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Lymphocyte transformation test for drug allergy detection

When does it work?

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ABSTRACT

Background: The lymphocyte transformation test (LTT) is an in vitro test system for the detection of a sensitization in the context of allergies to drugs. Its reported sensitivity varies largely and seems to be affected by different parameters. In review articles, the average LTT performance was often calculated by combining overall mean sensitivities of various published studies, but without considering different patient characteristics or varying patient numbers per publication.
Objective: To investigate the impact of different patient-specific and methodological parameters on the sensitivity of the LTT based on data on the level of the individual patient extracted from single studies.
Methods: We performed an advanced literature search in PubMed and screened the identified publications according to previously defined inclusion criteria. In total, individual patient data from 721 patients were extracted from 30 studies. Random-effects meta-regression analyses were performed.
Results: The analysis indicate that the enzyme-linked immunosorbent assay–based read-out is more sensitive compared with the classical radioactivity method (enzyme-linked immunosorbent assay: 80% vs radioactivity: 66%; $P = .08$). Interestingly, drug reaction with eosinophilia and systemic symptoms/drug-induced hypersensitivity syndrome is associated with a higher probability of a positive LTT test result compared with other investigated clinical phenotypes ("drug reaction with eosinophilia and systemic symptoms/drug-induced hypersensitivity syndrome" vs "bullous reaction"; odds ratio, 2.52; *P* value = .003). Our analysis also revealed an impact of the time to testing period after the occurrence of the allergic event ("< 2 weeks" vs "2 weeks-2 months"; odds ratio, 2.12; *P* value = .03).
Conclusion: The read-out method and relevant clinical parameters affect the sensitivity of the LTT. These findings are based on a meta-analysis providing a higher level of evidence than a single study or previous reviews not considering individual patient data.

Introduction

The clinical spectrum of drug hypersensitivity reactions ranges from harmless rashes to fatal reactions, such as allergic shock or toxic epidermal necrolysis.[1] A reliable detection and the exclusion of a drug allergic reaction are of huge relevance for the patients and the treating physicians. In consequence, many different in vivo and in vitro diagnostic tools with varying reliability are used for the detection of drug allergies.[2] One of these diagnostic tools is the lymphocyte transformation test (LTT), an in vitro test system for the detection of sensitization in the context of allergies caused by drugs or other substances.[3]

Since its introduction in the 1960s, the LTT has been used in many studies for the detection of a drug allergy. Over time, it became evident that a number of different patient-specific and methodological parameters may affect the outcome of the LTT.[4]

According to Mayorga et al,[5,6] the sensitivity of the LTT determined in various publications varies enormously from 25% to 89%, which can at least partly be explained by the fact that some studies focused on specific parameters by analyzing only one clinical phenotype or drug. An additional fundamental issue is that the number of patients within a study might not be sufficient to draw a final

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conclusion about the LTT performance.[7-9] The issue of small patient numbers was addressed by some review articles calculating the average LTT performance by combining overall mean sensitivities of many LTT publications, but without considering different patient characteristics or varying patient numbers per publication.[5,10-13] Therefore, the specific impact of these parameters on the LTT sensitivity remains elusive.

This meta-analysis aims to provide a comprehensive overview about the sensitivity of the LTT based on individual patient data extracted from 30 publications to ensure that different patient characteristics and varying patient numbers per publication are adequately considered. We also investigated the performance of different LTT read-out methods and the association between parameters of special relevance from a clinical point of view such as "clinical phenotype," "allergy-inducing drug," and "time to testing" with the performance of LTT.

Methods

The methods of this meta-analysis were previously described in an internal study protocol before the literature search was performed. The meta-analysis was not officially registered. The Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines were followed for reporting findings.

Literature Search

A literature search was performed in PubMed using the advanced search mode. The following search terms marked in bold and quotation marks were used and combined with the standard boolean operators:

- "**lymphocyte transformation test**" and "**drug or drugs**" and "**hypersensitivity or hypersensitivities**" not "**metal**" not "**Review** (publication type)" not "**Case reports**" (publication type) or;
- "**lymphocyte proliferation test**" and "**drug or drugs**" and "**hypersensitivity or hypersensitivities**" not "**metal**" not "**Review**" not "**Case reports**" or;
- "**lymphocyte stimulation test**" and "**drug or drugs**" and "**hypersensitivity or hypersensitivities**" not "**metal**" not "**Review**" not "**Case reports**" or;
- "**LTT**" and "**drug or drugs**" and "**hypersensitivity or hypersensitivities**" not "**metal**" not "**Review**" not "**Case reports.**"

Inclusion Criteria

The publications had to fulfill the following criteria to be included in the meta-analysis:

- A peer-reviewed publication in English;
- Published between January 1, 1995, and May 1, 2020;
- The publication comprised original experimental data with regard to drug detection in humans by means of the LTT;
- At least 5 patients were analyzed with the LTT;
- The publication allowed a retrospective analysis of the data by listing individual results from patients and patient characteristics defined in the next item;
- "Classical" LTT performance without improvements such as stimulation with cytokines or CD3-C28 antibodies, addition of antigen-presenting cells, or depletion of regulatory cells;
- Only patients with a reliable workup for the allergy detection (eTable 1);
- The following essential patient-specific characteristics had to be listed in the publication:
  - Clinical phenotype (eg, Stevens-Johnson syndrome, maculopapular exanthema [MPE]);
  - Allergy-inducing drug (eg, amoxicillin, carbamazepine);
  - Read-out parameter of the LTT (eg, radioactive [3H-thymidine incorporation], enzyme-linked immunosorbent assay [ELISA]);
  - Result of the LTT (positive or negative);
  - Reference standard applied for allergy detection (eg, medical history for the drug allergic reaction, patch test).

Data Extraction

The relevant data of the included publications were extracted and transferred into an Excel sheet. In addition, further patient characteristics were extracted from the publications defined as nonessential patient characteristics. These nonessential patient characteristics comprised age, sex, and the time to testing period after the allergic event.

Read-Out Parameters

In the publications, cytokine measurement by ELISA or ELISpot was mostly performed by determination of 3 different cytokines, such as interferon gamma, interleukin-5, and interleukin-10. However, some studies calculated sensitivities by analyzing each cytokine on its own (eTable 1). Alternatively, in other publications, the results of all 3 cytokines were pooled and only 1 of 3 had to be positive to account for a positive LTT result. For the meta-analysis, we chose the same method and pooled the cytokine results within a study. With this approach, we addressed the scenario that some allergic reactions may be associated with a skewed T cell response accompanied by a polarized cytokine profile representing for example a T_H1 or T_H2 response.

The multiplex approach used for cytokine detection by Lochmatter at al.[20] was included in the ELISA group because of the similarities between both methods. Basically, multiplex read-outs were included if these studies met our inclusion criteria.

With regard to the radioactivity read-out method, one of the tested drug concentrations had to be positive to account for a positive LTT.

The defined limits for a positive stimulation index or other stimulation calculations were used such as in the respective study.

Strategy for Data Analyses

At first, a descriptive analysis was performed to determine the number of studies and the number of individuals for which the characteristics of the patient (age, sex), the time to testing period, the allergy-inducing drug, the clinical phenotype, and a positive or a negative test result were reported.

