positive, false positive, true negative, false negative. This included papers with a range of different positivity thresholds for stimulation index (SI) (SI range between 2 to 3). Individual study values plotted and colour coded to reflect the assay type and size to represent number of participants. AUC 0.788, I^2 55.3% (27.9% - 72.4%), tau^2 = 0.252, p-value <0.0001

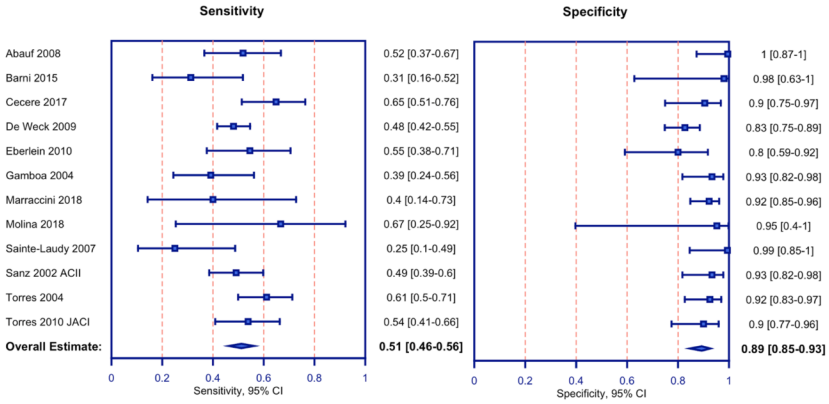


Figure Summary of sensitivity and specificity for 12 studies all with stimulation index (SI) positive threshold of 2, allowing an estimation of a summary point for sensitivity (0.51 (0.46 - 0.56) and specificity 0.89 (0.85 - 0.93),AUC 0.67, I^2 14.4%, tau^2 0.0, p=0.30

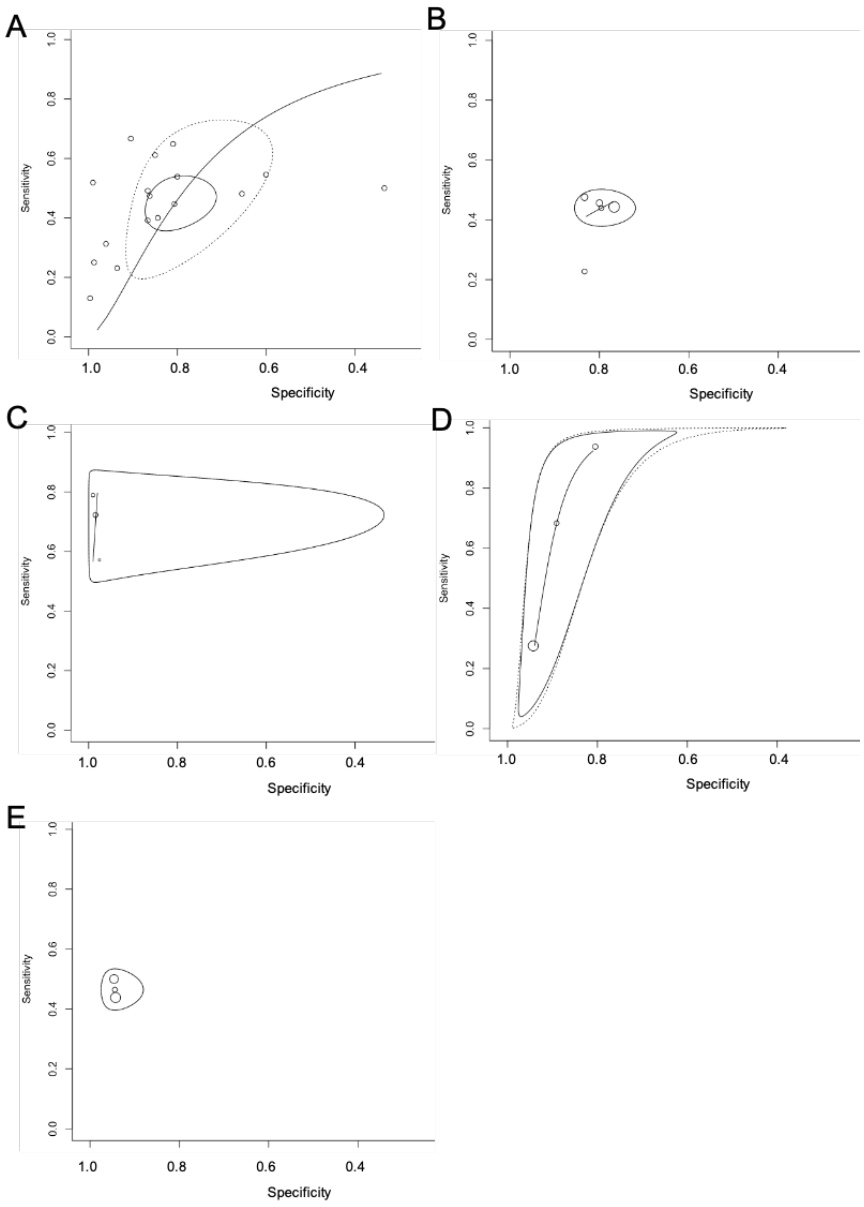
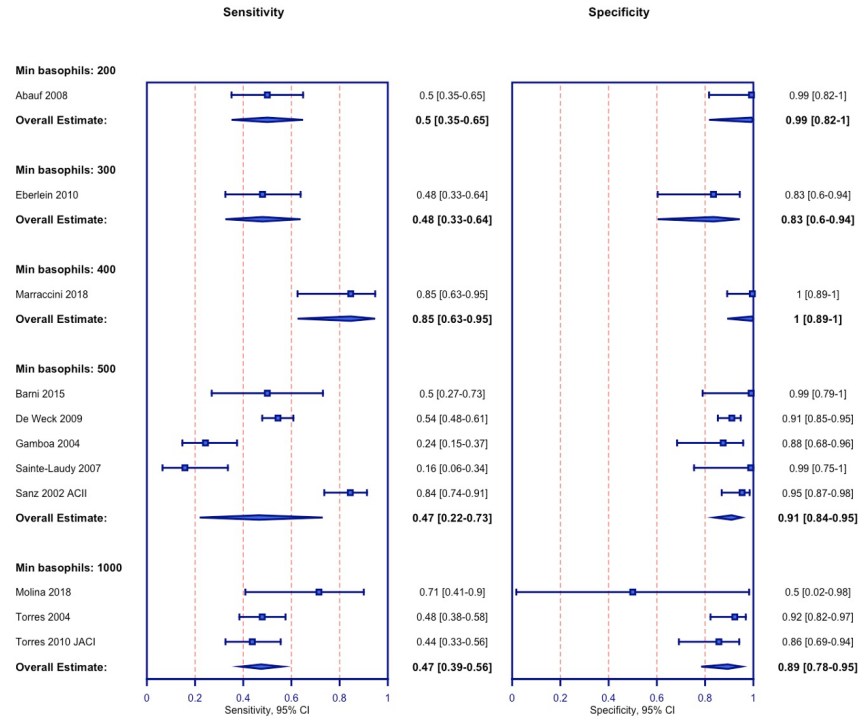
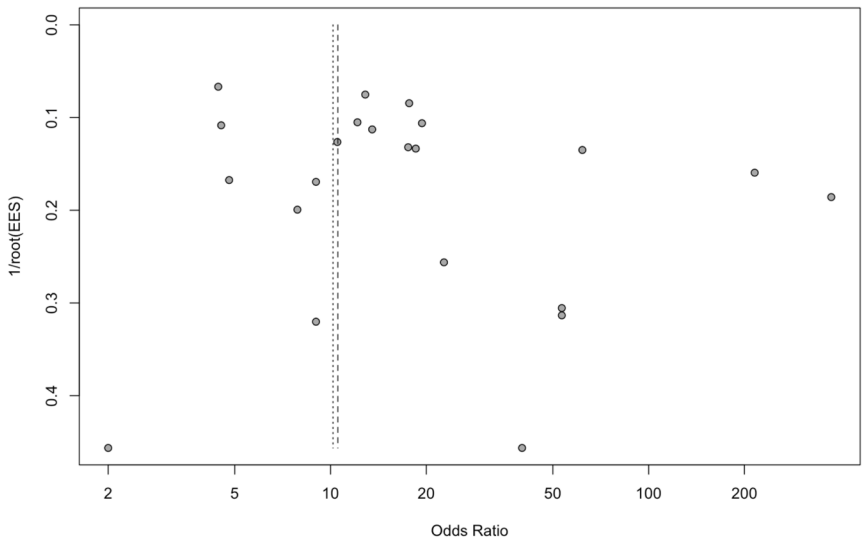


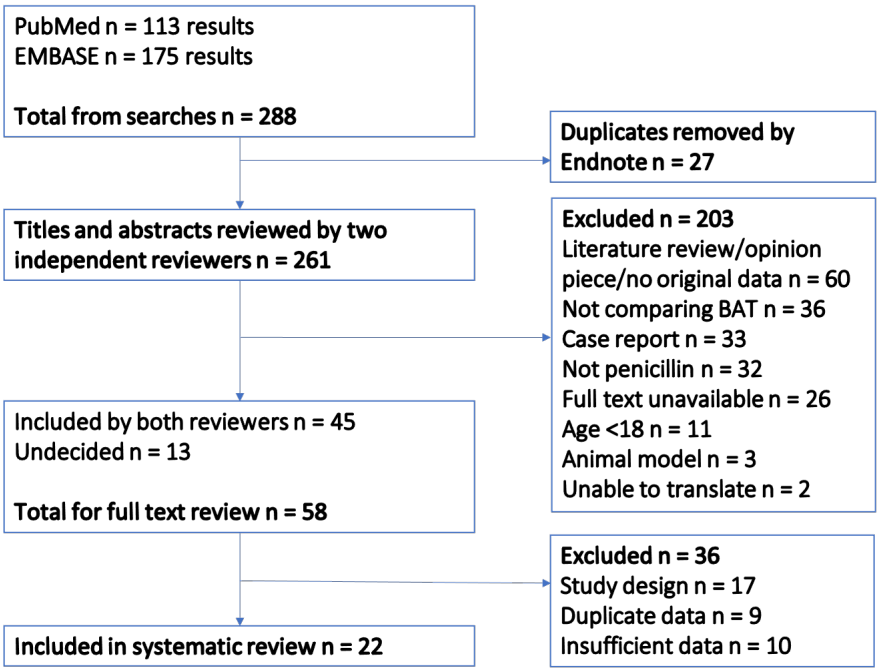
Figure Summary receiver operator characteristic curves (SROC) from all 22 papers including results on two assay types from six of the paper, so 28 results in total. Note we have only presented SROC curves as different stimulation index (SI) thresholds for a positive value were used across all studies. A - studies immediately analyzing whole blood with flow cytometry, n=17, I^2=59.78% (95% CI 30.3 – 76.7%), $tau^2$0.292; B – studies measuring sulfidoleukotriene release, n=4, I^2=26.9% (95% CI 0 – 72.5%), tau^2 0.0; C – studies