Performance targets for quality assessment of total and allergen-specific IgE and total tryptase in allergy and anaphylaxis: multicentric study and recommendations

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To the Editor,

Precision medicine is increasingly used as an approach to the management of allergy and anaphylaxis, thanks to progress in diagnostic tests and biomarkers now allowing thorough characterization of a patient's endotype[1]. Probability-based risk assessment and diagnostic algorithms have entered the allergists' toolbox[2-4]. Allergy tests must therefore offer reliable, robust, and proficient results in each patient. Focusing on*in vitro* diagnostics, these requirements have led to the development of quality assurance (QA) programs for allergy laboratory assays and their implementation in virtually all clinical laboratories performing allergy assays. However, full performance targets for allergy assays have not yet been established, leaving allergists and clinical scientists without a common body of recommendations for the three routine assays, namely total serum IgE (tIgE), allergen-specific serum IgE (sIgE), and serum total tryptase. As an example, not only do recommendations on the acceptable bias and uncertainty of measurement (UM) of allergy assays miss from available literature, but there is also a complete lack of published recommendations on tryptase QA criteria. The multicentric French network of public clinical laboratories had previously documented a single-analyte QA strategy and recommendation for sIgE[5]. Hence, we set out to define QA criteria for intra- and interassay variation, analytical accuracy, and UM for sIgE, tryptase, and tIgE. QA data from 24 French centers were collected, analyzed, and compared to available literature, prior to issuing recommendations for QA management programs in allergy testing.

Data were collected from 2016-2018 intralaboratory (internal) QA controls (IQA) and interlaboratory proficiency testing programs (external quality assurance, EQA) completed by the participant centers[6]. A literature search for English and French recommendations for allergy assays was performed, including scientific publications, statements of scientific societies, QA management schemes from independent QA organisms, and manufacturer documents. According to the regulated (tIgE) or nonregulated (sIgE, tryptase) analyte status[7], the current work applies to any tIgE system, but for sIgE and total tryptase it is limited to the ImmunoCAP assay system, which is in use in all participant centers, is currently perceived as the reference *in vitro* diagnostic method for allergy[2], and offers the only EU-cleared tryptase determination method. Briefly, IQA programs were performed with control samples provided by the manufacturer and with internal serum pools, particularly for tryptase determination. EQA programs were from UK NEQAS (UK National External Quality Assessment Services), Thermo Fisher Scientific (Uppsala, Sweden), ProBioQual (Lyon, France), and CTCB (Toulouse, France). All participant laboratories had subscribed to at least one EQA for each assay. Data analysis was performed stepwise: (1) definition of three concentration levels (low, medium, and high) within the dynamic range of each analyte and assignment of measurement results from each center to the corresponding level; (2) computation and analysis of intra- and interassay coefficient of variability (CV), bias from analytical accuracy, and UM for each analyte, concentration level, and participant; (3) comparison of assay performance of participant centers with extant recommendations, outlier identification and establishment of recommendations. Performance evaluation criteria were defined as follows: CV = 100xSD/mean (SD, standard deviation), bias = 100x[(participant result) – (peer group target result)]/(peer group target result), UM = [?] [u^2(IQA) + u^2 (IQA)], with u2(IQA) denoting the variance (square SD) of all IQA results of the same concentration level, and u2(EQA) denoting the variance of corresponding EQA results[8].

Comparison of participant centers' results and available recommendations (**Table 1**) revealed that actual tIgE assays outperformed most intra- and interassay CV recommendations, but were in line with bias recommendations. Actual sIgE assay performance for intra-and interassay CV matched the available non-manufacturer recommendations from CLSI (Clinical and Laboratory Standards Institute)[9], but inconsistently attained UK NEQAS standards (**Table 1**). Intra-and interassay CV for total tryptase determination could only be compared to manufacturer recommendations, which appeared too stringent for inter-assay CV. Similarly, actual accuracy bias for tryptase determination was less performant than the available UK NEQAS standards, designed for low concentration levels (**Table 1**). For the three analytes and each concentration level, UM was calculated but due to a complete lack of available recommendations it could not be evaluated outside the peer group. Moreover, due to the lack of adequate EQA for each tryptase level, the UM for low (< 8 µg/L) and medium (8-20 µg/L) could only be computed for a combined low and medium concentration level up to 20 µg/L (**Table 1**).