Funnel plots were created for each LTT method. In addition, each LTT method was analyzed for potential publication bias by funnel plot asymmetry using the trim and fill method. In this method, potentially missing studies (eg, studies with small effects) are imputed to create symmetrical funnel plots.

First, each LTT read-out was analyzed separately. For each of the read-out methods, sensitivities were pooled by inverse-variance weighted random-effects meta-analyses. Between-study heterogeneity was assessed by the heterogeneity variance τ^2, Higgins and Thompson's I^2, Cochran's Q, and H^2 statistic. The following definitions of I^2 were used, I^2 = 25%: low heterogeneity; I^2 = 50%: moderate heterogeneity; and I^2 = 75%: substantial heterogeneity.[14,15]

Second, LTT read-outs were compared by a meta-regression model including all LTT read-outs. Results of meta-analyses are presented by forest plots.

Third, univariate and multiple logistic mixed effects regression analyses were conducted to analyze whether the covariables "allergy-inducing drug," "clinical phenotype," and "time to testing" may affect the positive test results of the LTT methods. To account for the intra-study dependencies, the study ID was included as a random effect in each model. However, time to testing period was not specified in every study. Thus, to avoid losing individual patient data, two data

Table 1
Patient Characteristics

Parameter	Characteristics of ELISA subgroup: number of studies (n = 5) and individual patients included (n = 79)	Characteristics of ELISpot subgroup: number of studies (n = 4) and individual patients included (n = 94)	Characteristics of flow cytometer subgroup: number of studies (n = 1) and individual patients included (n = 19)	Characteristics of radioactivity subgroup: number of studies (n = 25) and individual patients included (n = 529)
	Number of individuals (proportion in subgroup)	Number of individuals (proportion in subgroup)	Number of individuals (proportion in subgroup)	Number of individuals (proportion in subgroup)
Female	38 (48.1%)	57 (60.6%)	15 (78.9%)	190 (35.9%)
Male	27 (34.2%)	35 (37.2%)	4 (21.1%)	137 (25.9%)
Individual data not available[a]	14 (17.7%)	2 (2.1%)	—	202 (38.2%)
Mean age	48.5	56.7	52.7	46.5
Median age	51.0	58.0	52.0	45.0
Individual data not available[a]	14 (17.7%)	2 (2.1%)	—	202 (38.2%)
Outcome of test methods				
Positive	67 (84.8%)	48 (51.1%)	13 (68.4%)	333 (62.9%)
Negative	12 (15.2%)	46 (48.9%)	6 (31.6%)	196 (37.1%)
Time periods until testing after allergic reactions (=“time to testing”)				
<2 wk	2 (2.5%)	45 (47.9%)	2 (10.5%)	52 (9.8%)
2 wk-2 mo	15 (19.0%)	7 (7.4%)	10 (52.6%)	93 (17.6%)
2 mo-12 mo	16 (20.3%)	17 (18.1%)	4 (21.1%)	130 (24.6%)
12 mo-36 mo	4 (5.1%)	18 (19.1%)	—	40 (7.6%)
>36 mo	6 (7.6%)	7 (7.4%)	3 (15.8%)	76 (14.4%)
Period not stated	36 (45.6%)	—	—	138 (26.1%)
Clinical reactions grouped by clinical phenotype or pathophysiological considerations (= “clinical phenotype”)				
Delayed-type reactions				
Maculopapular reactions	28 (35.4%)	26 (27.7%)	7 (36.8%)	190 (35.9%)
Bullous reactions	22 (27.8%)	36 (38.3%)	3 (15.8%)	109 (20.6%)
DRESS/DIHS	2 (2.5%)	32 (34.0%)	—	82 (15.5%)
Immediate type reactions	10 (12.7%)	—	6 (31.6%)	104 (19.7%)
Organ-specific reactions and others	17 (21.5%)	—	3 (15.8%)	44 (8.3%)
Tested drugs grouped by chemical properties (= “allergy-inducing drug”)				
Beta-lactam antibiotics	22 (27.8%)	29 (30.9%)	8 (42.1%)	215 (40.6%)
Other antibiotics	17 (21.5%)	3 (3.2%)	6 (31.6%)	90 (17.0%)
Other drugs	40 (50.6%)	62 (66.0%)	5 (26.3%)	224 (42.3%)
Aromatic anticonvulsant and drugs acting on the central nervous system[b]	16 (20.3%)	19 (20.2%)	2 (10.5%)	139 (26.3%)
Cardiovascular drugs and others[b]	12 (15.2%)	34 (36.2%)	3 (15.8%)	67 (12.7%)
NSAR, peripheral analgesics, contrast media, and morphine derivates[b]	12 (15.2%)	9 (9.6%)	—	18 (3.4%)

Abbreviations: DRESS/DIHS, drug reaction with eosinophilia and systemic symptoms/drug-induced hypersensitivity syndrome; ELISA, enzyme-linked immunosorbent assay; NSAR, non-steroidal anti-inflammatory drugs.
[a]Some studies only reported the ratio of female to male or average age.
[b]These 3 groups were grouped into “other drugs.”

sets were generated. One data set includes all individuals and the other data set includes all individuals with information of the time of testing. In multiple regression analyses, the covariables “allergy-inducing drug,” “clinical phenotype,” and “time to testing” (only for the second data set) were included in one model. Consecutively, because of the small sample size in some of the categories, subgroups were built (for description of subgroups refer to Table 1). Results are presented as odds ratios (ORs) with 95% confidence interval (CI).

A sensitivity analysis was performed by calculation of the adjusted sensitivities based on the funnel plots after imputation of potentially missing studies with the trim and fill method.[16]

Fourth, mean specificities were calculated for the LTT read-outs ELISA and ELISpot. For the radioactivity method, a summary receiver operating characteristic (SROC) analysis was performed. SROC analyses are used to calculated diagnostic test accuracies based on bivariate models. The SROC curve presents the sensitivities and the false-positive rate (1-specificity) of each study and reveals the relationship between sensitivity and specificity at various thresholds of the radioactivity method.

A *P* value less than .05 was considered statistically significant. No corrections for multiple testing were applied because this meta-analysis was planned as an explorative study. Therefore, the number of tests and the corresponding correction factors were not defined at the beginning of the project.

Results

Search Results

We identified 93 potential publications in our database search and 22 potential publications in the review article from Mayorga et al.[5] This review article was used to identify further publications, which were not found by the literature search because of its very comprehensive and detailed list of various LTT studies in the context of drug allergy. Overall, 30 publications fulfilled the inclusion criteria (Fig 1).

It should be emphasized that some LTT publications could not be considered because one or more inclusion criteria were not specified on an individual patient level. However, we would like to point out that the inclusion criteria are not equatable to any fundamental quality assessment of these studies but ensuring a consistent data analysis regarding specified clinical parameters.