measuring histamine release , n=2, I^2=15.8%, $tau^2$0; D studies using a direct method of observing basophils, n=2, I^2=91.8% (95% CI71.7% - 97.6%), $tau^2$3.3713; D studies using an indirect method of observing basophils, n=2, I^2=0%, $tau^2$0 .

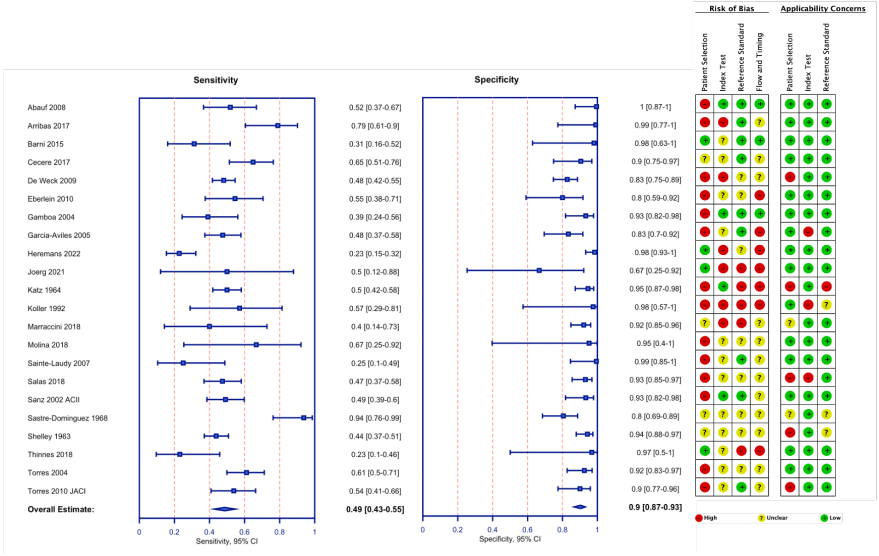


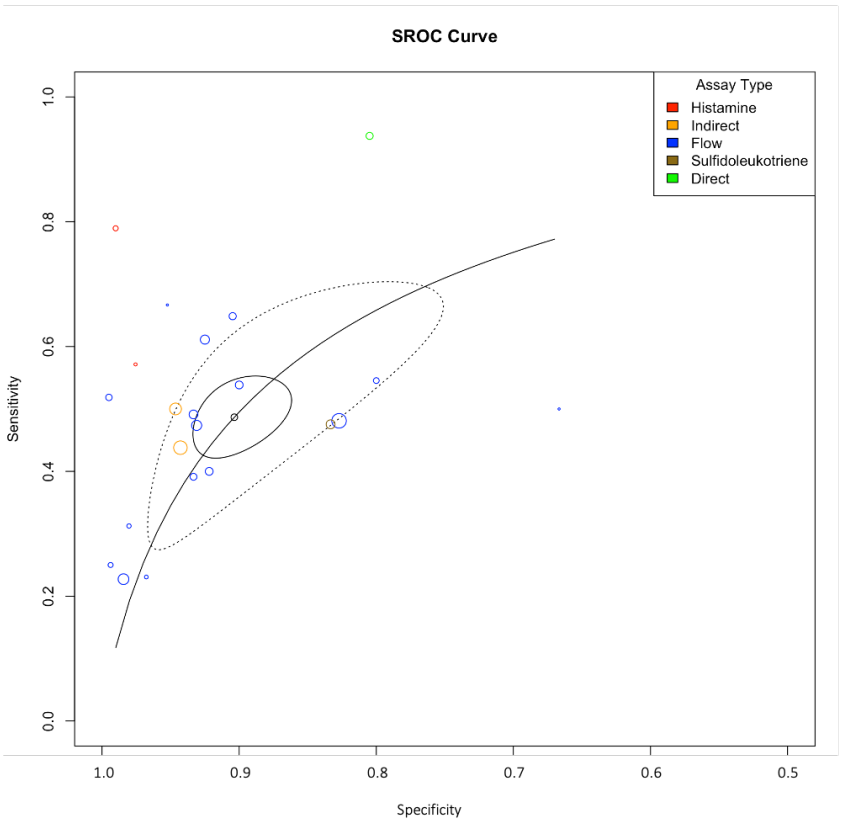


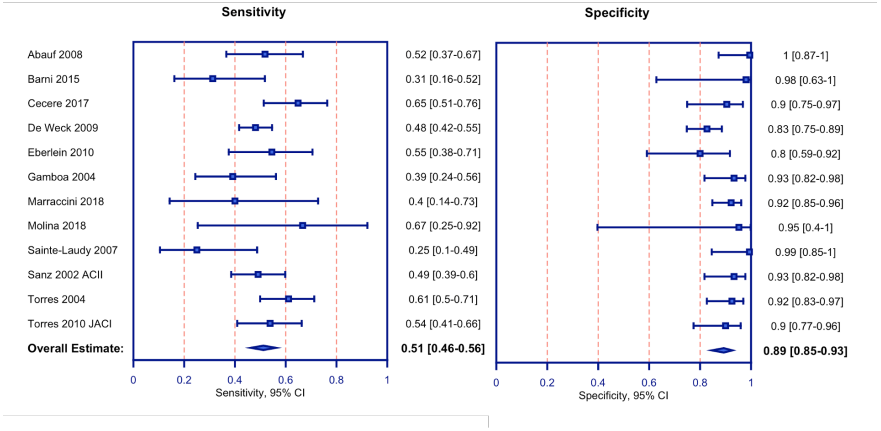
Appendix 1

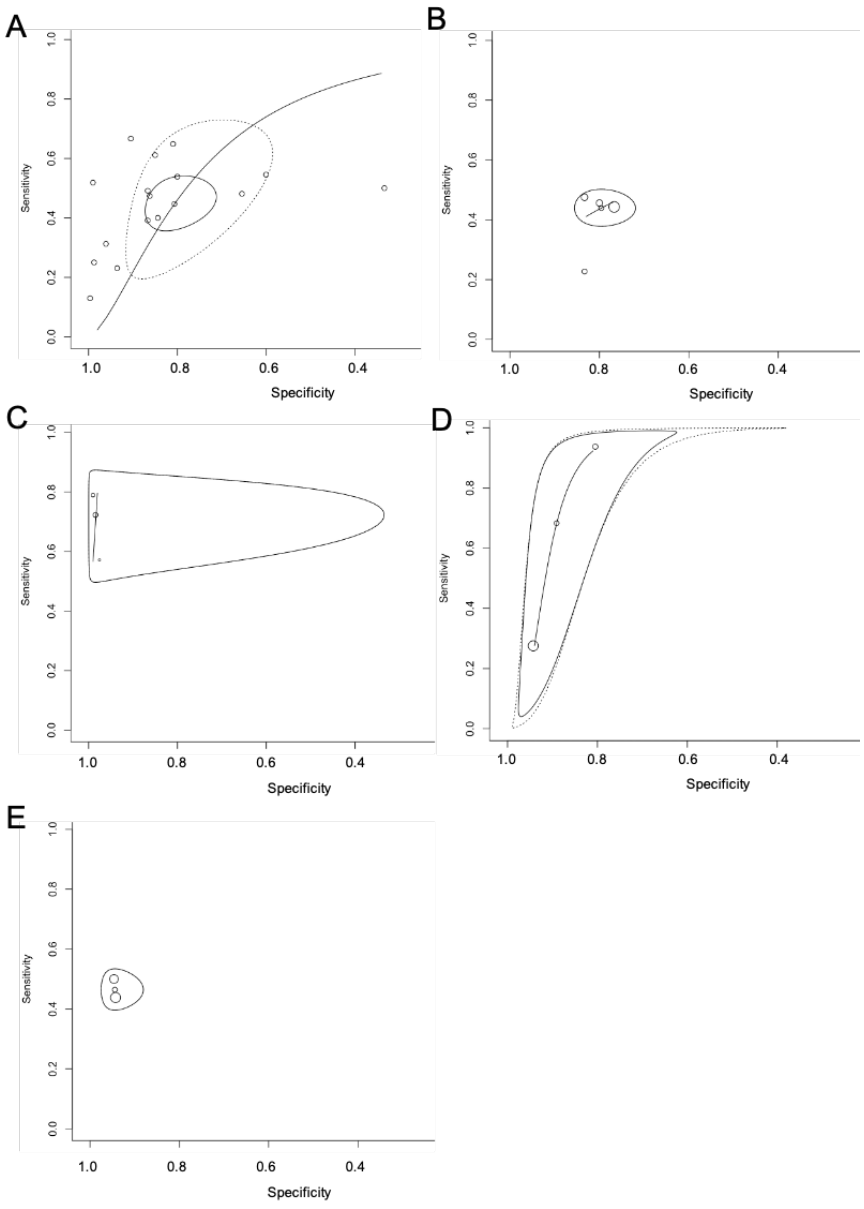
Search example from Ovid EMBASE, accessed 03/05/2022

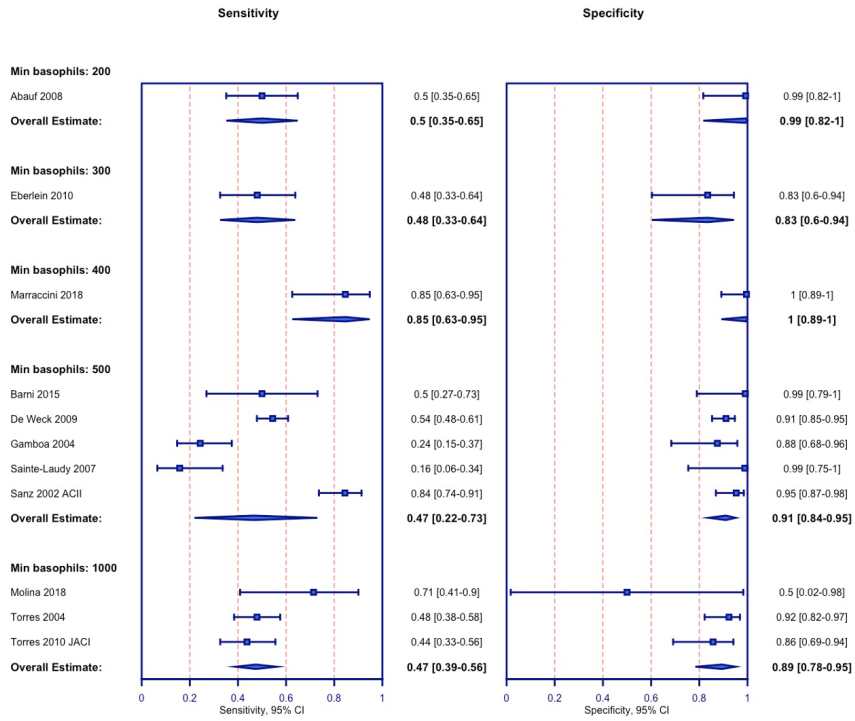


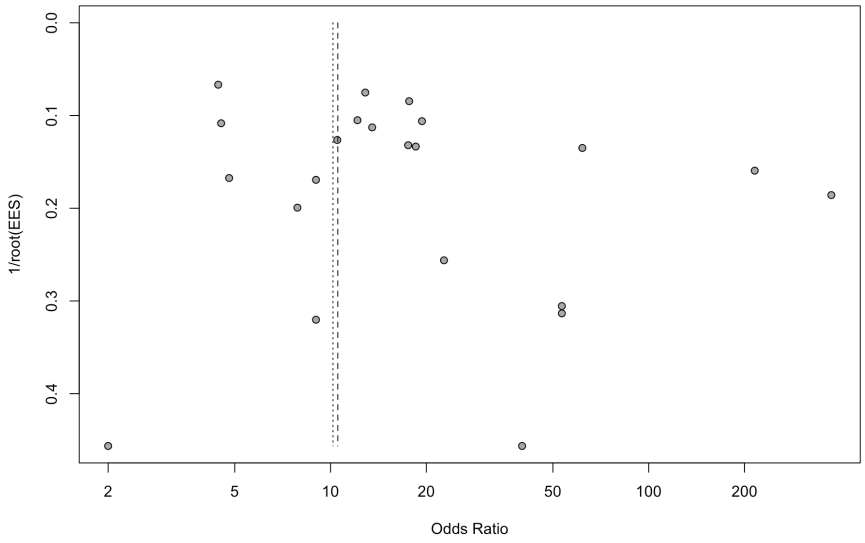


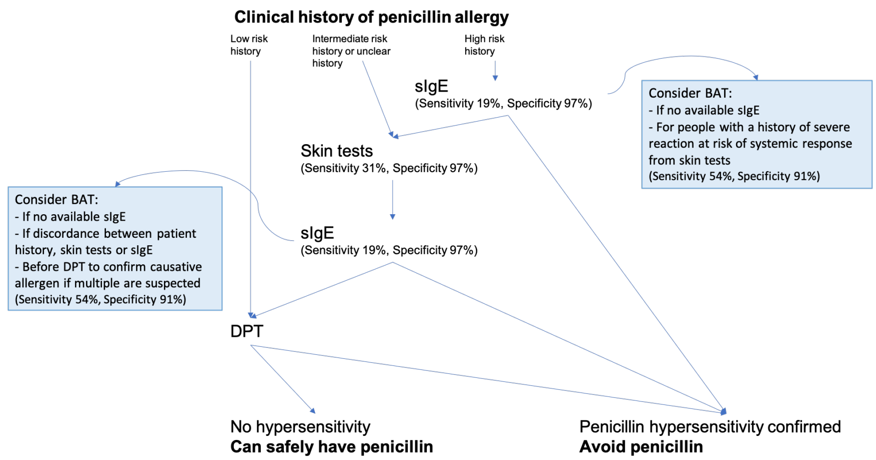












	Data Source	Total positive cases	Country (Setting)	Reaction types included	Penicillin Allergy definition	Control group	Definition of immediate penicillin allergy; time to reaction	Time since last reaction median(range)	Time from samples collection to processing, hours	All BAT methods used	Penicillin used in assay	Marker of basophil activatoin	SI threshold for positive result	Minimum number of basophils per test
Abauf 2008	Clinical and Experimental Allergy	27	France (Allergy Centre)	20/27 anaphylaxis, 7/27 urticaria and angioedema	13 positive on SPT, 14 positive on IDT to Amoxicillin/Ampicillin/Cefuroxime	14 NSAID allergy, 6 dealyed skin reaction to amoxicillin	<1 hours from ingestion to reaction	12		Flow cytometry	Amoxicillin, Ampicillin	CD63, CD203c	2	200
