EQAS
EXTERNAL QUALITY ASSESSMENT SCHEME

AINI EQAS 2024

FINAL REPORT

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On behalf of both the AINI Scientific and NINA Boards

ASSOCIAZIONE ITALIANA NEUROIMMUNOLOGIA

NINA
Network Italiano Neurologia Autoimmune

Con il contributo incondizionato di
ALEXION
AstraZeneca Rare Disease

Table of Contents

Introduction

As every year, the Italian Association of Neuroimmunology (AINI), and the Italian Network for the study of Autoimmune Neurology (NINA group) have organized an External Quality Assessment Scheme (EQAS) to promote quality and standardization in neuroimmunology laboratory diagnostics in Italy and in Europe.
In the evolving scenario of the neuroimmunology diagnostics, these schemes are an essential tool to promote self-evaluation, highlight critical assays, and identify issues to be tackled for continuous improvement.
Moreover, the recent rise of interest in many neuroimmunological disorders, mainly driven by the evolution of the therapeutic scenario, has made the standardization and optimization of laboratory diagnostics even more relevant to clinicians.

The results of the current EQAS are not intended as an exam for the participating laboratories, and the comparison with the reference result (the one codified as "sent as" in the present report) should always be interpreted cautiously, and not necessarily looked at as a "true value". For each scheme, we provide a comment with a possible interpretation, but we would be happy to hear your own comments and feedbacks. In the future, if you face critical results during the testing process, we invite you to **take pictures** of your results and sharing them with our community.

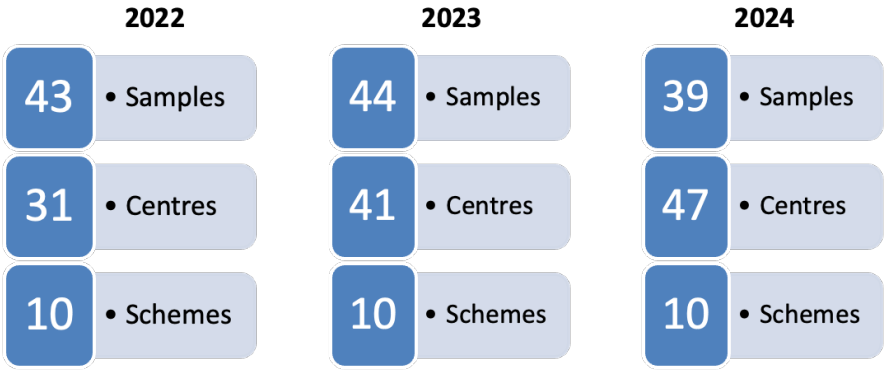
The results of the current EQAS have been preliminarily presented, as every year, during the annual AINI conference in Cagliari, and are now available for consultation on the AINI website (www.nina.aini.it) not just for the participating laboratories, but also for everybody interested in this area.

We thank in advance all the people that contributed to support and organize the current EQAS, and all the participating laboratories.

We hope that the results presented in this report will be of help to the participating labs, as well as to the AINI community.

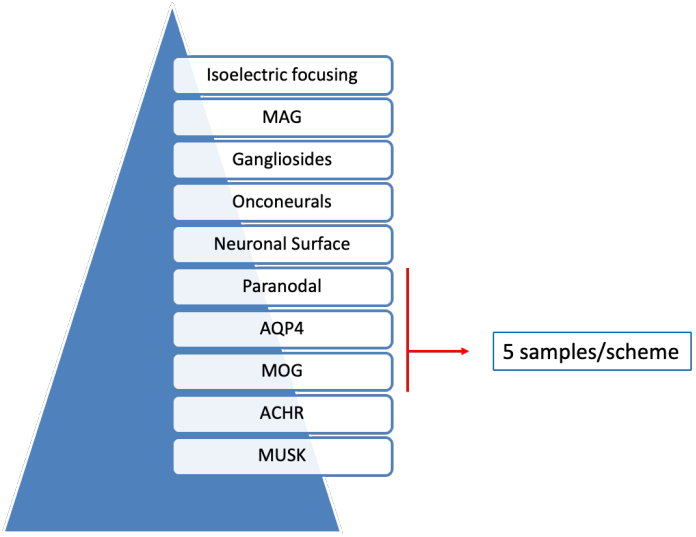
General Data of the 2024 AINI EQAS

The numbers of the 2024 AINI EQUAS



This year the number of schemes, and the consequent number of samples used has remained very similar compared to the previous years. We observed a further increase in the number of participating laboratories from 41 to 47, but only 42 provided results and were therefore included in the final report.

The schemes included in the 2024 AINI EQAS



The graph shows all the shemes of the EQAS. All schemes included 3 samples, except for AQP4, MOG and Paranodal antibodies, which included 5. The latter scheme on paranodal antibodies is particularly relevant this year, as a commercial kit is now available and has made these tests more accessible. However, no clear data on its analytic performance are available yet.

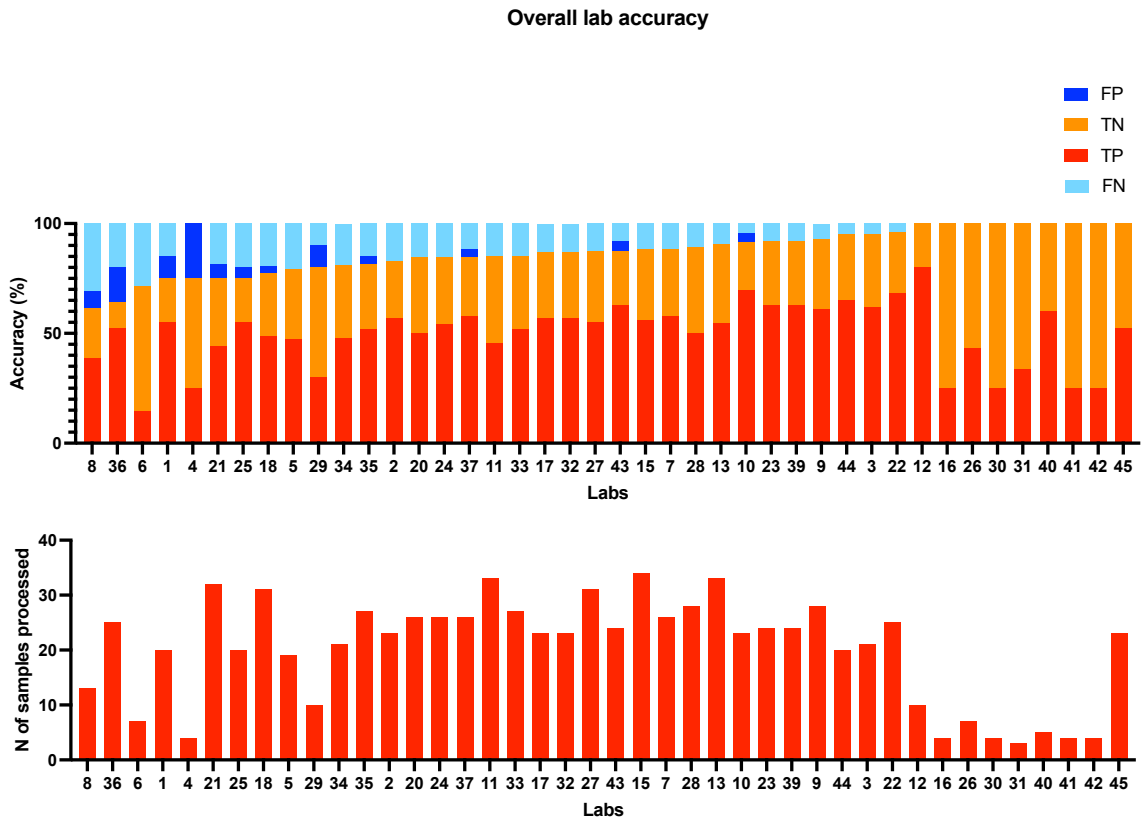
Participants to the AINI EQAS 2024

As in the previous editions, along with a long list of Italian collaborators that have participated to the EQAS for several years, we invited several labs from all around Europe. Here is a list of the participants:

Participating laboratory	City	Collaborator
Hospital Sant Pau	Barcelona	Cinta Lleixà
laboratorio analisi e biochimica clinica ospedale Sant'Andrea Roma	Roma	Vittoria Polidori
SC SmeL 2 analisi chimico cliniche - ASST Papa Giovanni XXIII	Bergamo	Previtali Giulia
Laboratorio Foggia	Foggia	Sernia Giorgia
Laboratorio di Patologia Clinica - Azienda Ospedaliera Universitaria Pisana	Pisa	Laura Caponi
Clinical Neurochemistry Laboratory, Santa Lucia Foundation	Roma	Giulia sancesario
Laboratorio analisi Ospedale San Giovanni Bosco Torino - ASL Città di Torino	Torino	Cicilano Matteo Alessandro
Laboratorio Analisi Borgo Trento Verona	Verona	Maddalena Marini
Medicina di Laboratorio ASST-VALLEOLONA P.O. Gallarate	Gallarate	Dr. Sferrazzo Annarita
DOMP Laboratorio di neurobiologia Torino	Torino	Cristiana Atzori
laboratorio specialistico Patologia Clinica 22034, Istituto Besta	Milano	Elena Corsini
Istituto Clinico Humanitas	Milano	Claudia Giannotta
U.O.C Patologia Clinica, Ospedale San Filippo Neri - ASL Roma1, Rome	Roma	Laura Cuomo
Division of Neuropathology and Neurochemistry , Department of Neurology, Medical University of Vienna	Vienna	Romana Höftberger and Verena Endmayr
Laboratory of Clinical Patology, AOU Sassari	Sassari	Giovanni Andrea Deiana
Laboratorio di Neuroimmunologia Modena	Modena	Roberta Bedin
Lab Liquor Clinica Neurologica IRCCS Policlinico San Martino, Genova	Genova	Davide Visigalli
Autoimmunità - Ospedale di Udine	Udine	Martina Fabris
Laboratorio di Riferimento, Euroimmun Italia	Padova	Piera De Gaspari
settore demenze e neuropatie autoimmuni A.O.U Sangiovanni di Dio e Ruggi D'Aragona Salerno	Salerno	Luigi Gallo
UOC Medicina di Laboratorio AULSS8 Vicenza	Vicenza	Vlentina De Riva
Laboratorio di Immunopatologia, Azienda Regionale Ospedaliera San Carlo, Potenza	Potenza	Teresa Carbone
Laboratorio Analisi Spedali Civili Brescia	Brescia	Emirena Garrafa
laboratorio generale AOU Careggi	Firenze	Tiziana Biagioli
Neurochemistry Lab-Policlinico-University of Bari Aldo Moro	Bari	Maddalena Ruggieri; Concetta Gargano
Laboratorio di Autoimmunità, Allergologia e Biotecnologie Innovative"	Reggio Emilia	Lucia Belloni
Laboratorio Diagnostica Neuroimmunologica, Istituto Besta	Milano	Francesca Andreetta
U.O.C. Medicina di Laboratorio Azienda Ospedale - Università Padova	Padova	Giulia Musso
Laboratorio di Neuropatologia, Università di Verona	Verona	Sara Mariotto
SOS Patologia Clinica Prato	Prato	Annalisa Azzurri
Ospedale San Raffaele	Milano	Stefania Del Rosso
Laboratorio Analisi ULSS2 Marca Trevigiana	Treviso	Silvia Zago
Laboratorio diagnostico di autoimmunologia-IRCCS Ospedale Policlinico San Martino Genova	Genova	Federica Maria Bozzano
Patologia clinica Chieti	Chieti	Barbara De Laurentiis
Laboratorio di Autoimmunologia	Imperia	PAOLA RIVERA
Patologia Clinica "Santissima Annunziata" - Taranto	Taranto	Maria Rosaria De Cagna
IRCCS Istituto delle Scienze Neurologiche di Bologna	Bologna	Maria Pia Giannoccaro
Ospedale Policlinico Bambin Gesù, Roma	Roma	Giorgia Bracaglia
LUM AUSL	Bologna	Ana Gabriela Grondona
laboratorio neurobiologia	Orbassano	sala arianna
Neurologia Autoimmune, Istituto Policlinico Gemelli	Roma	Jacopo Morroni
University of Oxford	Oxford	Patrick Waters

Results' summary

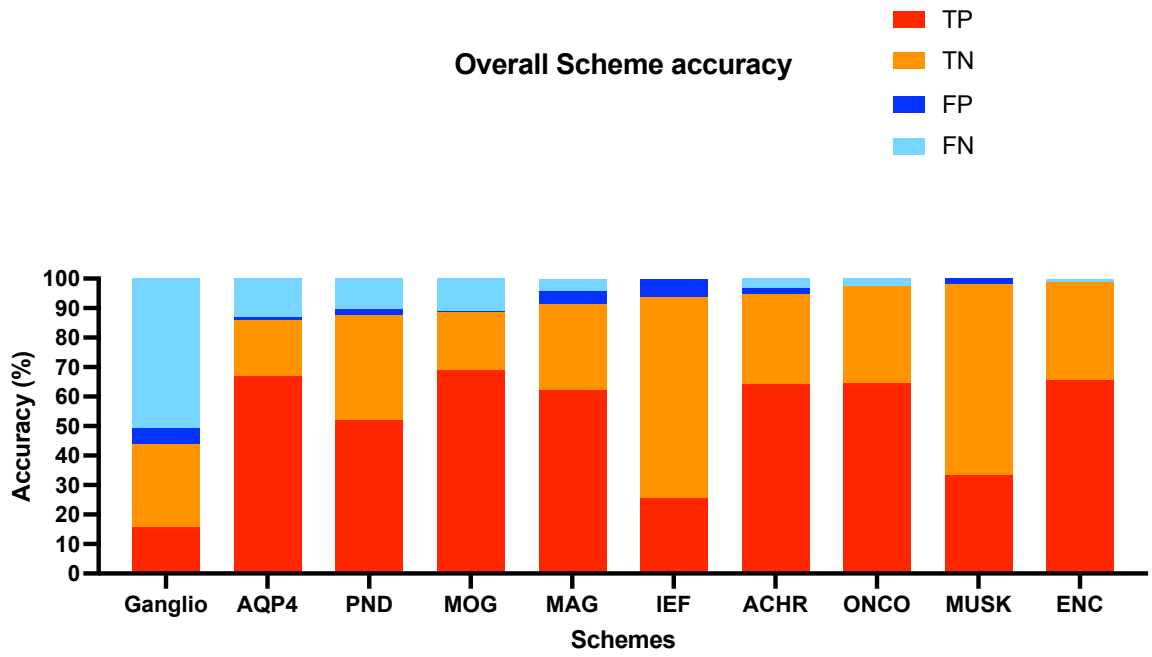
Overall accuracy of the laboratories



Overall accuracy can be estimated according to the % of samples tested that were concordant with the reference result ("sent as"). These are considered as true positives (TP, red) or true negatives (TN, orange). The performance is reported for each coded laboratory.

The accuracy was lower compared to that of the last years' EQAS, as only 40.5% of the laboratories has shown values ≥90%, and 78.6% ≥80%. However, these results are likely attributable to critical samples of specific schemes, whose results will be discussed below. The performance of each laboratory should be weighted according to the number of samples processed, that is shown at the bottom of the figure.

Overall accuracy of the schemes



In the graph are represented the performance in the 10 EQAS schemes. ENC= Neuronal surface antibodies; PND= paranodal antibodies; GANGLIO= ganglioside antibodies; IEF= isoelectric focusing; ONCO= intracellular neuronal antibodies. Four schemes had critical results with an accuracy lower than 90% (Ganglio, AQP4, PND and MOG). Since there is no objective criterion to define a "critical" scheme, we took into consideration both the proportion of discrepant results and the potential impact of inaccurate results on patients' management. In tests that have huge clinical implications, such as the AQP4 antibodies, an accuracy below 90% has worrying consequences for patient management and is not acceptable.