Quality Assessment of the Included Studies

A stringent quality assessment of the studies could not be implemented owing to their experimental character. In our opinion, there are no unique parameters that can be used for an objective quality assessment based on the provided information in the studies. Evident

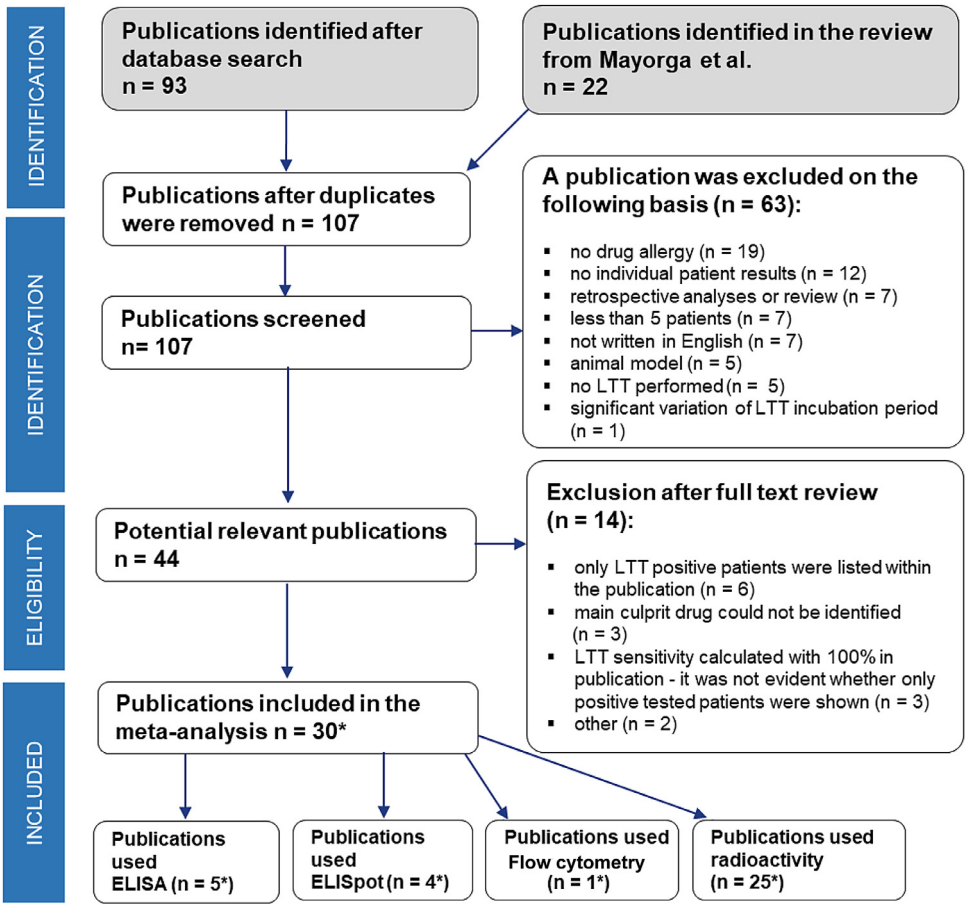


Figure 1. Flowchart of reference search. A total of 115 publications were identified by database search and the review by Mayorga et al[5] and analyzed whether the defined inclusion criteria had been met. Overall, 30 publications fulfilled the inclusion criteria. The asterisk represents some studies analyzed the same patients with 2 different read-out methods (n = 5). ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence activated cell sorting; LTT, lymphocyte transformation test.

parameters such as the number of included patients do not necessarily correlate with the quality of the LTT performance. In contrast, a reliable diagnosis of the causative drug with other allergy tests is extremely important if the LTT performance should be determined. However, different reference methods are most often used making a quality assessment of the causative drug determination challenging. Consequently, no quality assessment of the studies was done.

Patient Characteristics and Descriptive Analyses

In total, 721 patients formed the basis for the analysis. As expected, most of the studies used radioactivity as a read-out method for the LTT performance (25 studies, 529 patients) followed by ELISA (5 studies, 79 patients), ELISpot (4 studies, 94 patients), and flow cytometry (1 study, 19 patients) (Table 1).

A higher proportion of women compared with men was included in the studies (female: 41.6%, male: 28.2%, unknown sex: 30.2%). Most of the patients were analyzed relatively soon after the allergic event (< 12 months: 54.5% vs > 12 months: 21.4%). However, in 24.1% of the cases, the time to testing after the allergic event was not stated. Owing to the small sample size caused by the variety of different drugs and clinical reactions reported in the studies, these parameters had to be grouped into drug classes and according to the pathophysiological mechanism or clinical phenotype to increase the number of patients in each group (for read-out specific details: Table 1).

Overall, 38.0% of the involved drugs are beta-lactam antibiotics followed by "aromatic anticonvulsants and drugs acting on the central nervous system" (24.4%), other antibiotics (16.1%), and the more heterogenic groups of "cardiovascular drugs and others" (16.1%) and the cohort of "NSAR, peripheral analgesics, contrast media and morphine derivates" (5.4%) (eTable 2).

The "pathophysiological mechanism or clinical phenotype" (summarized as "clinical phenotype") comprises maculopapular reactions, which affected 34.8% of the patients, bullous reactions (23.6%), immediate reactions (16.6%), "drug-reaction with eosinophilia and systemic symptoms (DRESS)/drug-induced hypersensitivity syndrome (DIHS)" (16.1%), and "organ-specific and other reactions" (8.9%).

Statistical Meta-Analyses and Meta-Regression

Sensitivity of the Lymphocyte Transformation Test Depends on its Read-Out Method

First, we analyzed the sensitivity of the LTT in the context of the mainly used read-out methods comprising radioactivity, ELISA, ELISpot, and flow cytometry (Fig 2). This calculation was performed by meta-regression analysis for each method separately (Fig 3A) and included all methods in 1 model (Fig 3B). The results are presented as forest plot revealing all publications with the corresponding calculated sensitivity, which were ranked according to their contribution to the sensitivity proportion within the complete analyses (Fig 3A).

The highest sensitivity in the meta-regression analysis was calculated for the ELISA method with 80% (95% CI, 64-90). The classical and still frequently used radioactivity read-out had a lower sensitivity of 66% (95% CI, 58-72) compared with the sensitivity of the ELISA method (80% [95% CI, 64-90], *P* value = .08). The ELISpot technique had the