Arribas 2017	Journal of Investigational Allergology and Clinical Immunology	19	Spain (Allergy Units)	9/19 anaphylaxis, 7/19 urticaria,2/19 erythema, 1 unknown	sIgE to amoxicillin >0.35kUa/L	10 previously received amoxicillin with no adverse effect, negative ST and sIgE to amoxicillin	<90 minutes from amoxicillin exposure to reaction	3(2-8)		Passive histamine release test	APL(amoxicilloyl-octa-L-lysine), amoxicillin			
Barni 2017	Italian journal of Pediatrics	16*	Italy (Allergy Unit)	10 erythema, 9 LOC, 7 itching, 6 urticaria, 3 angioedema, 3 dyspnoea, 2 confusion, 1 throat tightness	According to ENDA criteria; 2 skin prick positive , 11 intradermal testing positive, 1 positive sIgE, 1 positive drug provocation test	5 negative on DPT	<1 hour after antibiotic; in the first 24–36 h of treatment	4(2-6)	<24	Flow cytometry (FAST)	Amoxicillin, amoxicillin/clavulanate potassium	CD63	2	500
Cecere 2017	Journal of Gerontology and Geriatrics	37	Italy (Immuno-Allergy Unit)	35/37 urticaria/agioedema, 237 anaphylaxis	Clinical history and skin tests according to EAACI criteria	21 Non allergic exposed subjects	Within 2 hours after taking the drug	(1-24)		Flow cytometry (Flow Cast kit)	Penicillin V, penicillin G, amoxicillin, ampicillin, PPL, MDM, cefuroxime	CD63	2	
