Report on survey ANA EQA schemes carried out in November 2024

EQALM Immunology WG

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The Immunology WG thanks all the EQA providers for their participation in this survey.

Purposes:

In the field of autoimmunity, the variability of results and the lack of standardisation are known. The EQALM Immunology WG decided to take actions to move towards an improvement in the situation. A first survey was carried out among laboratories participating in the EQA programs of PT providers which are members of EQALM (manuscript submitted for publication in 2025). The survey results showed variability in the use of methodologies and an inconsistent use of the ANA ICAP nomenclature. The WG proposed that harmonisation could be supported further via the EQA providers, in particular via the method of reporting results to their EQA programs (type of data collected, use of ANA ICAP nomenclature, ...). The aim of this second survey was to collect data on the current design of EQA programs provided by EQALM members. This information will assist in the production of a guideline which could be used by EQA providers wishing to harmonise elements of their autoimmunity EQA programs to assist with harmonisation within the field of autoimmunity.

Conclusion:

The results of this survey show a lack of harmonisation between the programmes of the 16 PT providers, across all areas studied (sample types, statistical plan, reporting of results, use of the ANA ICAP nomenclature and evaluation of results). The working group therefore decided to draw up a guideline for the implementation of an ANA EQA program. This guideline, which will benefit PT providers, should indirectly encourage medical laboratories to use the international nomenclature for reporting results to patients. In addition, the guidance will highlight the importance of harmonisation of results within autoimmunity testing and the need for a suitable EQA/PT program for the detection of any assay related issues such as lot to lot variation.

I. Information on the EQA organization (EQAO)and associated autoimmunity programs

PARTICIPANT EQA Providers:

16 EQA Providers have participated: Biologie Prospective, Controllab, Centro Regionale di Riferimento per la VEQ-Firenze, Centro Regionale di Coordinamento della Medicina di Laboratorio, CTCB, Equalis, IfQ-Lübeck, Instand, Labquality, Öquasta, PNCQ, RfB (SPM D),SEKK, SKML, SNEQAS, UK NEQAS.

The number of countries represented is 11 **(figure 1):** Austria, Brasil, Czech Rep, Finland, France, Germany, Italy, Netherlands, Slovenia, Sweden, UK.