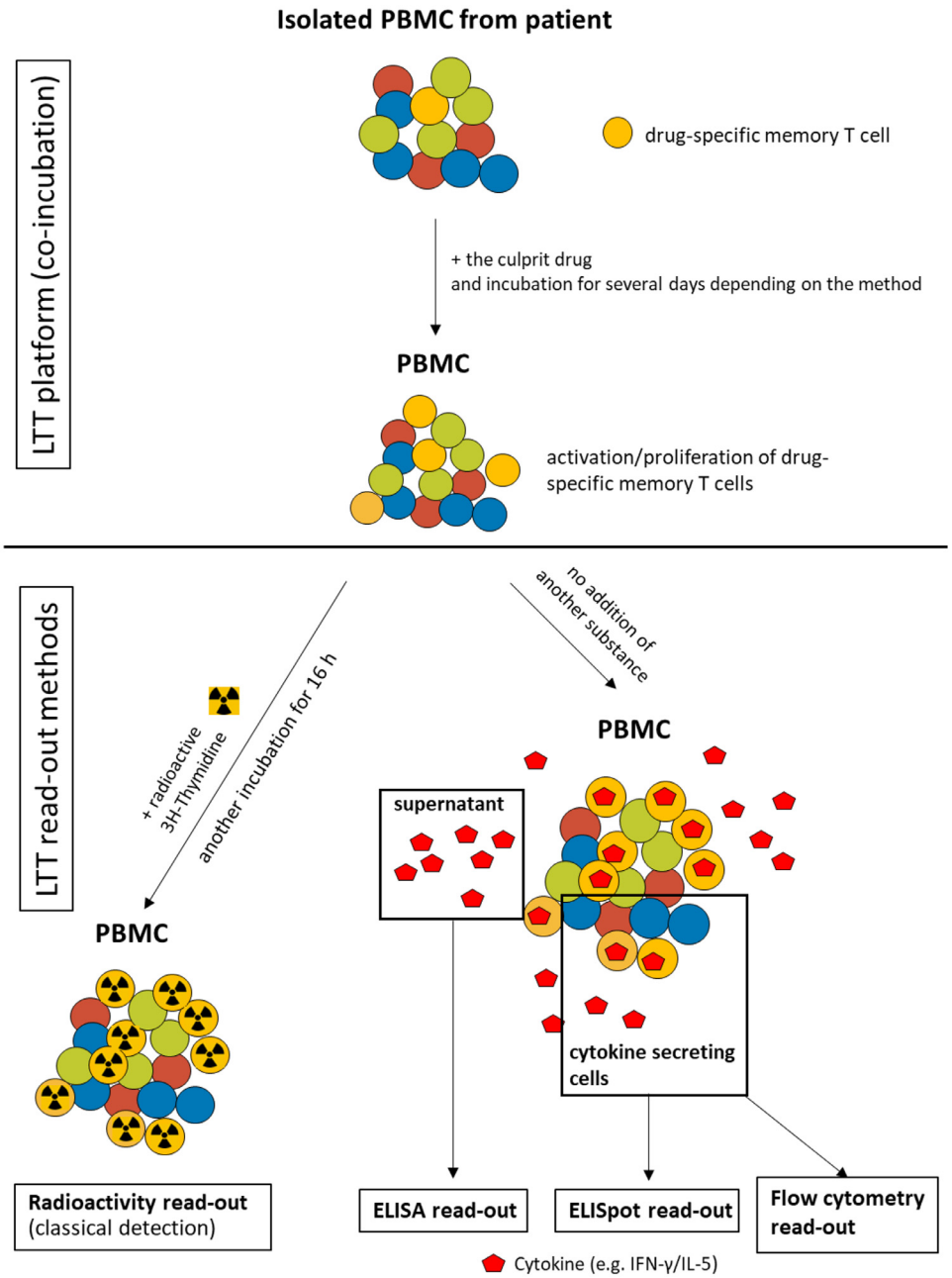


Figure 2. Basic principle of the LTT illustrating the differences of the read-out methods analyzed by this meta-analysis. A detailed description of the LTT performance can be found in review articles.[3] ELISA, enzyme-linked immunosorbent assay; LTT, lymphocyte transformation test; PBMC, peripheral blood mononuclear cells.

lowest sensitivity of 51% (95% CI, 33-69) compared with ELISA (difference, 29%; *P* value = .01). Of note, the third cytokine detection method by flow cytometry had a sensitivity of 76% (95% CI, 38-94) comparable with the sensitivity of the ELISA technique (Fig 3B).

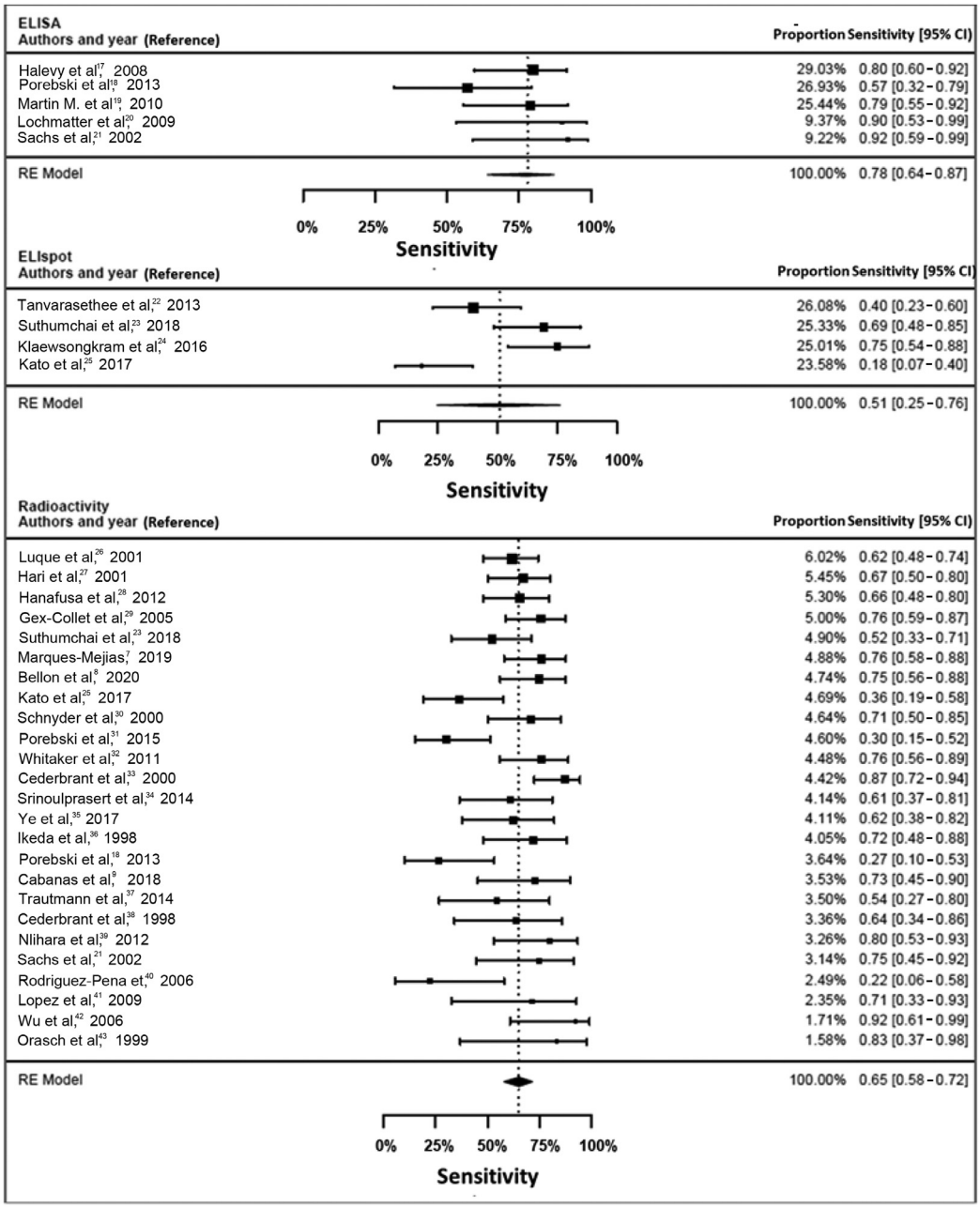
The sensitivity results of the meta-regression for each of the methods separately and the meta-regression analyses of all methods in one model differ only marginally (Fig 3A and B).