De Weck 2009	Journal of Investigational Allergology and Clinical Immunology	158	European (Allergology Centres)	118/178 anaphylaxis, 28/178 angioedema, 58/178 urticaria, 4/178 morbilliform exanthema	ENDA criteria; 132 skin tests, 19 DPT or history of reaction documented by a physician of multiple clinical events, 40 sIgE	81 control patients, 76/81 no history of allergic reaction, 5/81 allergic to other drugs		(1-300)	<24	Flow cytometry (Flow-CAST) and sulfidoleukotriene	BPN, PPL, MDM, amoxicillin, ampicillin	CD63	2	500
Eberlein 2010	Clinical and Experimental Allergy	22*	Germany (Allergy Service)	4 anaphylaxis, 15 urticaria, 6 angioedema	History of type-I allergic reaction and positive skin test to beta-lactams (except one patient). 14 positive skin prick test, 17 positive IDT, 4 sIgE	16 individuals with negative history for antibiotic allergy, negative skin tests, negative sIgE		56(1-204)	<24	Flow cytometry (Flow-CAST and Flow2 CAST)	Penicillin G, penicillin V, PPL, MDM, Ampicillin, Amoxicillin, Cefuroxime	CD63	2	300
Gamboa 2004	Journal of Investigational Allergology and Clinical Immunology	23	Spain (Allergy Service)	10 anaphylaxis, 13 urticaria	History of two or more episodes of urticia/angioedema after beta-lactam administration or positive sIgE or positive drug provocation test.	30 non beta lactam allergic adults with negative skin test and DPT.	Within 30 minutes after administration	15(1-120)	<24	Flow cytometry (FAST), antigen specific sulfidoleukotriene	Penicillin G, PPL, MDM, Ampicillin, Amoxicillin	CD63	2	500