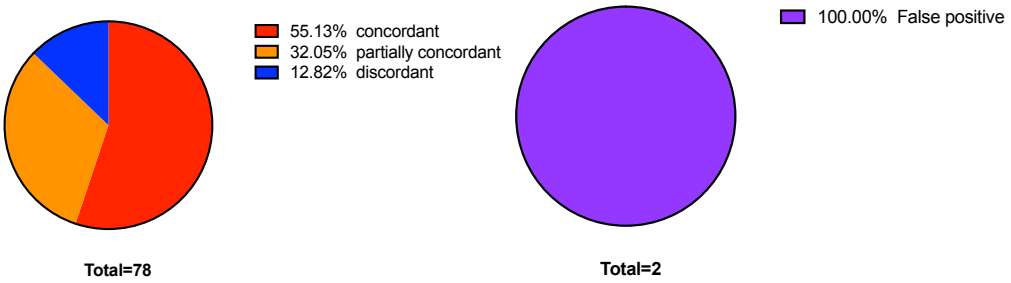
Isoelectric focusing (IEF) scheme

Participants: 20
Samples: 4 sera+4 cerebrospinal fluids (pairs)
Judgment: satisfactory

Results

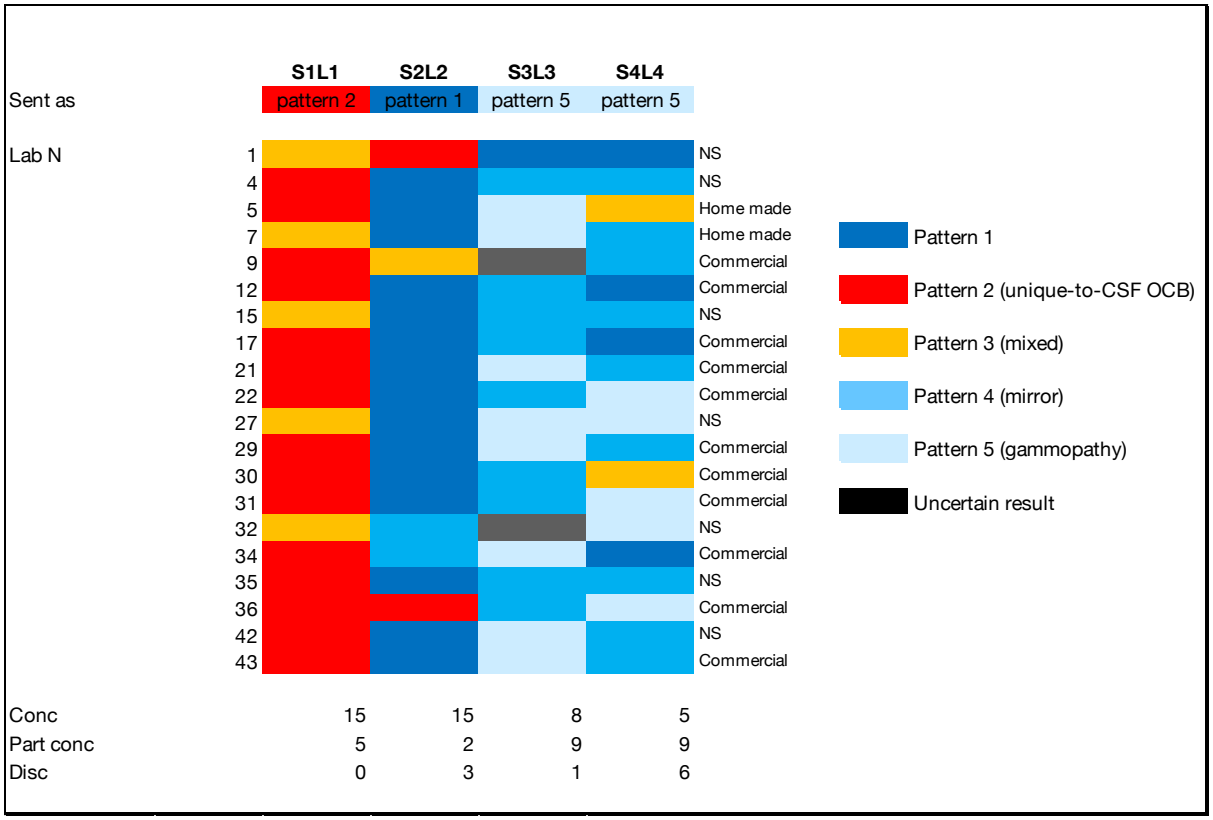
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

The concordance was assessed by considering the presence or absence of unique-to-CSF OCBs, which is the only parameter that has actual clinical implications. The overall accuracy was 87.2%, and the only inaccurate results were two false positives. All laboratories were able to detect CSF OCBS in the only positive sample, even though distinction between pattern 2 and pattern3 was difficult. However, since this distinction should not affect substantially the patient management, we considered these results as accurate. The scheme confirms the well-known difficulties in distinguishing pattern 4 (mirror) from pattern 5 (monoclonal gammopathy), which, in routine and even in future schemes, could be easily solved out by testing sera with agarose electrophoresis. The overall results represent an improvement compared to those critical reported in the past years.

AQP4 antibody scheme

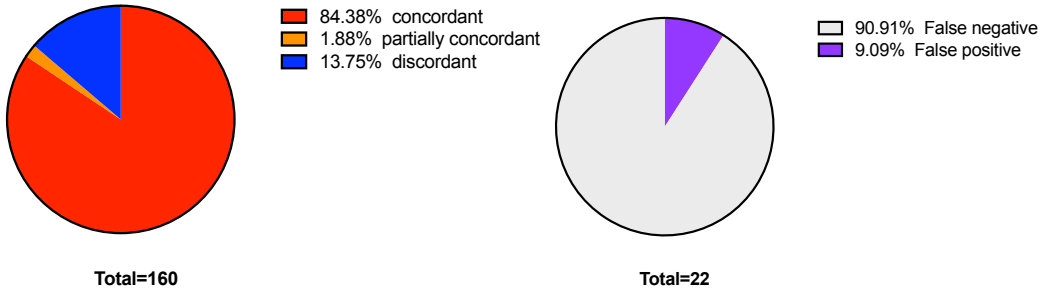
Partecipants: 32
Samples: 5 (3 strong positive, 1 weak positive and 1 negative; all positive samples were positive on both in-house LCBA and commercial FCBA in the reference laboratory)
Judgment: highly critical

Methods		
Assay	N of centres	Description
LCBA	6/32 (84.4%)	Live cell based assay with M23 AQP4 isoform; assessment with fluorescent microscope or flow cytometry (in-house)
FCBA	29/32 (90.6%)	commercial fixed CBA

Results

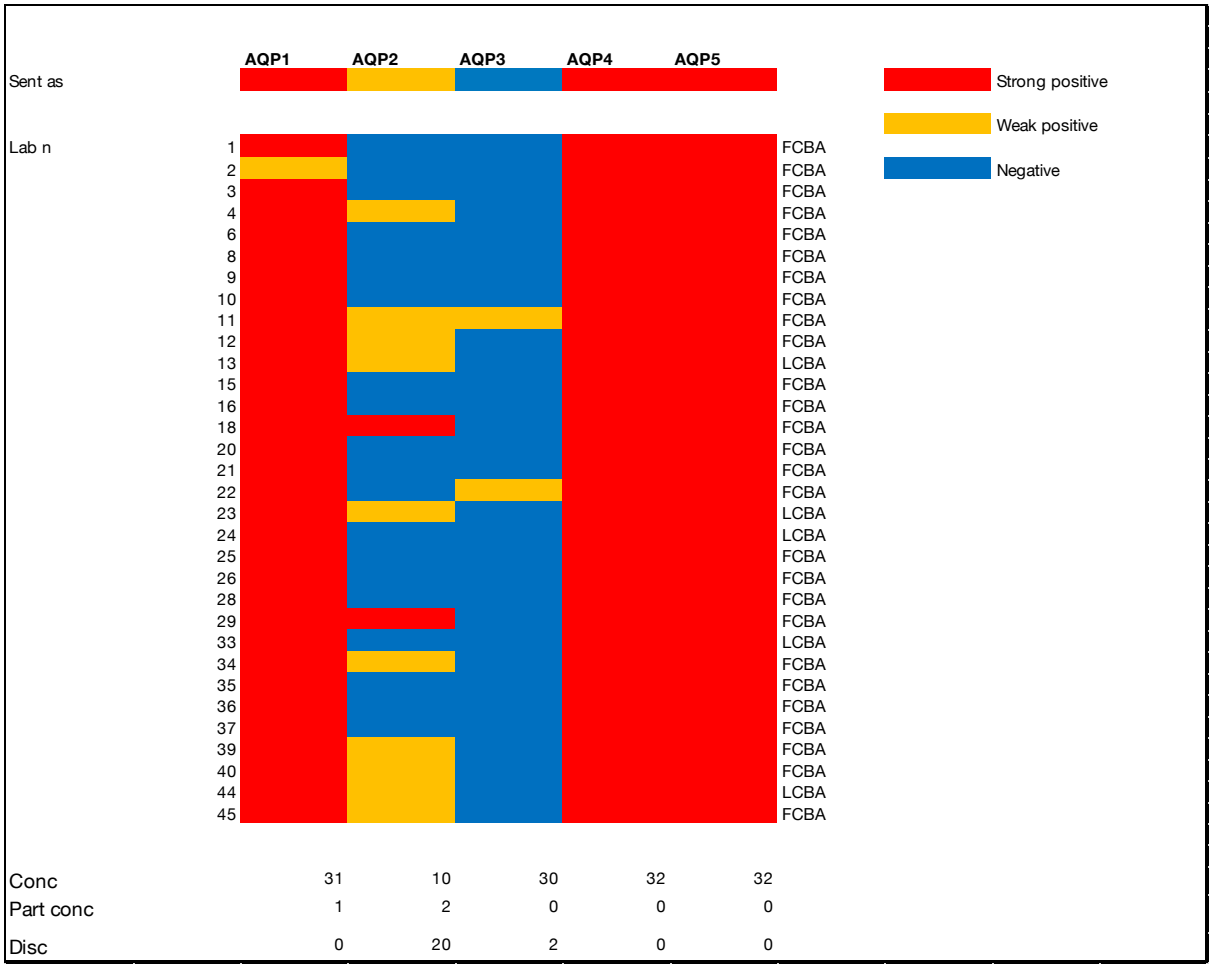
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