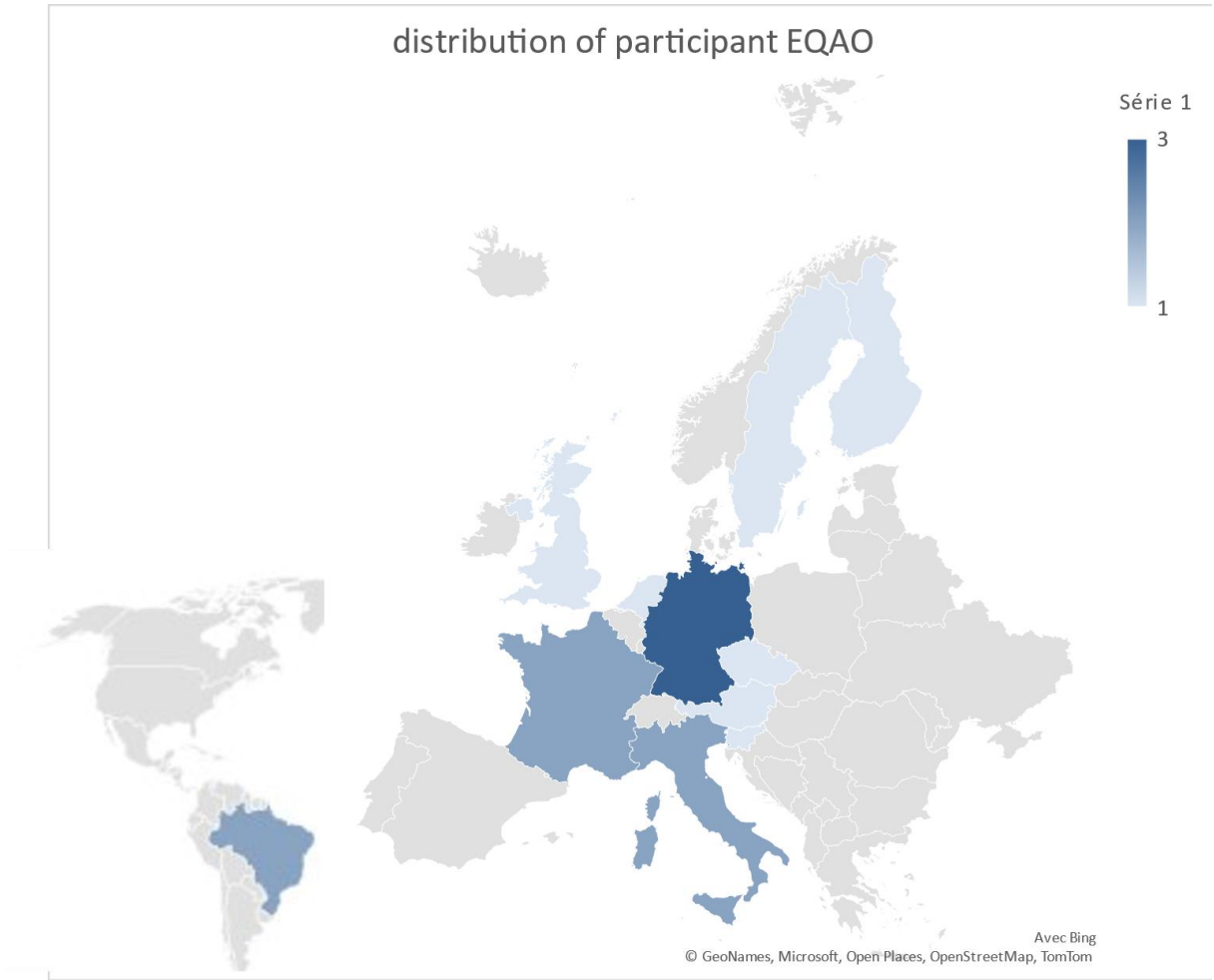

Figure 1. Distribution of participants : number of EQA Providers by country

11 EQA Providers are accredited to ISO 17043, and for one EQA Provider the process is underway.

EQA SCHEMES :

All the participant EQA Providers provide an ANA EQA program (EQAp) (see figure 2)

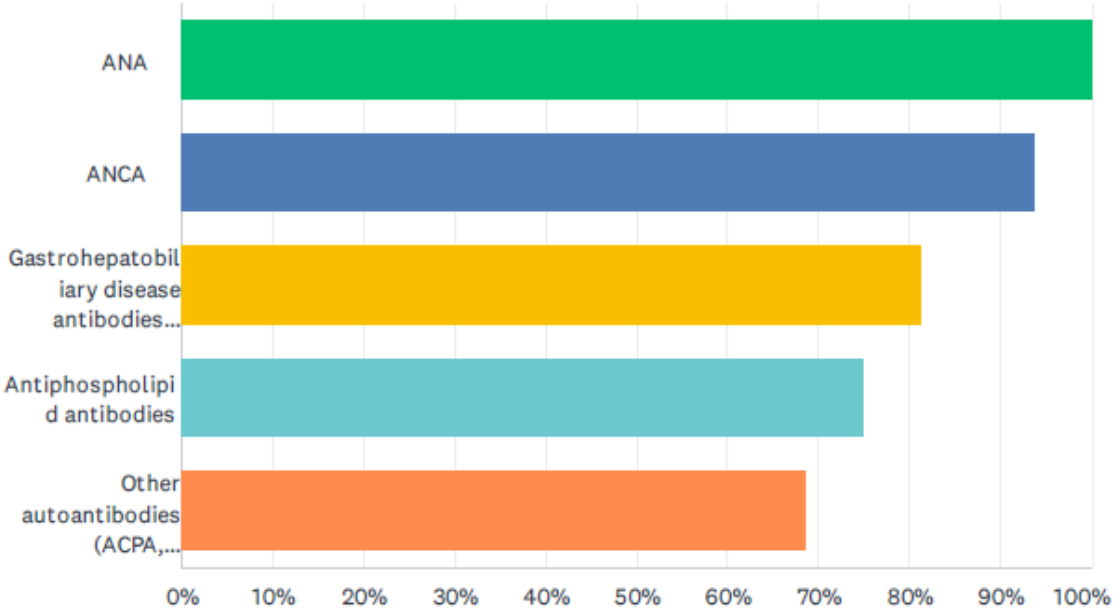

Figure 2. Programs proposed by EQA Provider

We observed a wide range of the number of participants within each ANA program (from 5 to 800 labs) and half of them are between 40 and 100 participants (table 1)