Garcia-Aviles 2005	Journal of Investigational Allergology and Clinical Immunology	61	Spain (Allergy Service)	44 anaphylaxis, 17 urticaria	History of anphylaxis, urticaria or angioedema and positive skin test to at least one beta-lactam	30 adult patients negative skin test and DPT.	<1 hour after administration	2 (1-11)		Antigen specific sulfidoleukotriene	Penicillin G , ampicillin, amoxicillin, MDM, PPL, cefuroxime, cefazolin		3	
Heremans 2022	Journal of Allergy and Clinical Immunology: In Practice	66	Belgium (Allergy Service)		EAACI/ENDA Guidelines. History and skin prick or intradermal test positive, or DPT positive	70 in whom diagnosis of AX allergy was excluded after complete negative workup		54(0-540)		Flow cytometry		CD63, CD203c		
Joerg 2021	World Allergy Organisation Journal	2	Switzerland (Allergy Centre)	2 anaphylaxis	History of anaphylaxis according to EAACI/ENDA standard and and skin test positive	3 IDT skin test negative		4(2-60)	<24 hours	Flow cytometry	Amoxicillin, Amoxicillin/Clavulanic acid	CD63	2.5	
Katz 1964	Journal of the American Medical Association	100	US (Naval Hospital)		History (questionnaire)	56 no history of penicillin allergy			1-150 days	Indirect techneque	Penicillin G, (PPL and 6-aminopenicillenicacid)			20