Analysis of data from participant centers and comparison with international standards (when available)

allowed the establishment of recommended targets for performance evaluation, defined as the 95th percentile of the participants' results (**Table 2**). It is noteworthy that UM, a performance criterion that should be considered whenever clinical interpretation and decision rely on quantitative results, needs improvement, both in terms of availability of adequate EQA samples spanning the whole range of analyte concentrations, and of results from participating centers. The first step to take is wider availability of IQA and EQA samples of paired concentration levels. As UM computation is based on the absolute value of variance, UM of low concentrations of an analyte is unfavorably impacted by the use of medium or high EQA sample results. In order to achieve the goal of using adequate pairs of EQA samples for each analyte level, in the absence of commercially available EQA programs, interlaboratory exchanges are a simple, cost-effective solution.

In conclusion, we report here the first experience-based performance results for the most usual *in vitro* allergy and anaphylaxis assays, their comparison with available recommendations, and the establishment of the first recommendations for total tryptase assays and for the uncertainty of measurement of the three considered analytes: total serum IgE, allergen-specific serum IgE, and total serum tryptase. Conceived as a working tool for allergists and clinical scientists, our report aims at incentivizing further improvement and better use of*in vitro* allergy assays for precision medicine.

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Conflict of interest

JV reports personal fees from Thermo Fisher Scientific, personal fees from Meda Pharma (Mylan), personal fees from Beckman Coulter, personal fees from Sanofi, outside the submitted work. The other authors have nothing to disclose.

Author contribution: AS and JV collected and analyzed data from all laboratories. All authors analyzed the results, performed the literature search, participated to the consensus recommendation, and drafted the manuscript. AS, RC, LG, and JV wrote the final manuscript.

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Table 1. Results from the multicentric AllergoBioNet network and 2009-2019 recommendations.

		Total IgE (kIU/L)	**Total IgE (kIU/L)**	**Total IgE (kIU/L)**	**Allergen-specific IgE (kUA/L)**	**Allergen-specific IgE (kUA/L)**	**Allergen-specific IgE (kUA/L)**	**Τοταλ τρψ-πτασε (μγ/Λ)**	**Τοταλ τρψ-πτασε (μγ/Λ)**
		Low (<100)	**Medium (100-400)**	**High (>400)**	**Low (<5)**	**Medium (5-10)**	**High (>10)**	**Low (<8)**	**Medium (8-20)**
Intra-assay variation (Repeatability)	**Results (median; range; sample size)**	**2.4** (0.9;4.0); 22	**2.1** (1.3;5.7); 23	**2.9** (0.9;7.5); 20	**3.1** (0.2;12.2); 24	**3.6** (1.1;8.6); 17	**3.5** (1.6;10.4); 19	**2.1** (0.4;3.3); 11	**1.9** (0.8;4.4); 15
	2009-2019 targets	3*;9§;10**;15	3*;6§;10**;15	3*;6§;10**;15	4*;10**	5*;10**	5*;10**	3*	3*
Interassay variation (Reproducibility)	**Results (median; range; sample size)**	**6.7** (3.0;14.9); 27	**6.9** (2.1;9.0); 17	**6.8** (3.0;9.2); 19	**6.8** (3.0;10.6); 26	**7.5** (4.2;11.5); 23	**7.7** (3.9;13.1); 21	**5.7** (2.8;13.0); 9	**6.2** (3.0;12.9); 16
	2009-2019 targets	5*;12§;20**	4*;8§;15**	7*;8§;15**	4*;20**	5*;15**	9*;15**	5*	6*
Bias (Accuracy)	**Results (median; range; sample size)**	**-1.0** (-18.0;14.8); 277	**0.5** (-15.3;14.1); 154	**-0.4** (-10.1;6.2); 21	**-0.4** (-28.2;26.3); 470	**-0.9** (-23.7;24.2); 273	**-0.4** (-22.3;25.5); 282	**-1.3** (-24.2;12.8); 28	**-2.4** (-16.9;17.7); 74
	2009-2019 targets	20¤;15§;20**	10¤;11§;15**	20¤;15§;20**	15#	15#	15#	8#	8#
Uncertainty of measurement (Precision)	**Results (median; range; sample size)**	**20.3** (13.0;50.0); 41	**15.8** (6.7;45.8); 35	**17.0** (6.7;22.9); 23	**21.0** (7.0;43.0); 52	**23.8** (6.8;42.4); 40	**23.9** (13.1;36.0); 37	**17.6** (13.0;24.6); 14	**17.6** (13.0;24.6); 14