Number of participants	Number Of EQA program
[0 ; 19]	1
[20 ; 39]	3
[40 ; 59]	3
[60 ; 79]	3
[80 ; 99]	2
[100 ; 499]	2
[500 ; 899]	2

Table 1. Number of participants at each EQA program

II. ANA survey Design

a. frequency

The number of samples sent per year ranges from 4 to 14 (see table 2).

Total number of samples per year	Number of EQAp	% EQAp
14	1	6,25
12	4	25
9	1	6,25
8	2	12,5
6	2	12,5
4	6	37,5

Table 2. Total number of samples per year

The frequency of surveys ranges from 2 to 14 with 44% of EQAO who proposed a frequency of 2 rounds (see table 3).

Frequency	Number of EQAp	% EQAp
2	7	43,8
3	2	12,5
4	5	31,3
6	1	6,3
14	1	6,3

Table 3. Number of rounds per year

b. Matrix

Concerning the matrix of the samples, serum is the most commonly used (see table 4). One EQA Provider specified that the diluted human serum is in fact a pool of patients. Another one specified that the serum is Defibrinated Human Plasma. The answer "other" specifies "human serum or lyophilized plasma".

Matrix	% of users	n users
Undiluted Human serum	62.50%	10
Diluted human serum	37.50%	6
Undiluted Human plasma	31.25%	5
Diluted Human plasma	25.00%	4
Human serum base with spike	6.25%	1
Virtual microscopy	12.50%	2
Other, specify in the free text box	6.25%	1

Table 4. Matrix of samples

EQA Providers who diluted matrix had to specify how. Only one of the 7 EQA Providers concerned uses PBS, the other use human serum or plasma (see table 5)

Diluent	n users
human serum	3
human serum or plasma	1
negative serum ou negative plasma	1
human plasma (negative)	1
PBS	1

Table 5. Diluents

c. Clinical case associated

3 EQA Providers give clinical notes accompanying each sample, two others do it for some samples. Eleven EQA Providers don't provide clinical notes.

d. Assigned value

Only 3 labs use participants consensus without any other confirmation. 8 EQA providers confirm the assigned value obtained by participants consensus with other data (experts' consensus or result of one expert or reference lab). Experts' consensus is used alone by 4 EQA providers. One EQA Provider used Supplier's values but confirm it by the participants' consensus (see table 5).

Assigned Value	n EQAO
By experts' consensus	4
By participants consensus	3
By experts' consensus + participants consensus	4
By participants consensus + reference lab	1
By participants consensus + expert opinion + reference lab	1
By participants consensus and also according to the pretesting.	1
By participants consensus + result obtained by the expert	1
Supplier's values (need to be confirmed by participants consensus)	1

Table 5. Determination of assigned value

III. Data collection

There is no consensus on the data collection (see table 6). The most frequently requested information is the "origin of substrate" (11 EQA Providers) and the least is the "reagent lot numbers" (1 EQA Provider). One EQA Provider provides a free text box for "batch numbers of reagent which are asked after the survey in case of IVD vigilance declaration"

Information collected	Number of EQAp	% EQAp
Reading mode (microscope/automated)	7	43.75%
Initial starting/screening dilution (e. g. 1/80…)	6	37.50%
Nature of the substrate (HEp2, HEp2000, HEp-20-10, rodent tissue…)	8	50.00%
Origin of substrate (name of reagent and of manufacturer)	11	68.75%
Nature of the Conjugate (IgG heavy chain specific, IgG heavy and light chain specific….)	3	18.75%
Origin of Conjugate (name of reagent and of manufacturer)	7	43.75%
Reagent lot numbers	1	6.25%