Koller 1992	Allergy	7	Austria (Allergy Service)	7 immediate systemic, 6 pruritus, 2 dyspnoea, 2 lip swelling, 1 rash	History of immediate allergy with positive sIgE or skin test positive	4 patients previously taken penicillin G or V and shown no hypersensitivity and no sIgE		2		Histamine release	PPL			
Marraccini 2018	International Archives of Allergy and Immunology	5	Italy (AllergyCentre)	58% angioedema and urticaria, 10%anaphylaxis, 32% no documentation*	History, positive sIgE or BAT, positive skin prick or intradermal test, drug provocation challenge.	24 negative sIgE, sin test and penicillin DPT		<2 years	Immediate	Flow cytometry	Penicilloyl G, penicilloyl V, amoxicillin, ampicillin, cefaclor	CD63	2	400
Molina 2018	Molecules	3	Spain	3 anaphylaxis	European Academy of Allergy and Clinical Immunology (EAACI)/European Network for Drug Allergy (ENDA) guidelines. History and skin test positive or sIgE positive	2 penicillin tolerant, cephalopsorin allergic		5(5-6)		Flow cytometry	BPO-De-An-G2, BPO-De-AN-G4, AXO-De-An-G2, AXO-DeAn-G4	GP53	2	1000
Sainte-Laudy 2007	Inflammation Research	12	France (Respiratory Pathologies, Allergology and Anaesthesia)	4 anaphylaxis, 4 angio-oedem, 4 urticaria	History alone or history and skin prick or intradermal tests.	16 tolerated beta-lactam in the last 6 months; 3 dealyed beta-lactam reaction, with negative skin tests, 2 negative DPT, 7 NSAID urticaria, 2 muscle relaxant allergy	< 30 minutes	(NA-12)	<2	Flow cytometry	Amoxicillin, ceftriaxone, penicillin G	CD63	2	500