The overall accuracy was 86.3%. However, considering the clinical relevance of AQP4 antibody detection, the scheme was considered highly critical. Most discrepant results were due to “false negatives” obtained with sample #2, which was sent as a weak positive. The sample was collected from a patient with NMOSD fulfilling Wingerchuck’s 2015 criteria, under treatment with rituximab, during a remission phase. For this sample, the discrepancies could not be clearly attributed to the type of assay performed by the testing laboratory. Indeed, “false negative results” were provided by 50% (3/6) of the laboratories performing a LCBA, and 66.7% (18/27) of those performing a FCBA. Similarly, it was not possible to stratify the results according to the laboratory experience (calculated as number of samples tested per year; data not shown). Puzzled by such a high level of inconsistency, we retrieved 5 leftover aliquots of the sample #2 that were prepared at the same time of the aliquots sent for the EQAS, and stored afterwards at 4°C. Upon retesting, 4/5 aliquots were positive, but 1/5 resulted negative.

The results from our EQAS highlight how very low titre samples can be critical thus providing inter-, and even intra-laboratory disagreement. This can be due to several factors, including pre-analytical or analytical (results interpretation) issues. Even though this EQAS represents an “artificial setting”, it is likely that these inconsistencies also occur in routine practice, especially when low-titre samples are involved. This should highlight that discrepancies are possible (even in highly specialized laboratories), and that clinicians should always consider retesting, when facing a negative result in a patient with a highly suggestive clinical phenotype. This is particularly relevant for AQP4-IgG, where the risk of false positives using CBA seems to be extremely low.

MOG antibody scheme

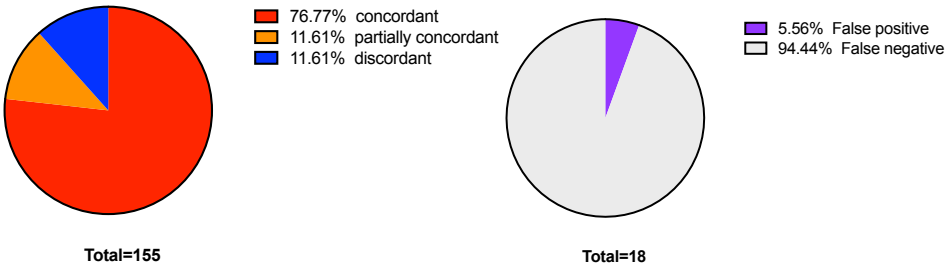
Partecipants: 31
Samples: 5 (3 strong positives, 1 low positive, 1 negative; all positive samples were positive on both LCBA for total IgG, LCBA for IgG1, and FCBA in the referral laboratory; all patients fulfilled the current diagnostic criteria for MOGAD)
Judgment: highly critical

Methods		
Assay	N of centres	Description
LCBA	6/31 (19.4%)	Live cell-based assay with human full length MOG isoform (in-house). One Lab used FACS for analysis.
FCBA	25/31 (80.6%)	Commercial fixed cell-based assay with full length human MOG isoform; human anti-Fc total IgG secondary ab; assessment with fluorescence microscopy

Results

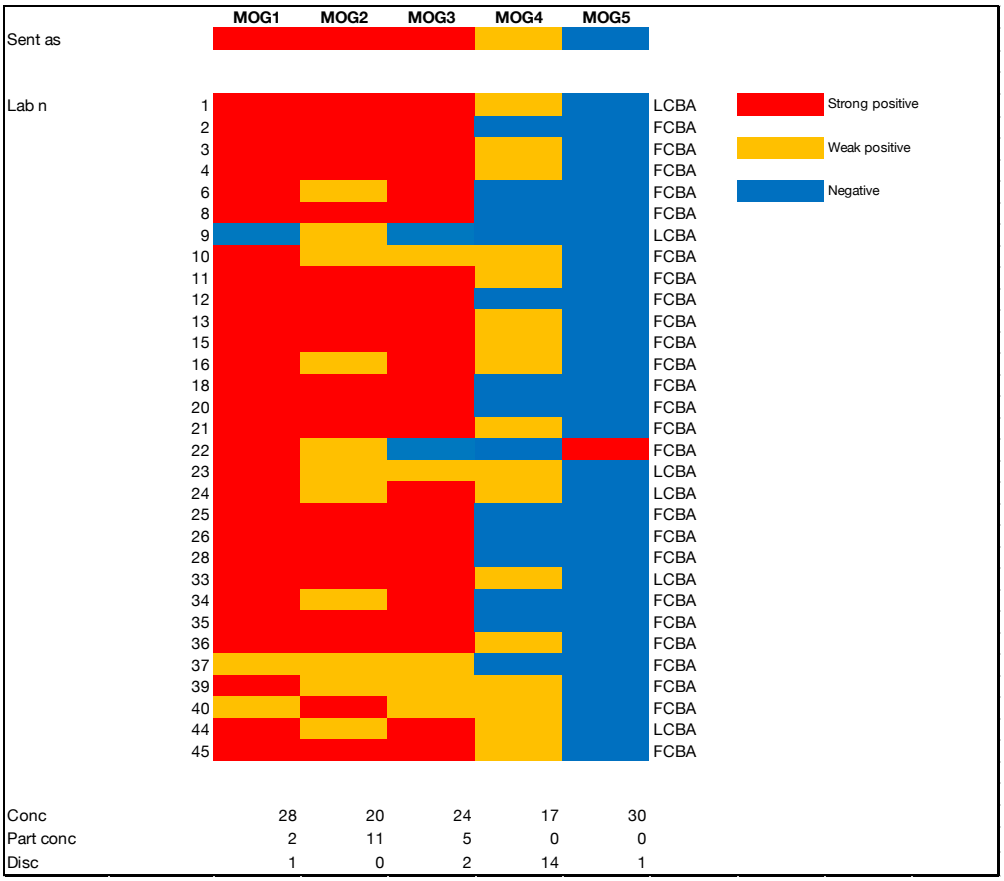
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

The overall accuracy was 88.5%. Despite the relatively good accuracy, the scheme was still considered highly critical due to the high number of laboratories (14/31) that provided at least one inaccurate result. The most critical results were “false negatives” concerning sample #4, that was sent as a “weak positive”. Differently from the AQP4 antibody scheme, the type of assay performed seems relevant here, as 83% (5/6) of laboratories performing a LCBA provided concordant results vs only 52% (13/25) of those using a FCBA. Therefore, along with the same pre-analytical and analytical issues addressed for AQP4 antibodies, these results still suggest more accurate analytical performance of the LCBA, in line with the literature data. When facing critical results, such as suspected false negatives or false positives, laboratories performing only a FCBA should refer to laboratories performing LCBA. A service supporting referral on critical samples has been established by AINI and NINA (for more information visit https://nina.aini.it/nina-flow/).