Specificity is another parameter characterizing the overall performance of the LTT. Unfortunately, in most of the studies, the results of the control persons for the specificity calculation were given as an overall outcome of all control persons but not on an individual level such as that for the included patients. In addition, some studies did not mention any specificity calculations making statistical analyses even more difficult. Nevertheless, for the radioactivity technique, we were able to perform a SROC curve revealing a specificity of 91% (95% CI, 86-95) associated with a sensitivity value of 62% (95% CI, 53-71) (included studies n = 18) (eFig 1A). For the ELISA and ELISpot read-out, we listed the available specificity values for each publication ranging from 62% to 100% for the ELISA method (included studies n = 5) and from 85.7% to 100% for the ELISpot method (included studies n = 2) (eFig 1B).

Impact of Clinical Phenotype, Allergy-Inducing Drug, and Time Point of Lymphocyte Transformation Test Analysis on the Lymphocyte Transformation Test Performance

We analyzed the impact of clinical relevant covariables such as "clinical phenotype," "allergy-inducing drug," and "time to testing" on the performance of the LTT (Figs. 4 and 5). A mixed effects logistic regression model including the 2 covariables clinical phenotype and allergy-inducing drug based on all extracted patients confirmed the aforementioned lower sensitivity for the ELISpot and radioactivity read-outs compared with the reference method ELISA (OR ELISpot:

A)



B)

LTT read out method	Sensitivity	95% confidence interval	*p*-value
ELISA	0.80	0.64-0.90	-
ELIspot	0.51	0.33-0.69	.013 *
Radioactivity	0.66	0.58-0.72	.084
Flow cytometry	0.76	0.38-0.94	.776

Figure 3. LTT sensitivities depending on different read-outs. (A) Forest plot of overall sensitivities. All publications are listed with the corresponding sensitivity, the 95% CI, and the weighting within analyses. (B) Meta-regression analysis. No additional covariates were included in this analysis. The asterisk represents *P* value < .05 = statistically significant. CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; LTT, lymphocyte transformation test.

0.12 [95% CI, 0.03-0.38]; *P* value < .001; OR radioactivity: 0.25 [95% CI, 0.09-0.63]; *P* value = .003) (Fig 4). For the covariable "clinical phenotype," a DRESS/DIHS was associated with a higher probability for a positive test result compared with bullous reactions (OR DRESS/DIHS: 2.52 [95% CI, 1.38-4.66]; *P* value = .003). In contrast, for maculopapular reactions and immediate reactions, the LTT had a reduced performance (OR maculopapular reactions: 0.84 [95% CI, 0.46-1.49]; *P* value = .55; OR immediate type reactions: 0.63 [95% CI, 0.27-1.41]; *P* value = .28). Similarly, beta-lactam antibiotics seem to be the best performing group within the covariable "allergy-inducing drug" (OR beta-lactam antibiotics: 1.59 [95% CI, 0.81-3.12]; *P* value = .17) (Fig 4).

The parameter "time to testing" was not specified in all studies; therefore, the statistical calculation was done with a reduced number of patients as indicated in the corresponding Figure 5. The time period "2 weeks to 2 months" after the allergic event was associated

LTT Methods 35 studies; 721 individuals		
Model: covariables "read-out method" + "clinical phenotype" + "allergy-inducing drug"		
Names of covariables	OR (±; 95.0% cl)	p-value
ELISA	Ref	Ref
ELISpot	0.12 (0.03-0.38)	< 0.001*
Flow cytometry	0.39 (0.06-2.69)	0.322
Radioactivity	0.25 (0.09-0.63)	0.003*
Bullous reactions	Ref	Ref
DRESS/DIHS	2.52 (1.38-4.66)	0.003*
Maculopapular reactions	0.84 (0.46-1.49)	0.550
Organspecific reactions	1.18 (0.46-2.98)	0.719
Immediate type reactions	0.63 (0.27-1.41)	0.276
Other antibiotics	Ref	Ref
Beta-lactam antibiotics	1.59 (0.81-3.12)	0.173
Other drugs	1.15 (0.64-2.09)	0.635

Figure 4. Impact of the read-out parameter, clinical phenotype, and allergy-inducing drug concluded in one model, This mixed effects logistic regression model analyzed the different covariables based on data of 721 individuals. Reference covariables were defined. The asterisk represents *P* value < .05 = statistically significant. OR, odds ratio; CI, confidence interval. DRESS/DIHS, drug reaction with eosinophilia and systemic symptoms/drug-induced hypersensitivity syndrome; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence activated cell sorting; LTT, lymphocyte transformation test.

LTT Methods 25 studies; 547 individuals		
Model: covariable "time to testing"		
Names of covariable	OR (±; 95.0% CI)	*P*-value
<2 wk	Ref	Ref
2 wk - 2 mo	2.12 (1.06-4.30)	.03*
2 - 12 mo	1.75 (0.90-3.46)	.10
12 - 36 mo	1.08 (0.49-2.41)	.85
> 36 mo	0.86 (0.41-1.81)	.69

*P-value < 0.05

Figure 5. Impact of the covariable "time to testing." Data of 547 patients with information about the time to testing were analyzed. The OR and the corresponding 95% CI are presented for each covariable. The asterisk represents *P* value < .05 = statistically significant. CI, confidence interval; LTT, lymphocyte transformation test; OR, odds ratio.

with more positive results compared with the reference period (< 2 weeks) (OR: 2.12 [95% CI, 1.06-4.30]; *P* value = .03). This rate of positive results continuously decreased with later time to testing periods if compared with the reference period (OR 2 - 12 months: 1.75 [95% CI, 0.90-3.46]; *P* value = .10; OR 12 - 36 months: 1.08 [95% CI, 0.49-2.41]; *P* value = .85; OR > 36 months: 0.86 [95% CI, 0.41-1.81]; *P* value = .69).

Assessment of Publication Bias

We assessed the publication bias by various indicators, funnel plots, and imputation of studies with the trim and fill method correcting for potentially missing studies to get funnel plot symmetry for the ELISpot, ELISA, and radioactivity methods. According to the heterogeneity indicators τ^2, I^2, and H^2, publications using the ELISpot method revealed the greatest intrastudy heterogeneity regarding the LTT sensitivity (τ^2 = 1.137; I^2 = 83.95%; H^2 = 6.23), whereas publications analyzing the LTT by ELISA revealed less heterogeneity (τ^2 = 0.141; I^2 = 24.36%; H^2 = 1.32). The publications using radioactivity as read-out had a moderate intrastudy heterogeneity (τ^2 = 0.306; I^2 = 55.95%; H^2 = 2.27) (eFig 2A). The publication bias assessed by funnel plots are shown in (eFig 2B). Using the imputation of studies with the trim and fill method correcting for potentially missing studies yielded the following sensitivity values: ELISA: 73% (95% CI, 59-84) (prior: 78% [95% CI, 56-90]); ELISpot: no studies were added; radioactivity: 61% (95% CI, 53-68) (prior: 65% [95% CI, 38-85]) (eFig 2C).

Discussion

There are many publications and reviews dealing with the sensitivity of the classical, radioactive LTT and the modified LTT, which measure cytokines by means of ELISA, ELISpot, or flow cytometry.[5,6,10-12] However, most of these studies calculate the average LTT performance by combining overall mean sensitivities of many LTT publications and do not consider patient numbers or individual clinical parameters of the included patients, which might have a relevant impact on the LTT performance.