Salas 2018	Journal of Allergy and Clinical Immunology	57	Spain (Allergy Service)	13 anaphylaxic shock, 40 anaphylaxis, 4 urticaria/angioedema	EAACI/ENDA Guidelines. History and skin prick or intradermal test positive, or DPT positive	28 negative skin testing or DPT to penicillin but positive skin test or DPT to clavulanic acid		10(0-24)		Flow cytometry	Amoxicillin, amoxicillin/clavulanate potassium	CD63	2	500
Sanz 2002 ACII	Allergy and clinical immunology International	57	Spain (Allergy Service)	46 anaphylaxis, 11 urticaria-angioedema	History of immediate allergy with positive skin test	30 adults nonallergic adverse reactions to other drug. Negative skin tests, tolerates beta-lactam		36(1-360)	<24	Flow cytometry (FLOW-CAST), sulfidoleukotriene	Amoxicillin, benzylpenicillin	CD63	2	500
Sastre-Dominguez 1986	Allergologia et immunopathologia	16	Spain (Allergy Service)	12 urticaria, 1 anaphylactic shock, 8 angioedema, 2 pruritus	History of immediate reaction with either skin test or oral/parenteral drug provocation test	10 non atopic controls		22(2-96)		Flow cytometry	Benzyl-peniciloyl ovalbumin, Penicillin G			20