Intracellular neuronal antibody scheme

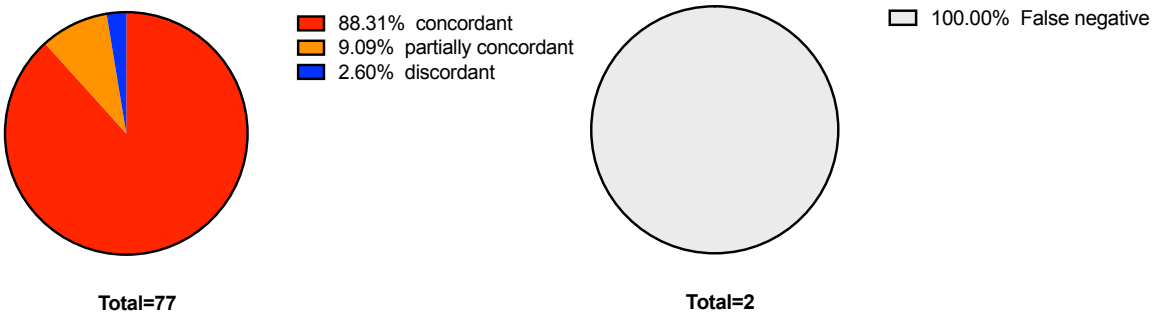
Partecipants: 26
Samples: 3 (1 positive for Ri, 1 positive for Ma2, and 1 negative)
Judgment: satisfactory

Methods		
Assay	N of centres	Description
TBA+line blot	13/26 (50.0%)	Included different type of commercial or in-house TBA
Line blot only	8/26 (30.2%)	Included different commercial line blots
Other	3/26 (11.5%)	Included 1 TBA only, 1 TBA+CBA, 1 TBA+Western blot
Unknown	2/26 (7.7%)	-

Results

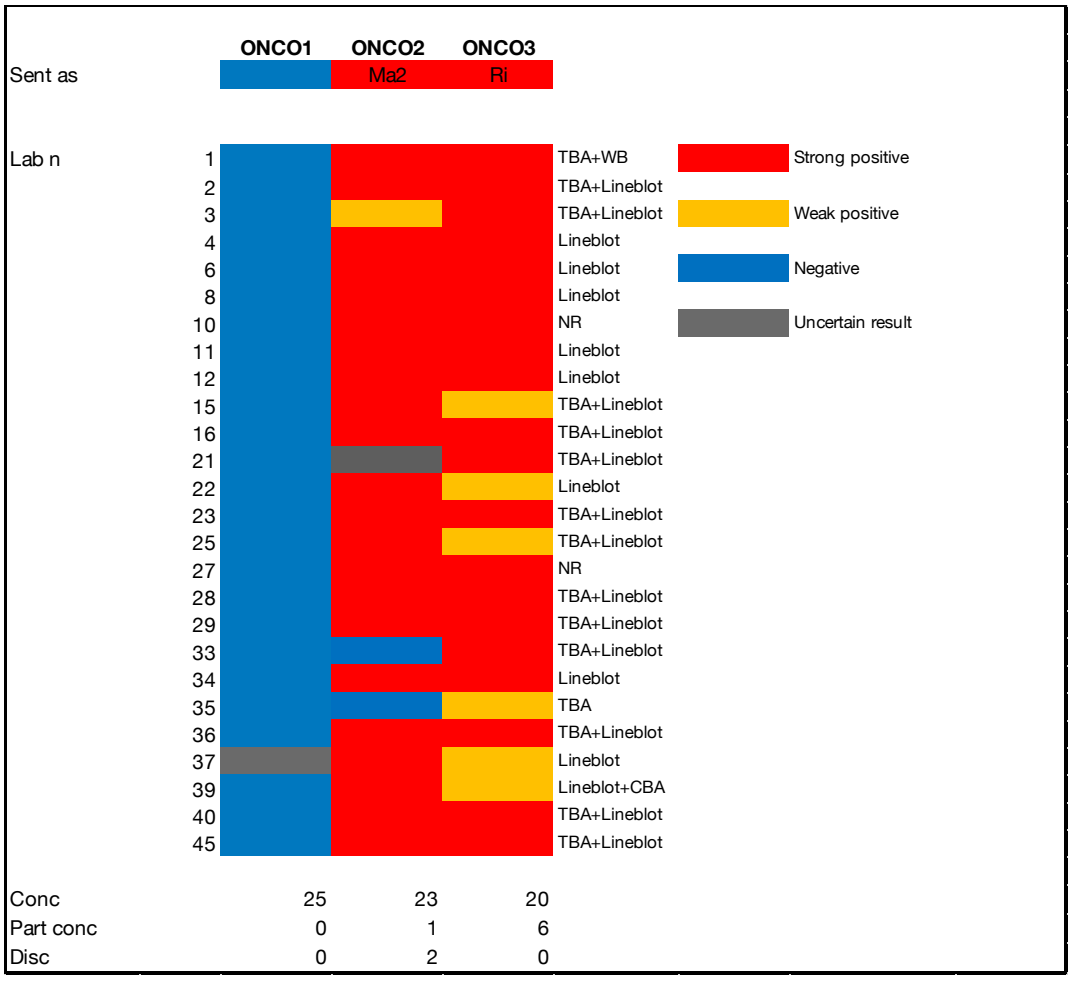
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

According to AINI recommendations, the most appropriate procedure for this scheme is the combination of TBA followed by a confirmation blot. However, a large proportion of laboratories (8/26) still used only line blots. The results were overall satisfactory, with an accuracy of 97.4%, in line with what reported the past years. Only 2 laboratories failed to identify a sample positive for Ma2, one using only a TBA. This could be due to challenges in recognizing the nucleolar pattern associated with Ma2 antibodies and strengthens the message of performing both tests (line blot and TBA) always.

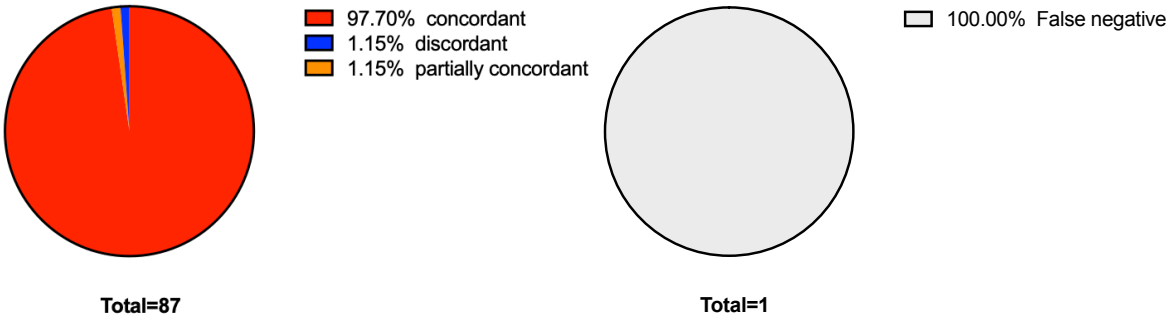
Neuronal surface antibody scheme

Partecipants: 29
Samples: 3 (1 CASPR2 positive, 1 NMDAR positive, 1 negative; all positive samples were identified with both in-house CBA and commercial CBA, and with in-house TBA)
Judgment: satisfactory

Methods		
Assay	N of centres	Description
FCBA	27/29	Commercial panel; one lab performed TBA in association
LCBA	2/29	CBA performed for each antigen separately; one lab performed TBA in association

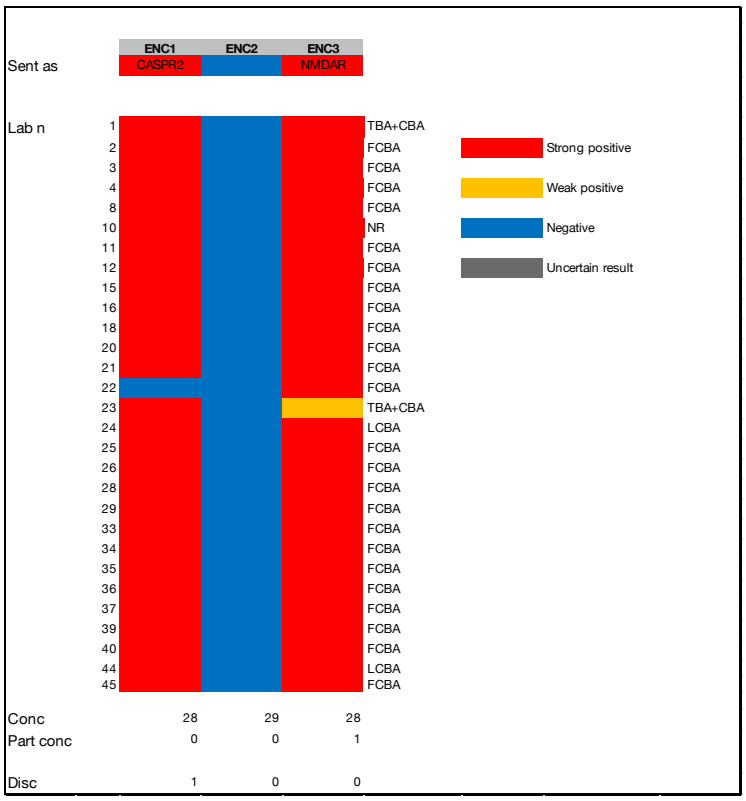
Results

Overall concordance of all tests performed



Heatmap

The graph represents the detailed results for each sample



Comments

The overall accuracy was 98.9%, and the only discrepant result was a “false negative” for a sample sent as CASPR2. This represents a huge improvement compared to the last year’s performance, likely due to the type of samples sent (only clear positives or negatives).