Read-Out Methods

We found indications for relevant differences of the LTT performance depending on the read-out parameters. Our results suggested that the ELISA is the most sensitive read-out technique compared with the classical radioactivity and ELISpot method. In line with this, a previously published review article also found ELISA to be the most sensitive method especially when different cytokines were determined in parallel.[5] The measurement of various cytokines may enhance the probability of a positive result compared with the

detection of only 1 parameter such as proliferation. However, it was unexpected that the ELISpot read-out reveals a clearly lower sensitivity than the ELISA although both methods detect cytokine secretion. This difference may reflect that in 3 of 4 studies using the ELISpot, read-out was based on only 1 cytokine, compared with 3 cytokines measured by 4 of 5 studies using the ELISA. More read-out parameters possibly increase the probability of a positive LTT result. Another reason for the low ELISpot sensitivity could be that nearly 50% of all patients analyzed with ELISpot were tested within the early phase after the allergic event (< 2 weeks), which was associated with less positive results compared with other time to testing periods. In contrast, for the ELISA and radioactivity read-out, only 2.5% and 9.8% of the respective patients were tested in this early phase. Nevertheless, a read-out–specific influence regarding the time to testing period cannot be excluded as all read-out techniques were pooled in our analysis.

Patients with drug-allergic reactions are often treated with corticosteroids, which may weaken the immune response in these patients leading to false-negative results. In fact, in 2 ELISpot publications, up to 50% of the patients were treated with systemic corticosteroids. These aspects may explain the low sensitivity of the ELISpot method in our analysis.

We also want to indicate that the calculation of the cutoff values for the ELISA, ELISpot and flow cytometry read-out, which define a positive or negative LTT, varies between the included studies. For the radioactivity method, a SI value of more than or equal to 2 or more than or equal to 3 (depending on the drug) is defined as a positive LTT result. In contrast, no consistent calculation of the cutoff values and no common cutoff value itself is determined for the ELISA, ELISpot and flow cytometry read-out so far. Unfortunately, we were not able to perform any analysis with our data to address this issue because of the raw data not being published in the studies (eg, results of the respective unstimulated and drug-stimulated sample).

Clinical Phenotype

Most of the included allergic reactions in our analysis belong to the type IV (lymphocyte-mediated, delayed type [74.5%]) or type I (immunoglobulin E [IgE]-mediated, immediate type reactions [16.6%]). Based on the detection principle of proliferating or cytokine-secreting immune cells, the LTT should be especially reliable for the detection of lymphocyte-mediated (=type IV) reactions.[3,44] In line with this, we could reveal that DRESS/DIHS, a delayed-type allergy, have the highest probability of a positive LTT result. However, for maculopapular reactions, one of the most common type IV allergic reaction, we observed a lower probability of a positive test result, which was comparable with that of immediate reactions. Overall, immediate reactions tend to be less reliable detectable by the LTT compared with all analyzed delayed-type reactions. However, the LTT is basically working in IgE-mediated allergic reactions as well.[26,45]

Interestingly, a well-performing LTT for DRESS/DIHS compared with bullous reactions (such as Stevens-Johnson syndrome/toxic epidermal necrolysis) and maculopapular exanthema was also found in previously published data from Porebski et al.[11,12]

Allergy-Inducing Drug

LTT results for more than 100 different drugs were extracted for this meta-analysis and were grouped into 3 different drug classes. We did not observe any association between a drug class and the LTT performance, which might be caused by the great chemical heterogeneity of the drug classes. In addition, some drug classes often induce nonallergic hypersensitivity reactions, which can be mixed up with allergies because of their similar clinical appearance. Because nonallergic (pseudoallergic or intolerance) reactions cannot be detected by the LTT, the inclusion of these drugs could reduce its sensitivity. For example, nonsteroidal anti-inflammatory drugs or contrast media often induce non–immune-mediated instead of immune-mediated drug hypersensitivity.[46,47] In addition, the recently discovered MRGPRX2 receptor may trigger direct, IgE-independent mast cell degranulation for some drug classes, such as neuromuscular blocking agents, fluoroquinolones, morphine, contrast media, or vancomycin.[6,48-50] Thus, for MRGPRX2-mediated reactions, the same impact on the LTT sensitivity may apply.

Nevertheless, we could find that beta-lactam antibiotics—representing the most homogenous investigated drug class—have the highest probability of a positive LTT result.

Time to Testing

The time to testing period after the allergic event is crucial and can have a substantial impact on the LTT performance.[4] In the very early phase after the allergic event, the immune cells are still activated so that a high background of proliferating cells or cytokine secretion even in the unstimulated control sample of the patient might be the consequence.[27] This high background reduces the maximum stimulation index, which is most often used for LTT assessment, causing less positive LTT results. In contrast hand, it is known that memory cells in the peripheral blood have a rapid turnover.[51] However, these memory cells are the key cells indirectly detected by the LTT.[4]

In our meta-analyses, we could confirm a relevant impact of the time to testing period on the LTT performance in accordance with the described biological principles. We found the periods of “2 weeks to 2 months” and “2 months to 12 months” to be associated with a higher probability of a positive LTT result compared with the very early phase of “< 2 weeks.” Interestingly, the LTT performance actually decreases with later time points especially after 36 months.

Owing to small size, we did not analyze whether the time to testing period depends on parameters such as the clinical phenotype, which may have an impact according to Kano et al.[52] They described a dependency of the clinical reaction on the time to testing period.

Strengths and Limitations

We believe that this systematic meta-analysis is one of the most detailed analyses concerning the use of the LTT for the detection of a drug sensitization in drug allergies. In contrast to many other LTT reviews, we extracted individual patient data and focused on relevant clinical parameters from 30 publications including more than 700 patients.

However, the study has some limitations, which should be taken into account for data interpretation. By nature, there is a huge heterogeneity of the data caused by differences between the included publications in terms of LTT performance itself (eg, number of seeded cells), definition of a positive result, no common reference standard regarding allergy detection, and included patient numbers. Moreover, only studies providing specific patient characteristics on an individual level were included in this meta-analysis resulting in an exclusion of several LTT studies. This bears the risk that our analysis might not correlate with all available LTT data. We addressed this issue by choosing a wide period of time (25 years) for our literature search to include as many publications as possible. Though, for the ELISA, ELISpot, and flow cytometry method, the included publications and patients remained relatively small.

Finally, because of missing data for the control persons, we were not able to calculate corresponding specificity values in the same detail such as for the sensitivity analyses, which would be helpful for

Table 2
Rough Classification of All Analyzed Parameters

Parameter	Best performing LTT	Intermediate performing LTT	Worst performing LTT
Read-out method	ELISA; flow cytometry	Radioactivity	ELISpot
Clinical phenotype	DRESS/DIHS	Organ-specific reactions; bullous reactions	Maculopapular reactions; immediate type reactions
Allergy-inducing drug		Beta-lactam antibiotics; other antibiotics; other drugs	
Time to testing period	2 wk-2 mo	<2 wk; 2 mo-12 mo; 12 mo-36 mo	>36 mo

Abbreviations: DRESS/DIHS, drug reaction with eosinophilia and systemic symptoms/drug-induced hypersensitivity syndrome; ELISA, enzyme-linked immunosorbent assay; LTT, lymphocyte transformation test.
NOTE. This classification is based on the calculated odds ratio values regarding the association between the analyzed parameters and the positive test result of the LTT methods and does not include any statistical significances, confidence intervals, or other study effects of the results. For LTT interpretation, the detailed results presented in Figures 3 to 5 have to be considered.

assessing the overall performance of the LTT. However, at least for the radioactivity method, we provided a SROC, which indicates an appropriate specificity of 91%.