Shelley 1963	Journal of the American Medical Association	138	USA (Allergy Service)		History of immediate allergy	70 adults recently tolerate penicillin* of these 66 TN, 4 FN on both direect and indirect fixation techneque				Indirect technique and direct fixation	6 -amino-penicilloic acid, potassium penicillin G, Sodium penicillin O, potassium phenoymethyl penicillin, potassium penicillin V, potassium phenethicillin, methicillin, oxocillin			20
Thinnes 2018	Cutaneous and Ocular Toxicology	9	Germany (Allergy Centre)	6 urticaria, 1 angioedema , 2 other	History of type I hypersensitivity reaction and skin prick or patch test or sIgE positive	3 negative skin test, sIgE and penicillin drug provocation test		137(2-360)		Flow cytometry	Amoxicillin, penicillin, ampicillin	CD63	3	500
Torres 2004	Clinical and Experimental Allergy	61	Spain (Allergy Service)	15 urticaria, 46 anaphylaxis	European Network for Drug Allergy (ENDA). Hisotry of immediate reaction and sIgE or skin testing or DPT positive	40 skin test negative patietns with toleranceto beta-lactams		8(1-24)		Flow cytometry (basotest)	PPL, MDM, penicillin G, amoxicillin, ampicillin, cefuroxime, ceflacor	GP53	2	1000
Torres 2010 JACI	Journal of Allergy and Clinical Immunology	39	Spain (Allergy Centre)		History of immedaite allergic reaction and positive skin test	30 good tolerance of amoxicillin/clavulanic acid				Flow cytometry (basotest)	Benzylpenicillin, amoxicillin, amoxicillin/clavulanate potassium	GP53	2	1000

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