Ganglioside antibody scheme

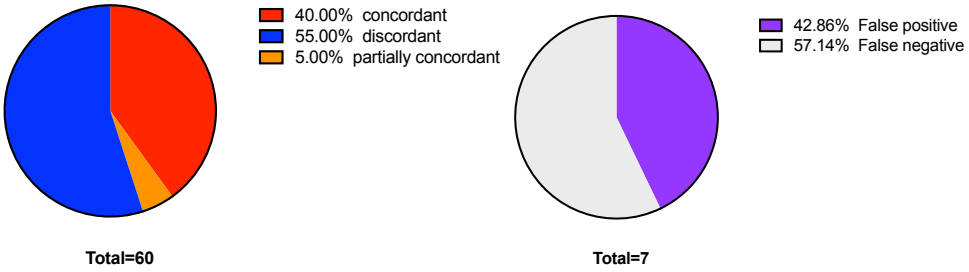
Partecipants: 21
Samples: 3 (1 GM1 IgG positive, 1 GM1 + GD1b IgG positive, 1 negative)
Judgment: highly critical

Methods		
Assay	N of centres	Description
Immunoblot	14/21 (66.6%)	This included different brands of immunoblots
ELISA	4/21 (19.0%)	ELISA: Buhlmann in 3 labs, home made in 1 lab
ELISA+immunoblot	2/21 (9.5%)	-
Unknown	1/21 (4.0%)	-

Results

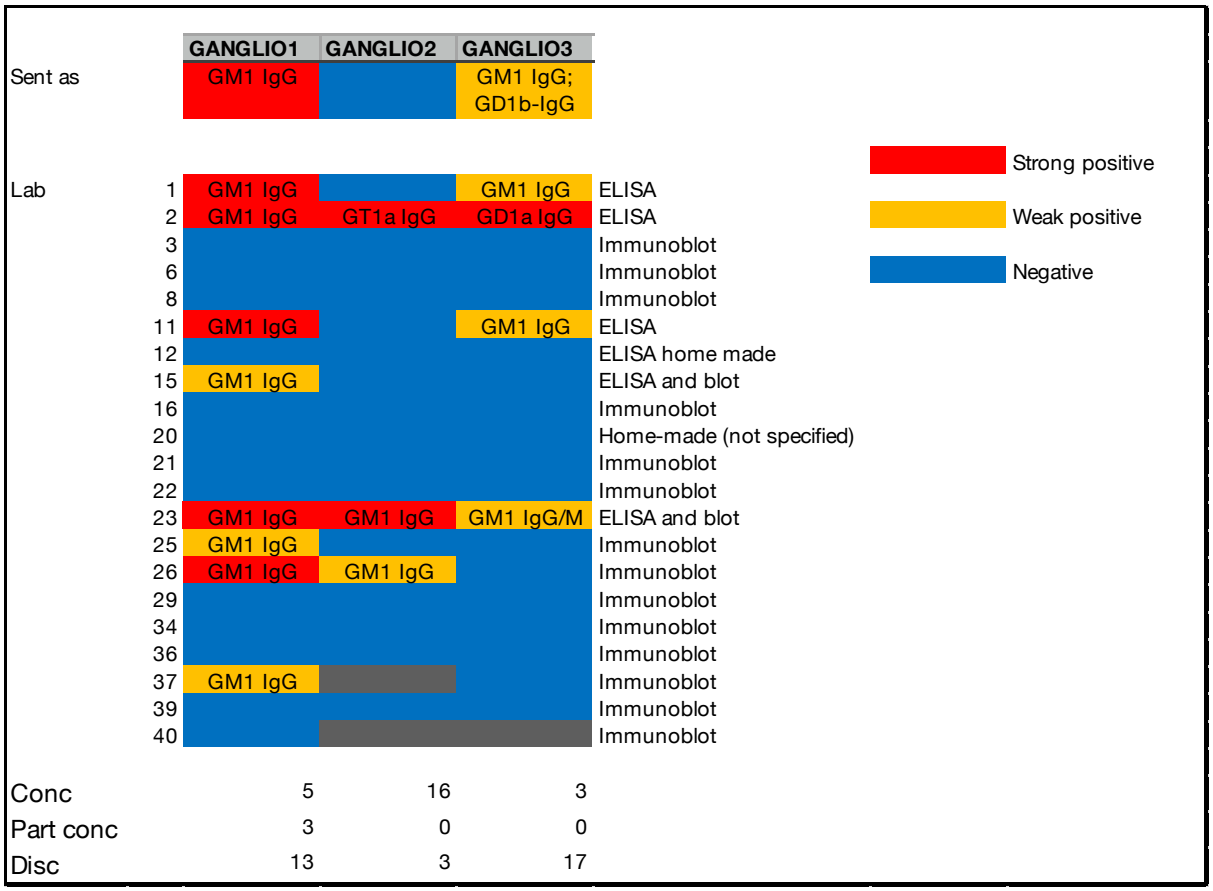
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

The overall accuracy was 45.0%, and was the lowest ever reported in the entire EQAS history. Only 3 laboratories used the Buhlmann ELISA, that is the recommended method according to both the INCAT and AINI guidelines. The discrepant results pertained both to false positives and false negatives. Given the large proportion of laboratories using potentially suboptimal tests, and the large number of non-relevant reactivities, we classified this scheme as highly critical, with the warmest recommendation to use ELISAs, as the state of the art suggests that to keep using blots means misdiagnosis and wasting money.

MAG antibody scheme

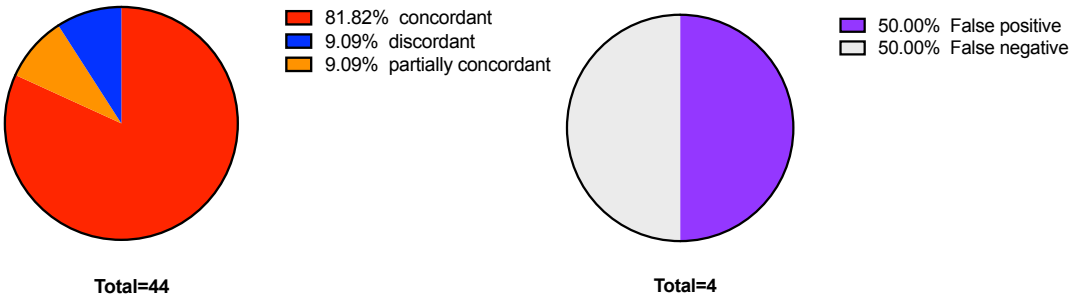
Partecipants: 15
Samples: 3 (2 strong positive, 1 negative)
Judgment: satisfactory

Methods		
Assay	N of centres	Description
ELISA	11/15 (73.3%)	11 ELISA Buhlmann, in one case not specified
IIF	4/15 (26.7%)	Indirect immunofluorescence on sciatic nerve (in one lab confirmed with immunoblot) – non-MAG specific assays

Results

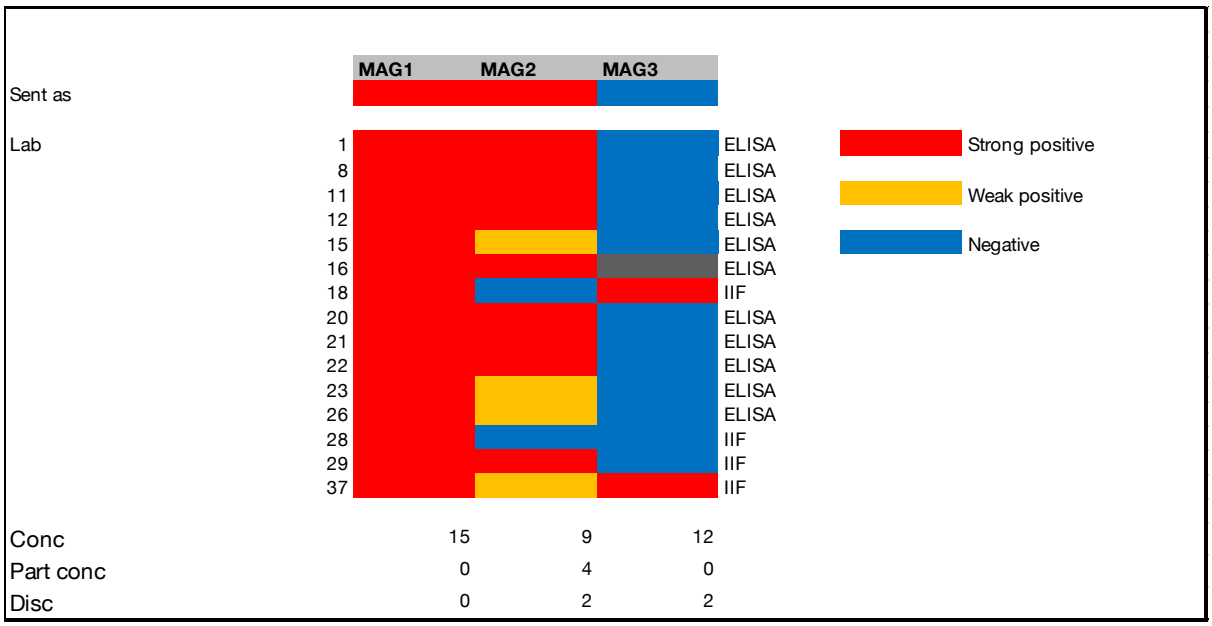
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