Nevertheless, owing to our diligent study design of extracting individual patient characteristics from 30 LTT studies, we believe that our study will be helpful to substantially extent the current knowledge of LTT performance with simultaneous consideration of important clinical parameters.

In conclusion, our analyses reveal a relevant impact of parameters, such as the LTT read-out method, the clinical phenotype of the allergic reaction, the allergy-inducing drug, and the time to testing period. From a clinical point of view, it can be concluded from our data that the LTT provides the best performance when the ELISA read-out is used. Moreover, a DRESS/DIHS, beta-lactam antibiotics, and a testing period of 2 weeks to 2 months after the allergic event are especially suitable for a well-performing LTT. An overview and rough classification of all analyzed parameters on the LTT performance is summarized in Table 2. These findings are based on a meta-analysis providing a higher level of evidence than a single study or previous reviews not considering individual patient data Table 2.

Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.anai.2022.06.014

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Supplementary Data

eTable 1
Type of Allergy Detection and Tested Cytokines

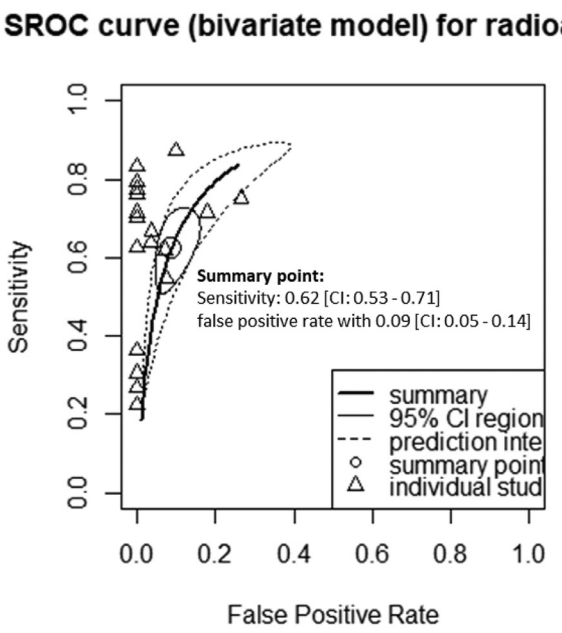
Method	Publication (year)	Reference diagnostic approach for allergy detection	Cytokines(if tested)
ELISA			
	Halevy and Grossman,[17] (2008)	provocation test **or** withdrawal test	IFN-γ
	Porebski et al,[18] (2013)	ALDEN Score ≥ 6	IFN-γ, IL-5, IL-2
	Martin et al,[19] (2010)	classification according to Nyfelder (1997), only category A	IFN-γ, IL-5, IL10
	Lochmatter et al,[20] (2009)	classical LTT	IFN-γ, IL-5, IL-2
	Sachs et al,[21] (2002)	classification according to Nyfelder (1997), only categorie A	IFN-γ, IL-5, IL10
ELISpot			
	Tanvarasethee et al,[22] (2013)	Naranjo adverse drug reaction probability scale, only "probable" and "definite"	IFN-γ, IL5, IL-10
	Suthumchai et al,[23] (2018)	Naranjo adverse drug reaction probability scale, only "probable" and "definite"	IFN-γ
	Klaewsongkram et al,[24] (2016)	ALDEN Score	IFN-γ
	Kato et al,[25] (2017)	medical history	IFN-γ
Radioactivity			
	Luque et al,[26] (2001)	medical history and skin test **or** RAST **or** provocation test (at least one test was positiv)	
	Hari et al,[27] (2001)	medical history	
	Hanafusa et al,[28] (2012)	medical history	
	Gex-Collet et al,[29] (2005)	not specified	
	Suthumchai et al,[23] (2018)	Naranjo adverse drug reaction probability scale, only probable and definite	
	Marques-Mejias et al,[7] 2019	medical history	
	Bellon et al,[8] 2020	ALDEN Score ≥ 4	
	Kato et al,[25] (2017)	medical history	
	Schnyder and Pichler,[30] (2000)	medical history	
	Porebski et al,[31] (2015)	medical history	
	Whitaker et al,[32] (2011)	medical history	
	Cederbrant et al,[33] (2000)	medical history	
	Srinoulprasert and Pichler,[34] (2014)	medical history	
	Ye et al,[35] (2017)	provocation test	
	Ikeda et al,[36] (1998)	medical history	
	Porebski et al,[18] (2013)	ALDEN Score ≥ 6	
	Cabanas et al,[9] 2018	ASPS: algorithm of the spanish pharmacovigilance system, only "probable" and "definite"	
	Trautmann et al,[37] (2014)	medical history and patch test **or** intracutaneous test **or** provocation test (at least one test was positiv)	
	Cederbrant et al,[38] (1998)	medical history	
	Niihara et al,[39] (2012)	medical history and patch test **or** provocation test **or** classical LTT (at least one test was positive)	
	Sachs et al,[21] (2002)	classification according to Nyfelder (1997), only categorie A	
	Rodriguez-Pena et al,[40] (2006)	intracutaneous test (delayed reaction)	
	Lopez et al,[41] (2009)	medical history and skin test **or** provocation test (at least one test was positiv)	
	Wu et al,[42] (2006)	not specified	
	Orasch et al,[43] (1999)	medical history	

Abbreviations: ELISA, enzyme-linked immunoassay; ELISpot, enzyme-linked immunosorbent spot.
NOTE. The allergy detection method used as reference in the corresponding publication and the tested cytokines (applicable for ELISA and ELISpot studies) are listed for each publication included in this meta-analysis.