		Total IgE (kIU/L)	Total IgE (kIU/L)	Total IgE (kIU/L)	Allergen-specific IgE (kUA/L)	Allergen-specific IgE (kUA/L)	Allergen-specific IgE (kUA/L)	Τοταλ τρψ-πτασε (μγ/Λ)	Τοταλ τρψ-πτασε (μγ/Λ)
	2009-2019 targets	none	none	none	none	none	none	none	none

Intra-assay variation, an estimate of repeatability, was calculated in each participant center as the coefficient of variation of 20 to 30 measurements of the same analyte, performed consecutively during the same day: CV = 100xSD/mean. Interassay variation, a measure of reproducibility, was calculated in each participant center as the coefficient of variation of 20 to 30 measurements of the same analyte, performed consecutively over 20 to 30 days. The measurement bias, an estimate of accuracy, was calculated as 100x[(participant result) – (peer group target result)]/(peer group target result). Finally, the uncertainty of measurement, an estimate of precision, was calculated as [?] [u^2(IQA) + u^2 (IQA)], with u2(IQA) denoting the variance (square SD) of all IQA results of the same concentration level, and u2(EQA) denoting the variance of corresponding EQA results. Outliers were not excluded from the presented data. The intra-assay and interassay sample size (n) denotes the number of studies (20-30 measurements each) performed by the participants, while the bias and UM sample size refers to the number of individual results obtained by the participants. CV, coefficient of variation; EQA, external quality assurance; IQA, internal quality assurance; SD, standard deviation; UM, uncertainty of measurement. Special symbols denote the origin of 200-2019 recommendations: * manufacturer (Thermo Fisher Scientific), ** CLSI 2009 (reference 9),*** CLSI 2016 (reference 7), § SFBC (French Society for Clinical Biology) 1999, # UK NEQAS (UK National External Quality Assessment Services) 2019,¤ AFSSAPS (French Agency for Health Security) 2010.

Table 2. AllergoBioNet network recommendations 2020, defined as the 95th percentile of observed performance in participants' results (adapted with permission from reference 6).

	Total IgE (kIU/L)	Total IgE (kIU/L)	Total IgE (kIU/L)	Allergen-specific IgE (kUA/L)	Allergen-specific IgE (kUA/L)	Allergen-specific IgE (kUA/L)	Τοταλ τρψ-πτασε (μγ/Λ)	Τοταλ τρψ-πτασε (μγ/Λ)	To τρ πτ (μ
	Low (<100)	**Medium (100-400)**	**High (>400)**	**Low (<5)**	**Medium (5-10)**	**High (>10)**	**Low (<8)**	**Medium (8-20)**	**Hi (>**
Intra-assay variation (%CV) (Repeatability)	10	10	10	10	10	10	5	5	5

	Total IgE (kIU/L)	Total IgE (kIU/L)	Total IgE (kIU/L)	Allergen-specific IgE (kUA/L)	Allergen-specific IgE (kUA/L)	Allergen-specific IgE (kUA/L)	Τοταλ τρψ-πτασε (μγ/Λ)	Τοταλ τρψ-πτασε (μγ/Λ)	Το τρ πτ (μ
Interassay variation (%CV) (Reproducibility)	15	15	15	15	15	15	10	10	10
Bias (Accuracy)	20	15	15	30	25	25	20	20	20
Uncertainty of measurement (Precision)	30	20	20	30	30	30	25	25	25