Table 6. Information collected with the results of ANA screening by IIF

IV. Results collection

a. Results returned by the participating labs

Qualitative results must be returned to the EQA Provider in all programs but only 69% of EQA Providers asked for the Titer and 81% for the nuclear patterns (see table 7)

Results returned by the participating labs	Number of EQAp	% EQAp
Titer (e. g. 1/320)	11	68.75%
Light intensity if automated	1	6.25%
Qualitative results (ANA Detected/ANA not detected)	16	100.00%
Nuclear patterns	13	81.25%
Cytoplasmic patterns	13	81.25%
Mitotic patterns	8	50.00%

Table 7. results of participants

b. Results scored within the assessment

Only 14 EQA providers answered this question see (table 8). All of them evaluate the qualitative results but less than 30% evaluate the quantitative results. Concerning the patterns, 57% score nuclear and cytoplasmic patterns.

Results scored	Number of EQAp	% EQAp
Titer (e. g. 1/320)	4	28.57%
Light intensity if automated	0	0.00%
Qualitative results (ANA Detected/ANA not detected)	14	100.00%
Nuclear patterns	8	57.14%
Cytoplasmic patterns	8	57.14%
Mitotic patterns	3	21.43%

Table 8. results of participants

c. Way of submitting patterns

14 EQA Providers ask labs to answer by choosing a pattern from a predefined list according to the International Consensus on Antinuclear Antibody Patterns (ICAP) and one EQA Provider from a predefined list according to historical usage in the country. One EQA Provider utilises a mixture of the two lists.

V. Data analysis and reports

a. data analysis

Only 15 EQA Providers answered the questions on the data analysis.

33% of EQA Providers use peer groups based on the substrate to analyse titers and qualitative (+/-) results. One EQA Provider stated: “In our experience, the IIFT results can be evaluated well - independently of the manufacturer. Screening results obtained with ELISA tests often show a heterogeneous picture.”

27% of EQA providers compare results obtained on classical microscope and automated microscope. Among these 4 participants, 2 EQA Providers saw no differences, one EQA Provider saw better performance on classical microscope and one better performance on automated microscope.

b. reports

Only 15 EQA Providers answered the questions on report:

- 7 provide clinical notes in the final report
- 13 provide a discussion or report commentary
- 5 complete their reports with photos for educational purpose(recognition of patterns)

One EQA provider stated : “Regarding the reported HEp2 patterns: we report the number of HEp2 patterns in total and define the strongest pattern only. We do not report ANA patterns with AC numbers according to ICAP nomenclature.”

c. IVD vigilance

In case of poor performance of a reagent, 4 EQA Providers from 15 make an IVD vigilance declaration to the competent authority.

V. EQA organizations cooperation

If the EQALM immunology working group makes recommendations regarding the organization of an ANA EQA, 11 EQA Providers agreed to harmonise their program. For the two who answered no, one stated that they are not totally against harmonising their program with the recommendations that could be made by the working group. They answered "no" because a discussion with their experts and an impact study will have to be carried out before they accept any modification of the program. The other EQA Provider explained the no response stating “The EQA programme is under professional supervision of the Czech Society of Allergology and Clinical Immunology”. For five EQA providers that answered they agree to harmonise their program, they expressed reservations:

- If it is applicable and in line with our requirements

- it depends on the recommendations

- needs to be discussed in the immunology steering group.

- after discussion with our local experts and in case they agree

- only if it makes sense and it is applicable

In the context of accreditation, the majority of EQA providers don’t need to discuss issues specific to the ANA program within the EQALM immunology working group . One has a question : “determining the assigned values with a large divergence of results depending on the reagent is a challenge: - how to demonstrate the validity of the choice of the value assigned to the auditors - Faced with such a dispersion of results, how can we respond to impartiality when we take the consensus of experts whose results were obtained with a specific reagent?”. Another organisation states : “we are not planning to accreditate this program since we are not giving a formal evaluation of performances (score and/or other methods of evaluation)”.