eTable 2
Drugs Included in Each Group

Beta-Lactam antibiotics: Amoxicilline, Ampicilline, Augmentine, Bacampicillin, Benzylpenicillin, Cefazoline, Ceftazidime, Cefepime, Cefotaxime, Ceftriaxone, Cefuroxim, Clavulanic acid, Imipenem, Meropenem, Penicillin, Penicillin G, Phenoxymethylpenicillin, Phenoxymethylpenicillin-PLL, Piperacillin/Tazobactam
Other antibiotics/anti-infectives: Azitromycin, Ciprofloxacin, Clarithromycine, Clindamycine, Cotrimoxazole, Doxycyline, Erythromycin, Ethambutolhydrochloride, Isoniazide, Levofloxacin, Minocycline, Moxifloxacin, Nifurtimox, Ofloxacin, Oseltamivir, Proguanil, Pyrazinamide, Rifampicin, Sulfadoxine, Sulfamethoxazole, Sulfapyridine, Sulfasalazine, Trimethoprim, Vancomycin
Aromatic anticonvulsants and drugs acting on the central nervous system: Carbamazepine, Chlorazepate, Citalopram, Fluvoxamine, Lamotrigine, Oxacarbazepine, Phenytoin, Phenobarbital, Sodium valproate, Thioridazin, Triazolam, Zonisamide
Cardiovascular drugs and others: Acetazolamide, Allopurinol, Amlodipine, Articaine, Atenolol, Benzalkoniumchloride, Bismuthate, Bupivacaine, Budesonide, Cinchocaine, Dexketoprofen, Dorzolamid, Enoxaparin, Fenoterol, Ferroglycine, Furosemid, Hydrocortisone, Indapamide, Lansoprazol, Lidocaine, Lignocaine HCL, Losartan, Loratadine, Mepivacaine, Metolazon, Nadroparine, NA-Perchlorate, Nifedipine, Nilvadipine, Omeprazole, Oxpurinol, Prednisolone, Procaine, Propafenone, Propranolol, Propylthiouracil, Ranitidin, Simvastatine, Thiamin, Ticlopidin, Torasemid, Vemurafenib, Verapamil, Vitamin B complex, Vitamin B1, Vitamin B6, Vitamin B12
NSAR, peripheral analgesics, contrast media, and morphine derivates: Acetylsalicylic acid, Celecoxib, Codeine, Diclofenac sodium, Dipyrone, Fentanyl, Ibuprofen, Iomeron, Iodixanol, Metronidazole, Mefenamic acid, Metamizole, Naproxen, Paracetamol, Tramadol

A)



B)

ELISA	Author	Year	Number of controls	Specificity in publication [%]
	Halevy	2008	11	62
	Lochmatter	2009	15	93.3
	Martin	2010	10	100
	Porebski	2013	18	95
	Sachs	2002	5	60

ELISPot	Author	year	Number of controls	Specificity in publication [%]
	Kato	2017	18	100
	Klaewsongkram	2016	21	85.7
	Suthumchai	2018	20	not stated
	Tanvarasethee	2013	20	not stated

eFigure 1. LTT specificity depending on different read-out parameters. (A) Specificity calculation by summary receiver operating characteristic curve for the radioactivity read-out method. The triangles represent the sensitivity and false positive rate of the included publications providing data of control persons (n = 18). Overall, the summarized sensitivity was calculated with 0.62 (95% CI, 0.53-0.71) and the false positive rate with 0.09 (95% CI, 0.05-0.14) indicated by the summary point. The false positive rate corresponds to a specificity value of 91% ([1-0.09]×100). (B) For the ELISA and ELISpot method the provided data of controls were not sufficient to perform statistical calculations. Therefore, the available data are listed in a table to enable interpretation of the sensitivity results. CI, confidence interval; ELISA, enzyme-linked immunoassay; ELISpot, enzyme-linked immunosorbent spot; LTT, lymphocyte transformation test.

A) Publication bias assessment by different indicators:

- τ^2 quantifies the variance of the true effect sizes
- $H^2 \leq 1$ no study heterogeneity; $H^2 > 1$ between study heterogeneity is present
- I^2 described the percentage of variation due to between-study heterogeneity
- Q is used to test if there is excess variation in our data, meaning more variation that can be expected from sampling error alone

B) Publication bias assed by funnel plots:

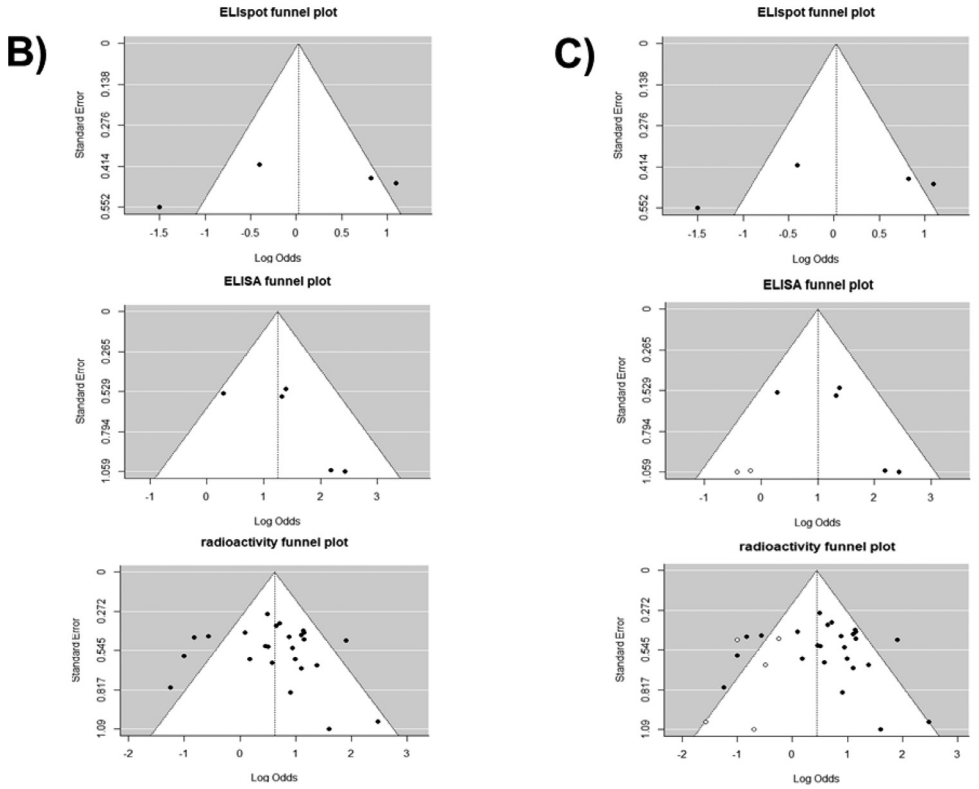
- ELISpot: In funnel plot two studies could be outliers but there seems to be no asymmetry.
- ELISA: The funnel plot shows asymmetry for the original 5 studies
- Radioactivity: The funnel plot shows outliers and potential asymmetry

C) Imputation of studies with the trim and fill method correcting for small study effects:

- ELISpot: No studies were added -> effect size was not affected
- ELISA: Two studies were added (white circles) -> effect size was getting smaller after imputation. Thus, the calculated effect size for ELISA might be overestimated. Effect size: 0.73 [0.59-0.84] compared to 0.78 [0.56-0.90] before imputation
- Radioactivity: Five studies were added (white circles) -> effect size was getting smaller after imputation. Thus, the calculated effect size might be overestimated. Effect size: 0.61 [0.53-0.68] compared to 0.65 [0.38-0.85] before imputation.

A)

	ELISA (5 studies)	ELISpot (4 studies)	Radioactivity (25 studies)
τ^2	0.141	1.137	0.306
I^2	24.36 %	83.95%	55.95%
H^2	1.32	6.23	2.27
Q p-value	5.294 0.259	16.975 0.0007	53.26 0.0005
Sensitivity + predicition interval	0.78 [0.56-0.90]	0.51 [0.08-0.92]	0.65 [0.38-0.85]



eFigure 2. Assessment of publication bias by indicators (A), funnel plots (B), and imputation of studies with the trim and fill method correcting for small study effects (C) for different LTT read-out parameters (ELISpot, ELISA, radioactivity).