Results are overall satisfactory, with an accuracy of 90.9%. Two laboratories failed to identify a sample sent as “strong positive”, and two reported as positive a sample sent as “negative”. Notably, all the discrepant results occurred in laboratories using the IIF as detection method. Our results suggest that ELISA remains the gold standard, as recommended by AINI guidelines, and that IIF on sciatic nerve alone should not be used for clinical purposes.

Paranodal antibody scheme

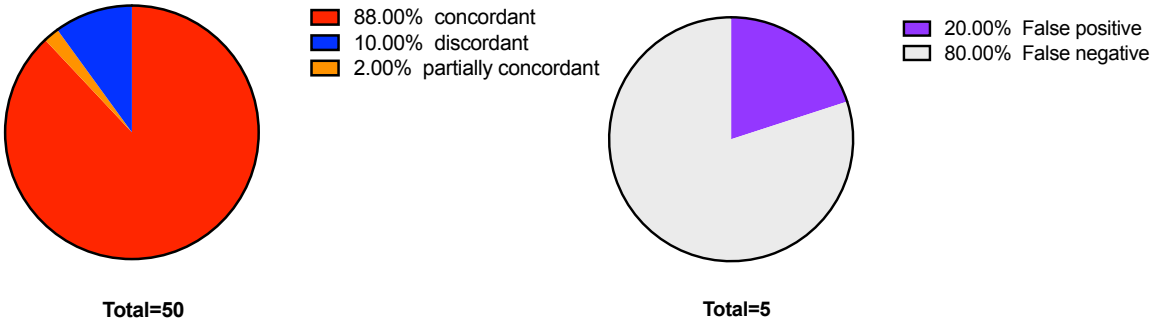
Partecipants: 10
Samples: 5 (2 CNTN1 positive, one NF155 positive, 2 negative)
Judgment: satisfactory

Methods		
Assay	N of participants	Comments
FCBA	6/10 (60%)	1 lab together with in house ELISA
LCBA	3	-
ELISA	1	In house

Results

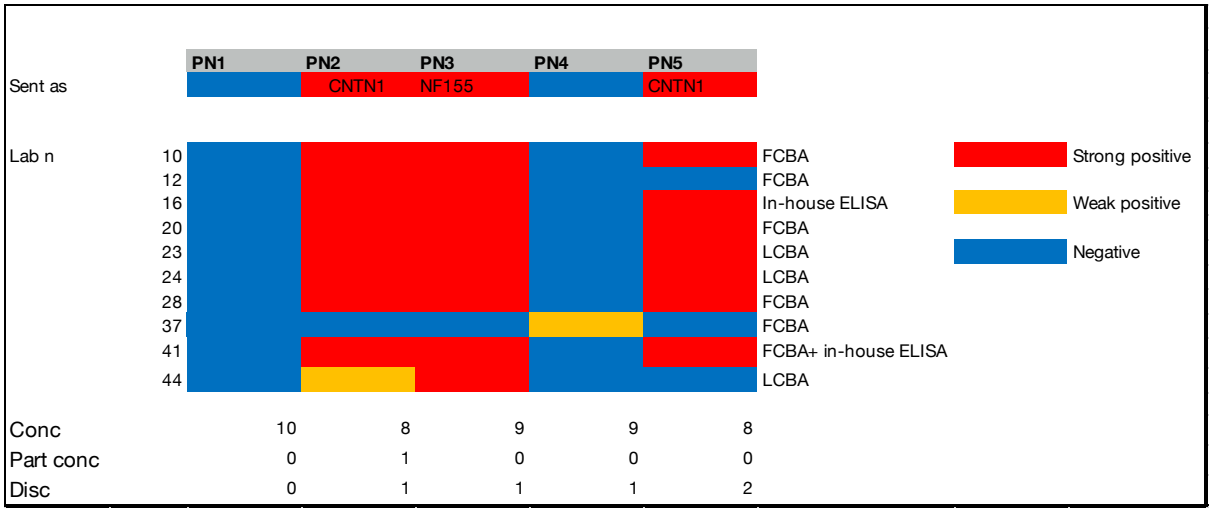
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

Compared to last year's EQAS, this scheme showed an increase in the participants likely reflecting the availability of a commercial FCBA for nodo-paranodal antibody testing. This increase was associated with a higher number of discrepant samples, with an overall accuracy of 90%. Notably, all the discrepant results were found in laboratories using the FBA, and 4/5 could be attributed to only one laboratory. Overall, this scheme provides preliminary evidence supporting the use of the FCBA for nodo-paranodal antibody detection. However, further validation studies are needed to assess its accuracy in routine diagnostics.

Nicotinic acethylcholine receptor antibody scheme

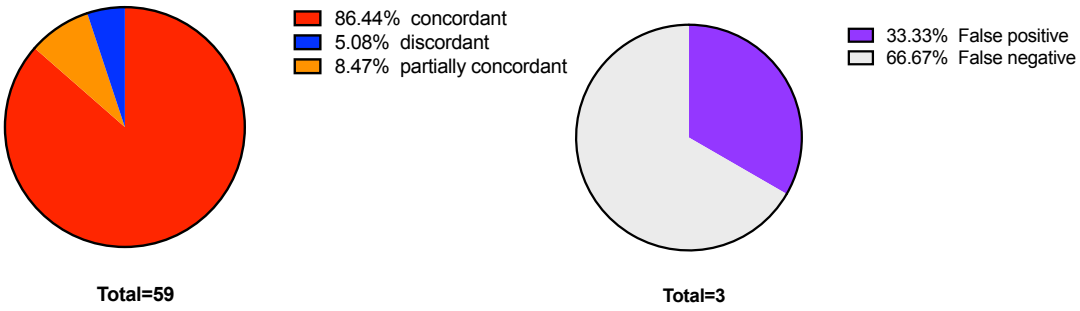
Partecipants: 20
Samples: 3 (1 low positive, 1 strong positive, 1 negative)
Judgment: satisfactory

Methods		
Assay	N of centres	Description
RIA	3/20 (15%)	Commercial RIA
LCBA	2/20 (10%)	-
FCBA	15/20 (75%)	-

Results

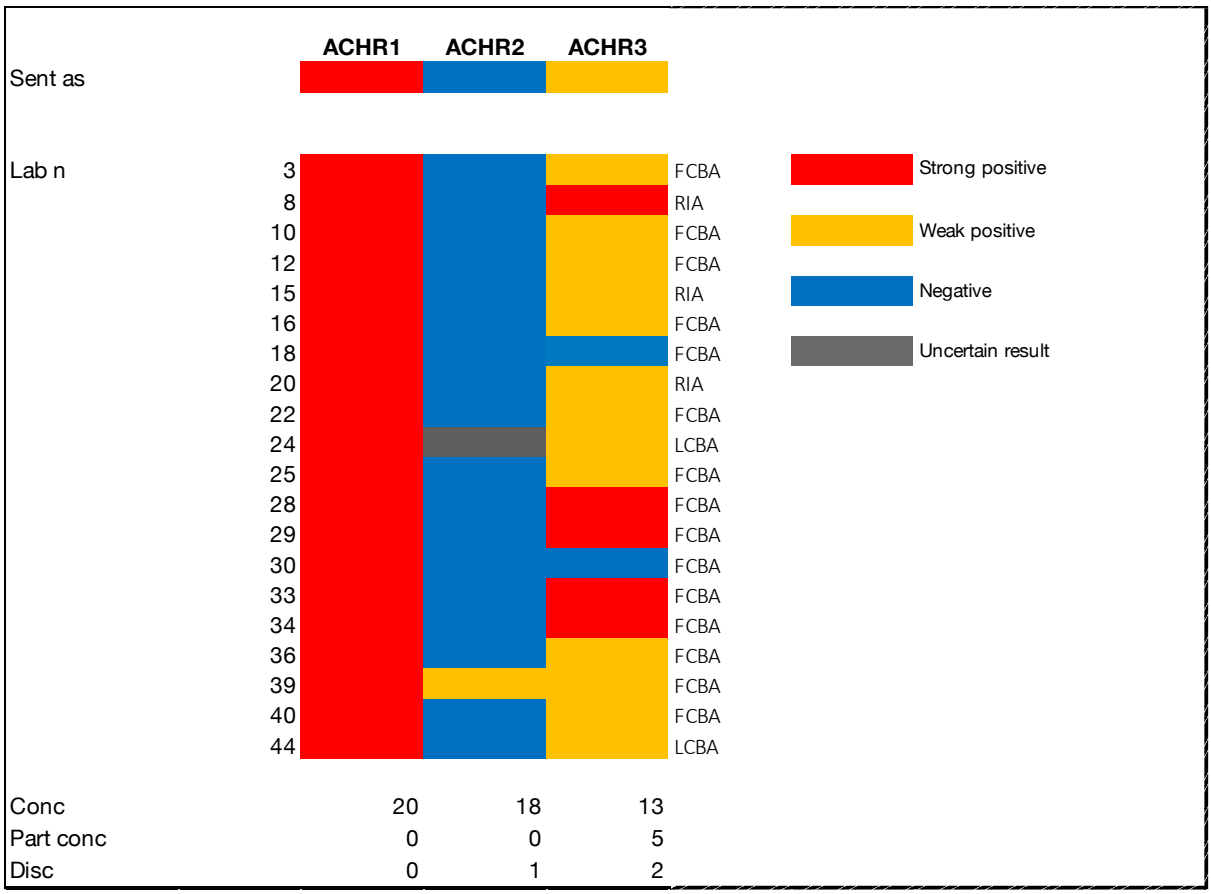
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

The assays used in this scheme reflect the modification of the diagnostic scenario. RIAs, currently considered the gold standard, were used only by 3/20 laboratories, whilst all the remaining ones converted to CBAs. Compared to last year's EQAS, none of the laboratories used ELISAs. The overall performance was satisfactory, with an accuracy of 94.9%, in accordance with what recently reported in the literature on this topic. All the discrepant results were provided by laboratories performing a FCBA.

MUSK antibody scheme

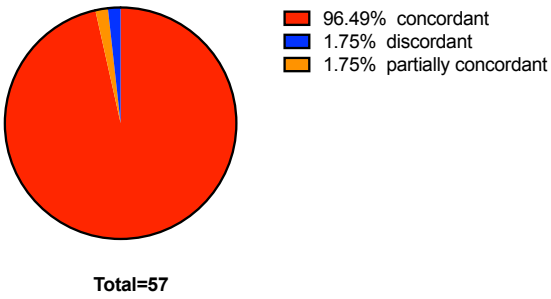
Partecipants: 19
Samples: 5 (2 strong positive, 1 low positive, 2 negatives)
Judgment: satisfactory

Methods		
Assay	N of centres	Description
FCBA	12/19 (63.2%%)	-
RIA	2/19 (10.5%)	Commercial RIA
LCBA	2/19 (10.5%)	-
ELISA	1/19 (5.2%)	-

Results

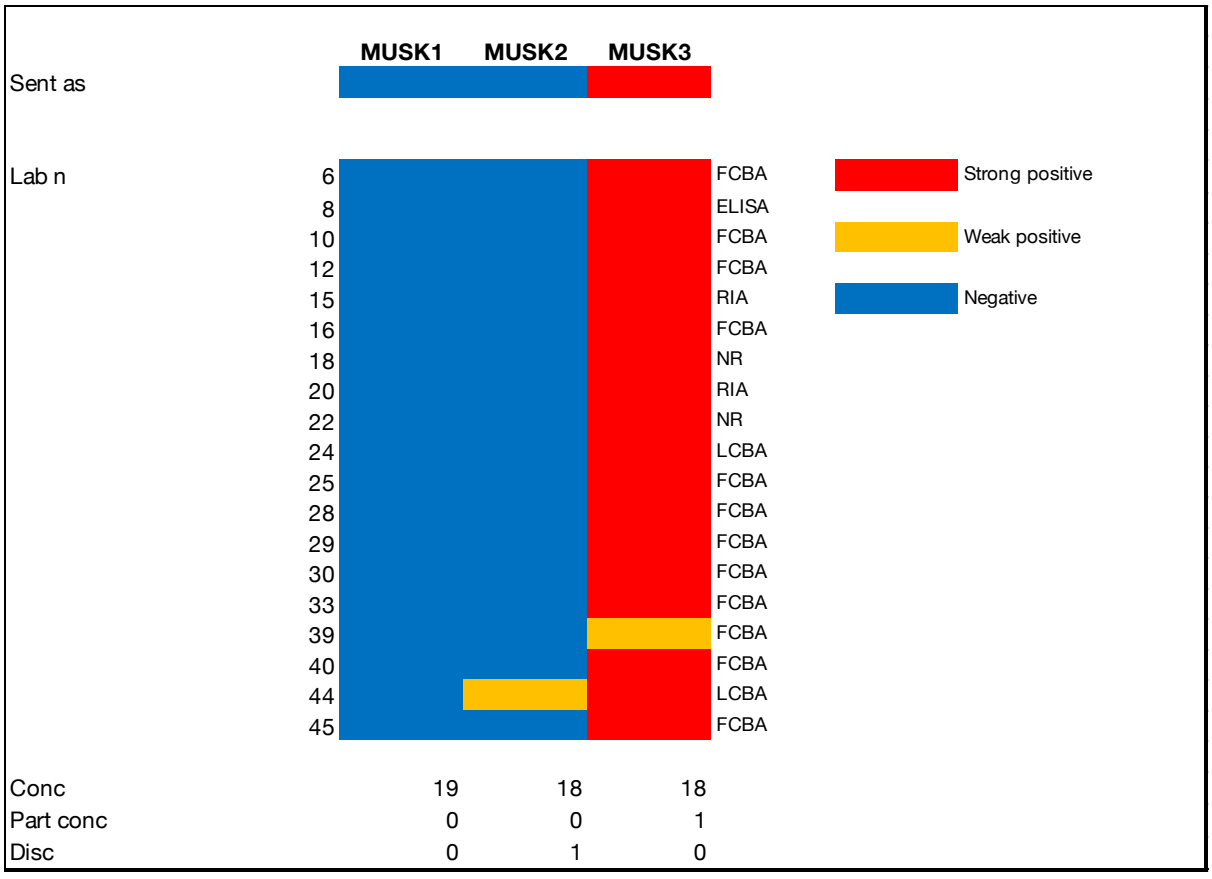
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

The assays used in this scheme largely reflect what has been described for the ACHR scheme. Again, results are excellent, with an overall accuracy of 98.2%. Only one laboratory reported a false positive in one sample using a LCBA.

Conclusions

The results of this EQAS point toward relevant issues in neuroimmunology laboratory diagnostics, especially concerning the AQP4, MOG and ganglioside antibody schemes. We hope that the data reported here might be useful to orient diagnostic strategies in the participating laboratories, but also to raise questions and favor discussion.

In the past years, AINI implemented two main strategies to address these issues. First, AINI organized specific theorico-practical courses focused on the laboratory diagnostics in neuroimmunology. Following this tradition, we are currently organizing the second 3-day course in December 2024 (Winter School of Laboratory Diagnostics in Neuroimmunology) that, by exploiting interactive teaching and practical activities on microscopes, will provide essential training to avoid common pitfalls in the routine diagnostic practice. This school, as many of AINI initiatives, is intended to attract both clinicians and people directly implicated in the laboratory diagnostics. More information will be available on the website www.aini.it.

Secondly, AINI has implemented the NINA-Flow project, a system for the referral of critical samples to specialized laboratories. This project, that is now active only for AQP4, MOG, ACHR and MUSK antibody diagnostics, will provide a tool to improve the diagnosis for patients with NMOSD, MOGAD and Myasthenia Gravis in Italy. In addition, we hope that this initiative will help to improve the performances of the participating laboratories. More information can be found on the website www.nina.aini.it.

We would like to thank all the Italian and European participants to this EQAS for their valuable contributions. Please feel free to contact us for any queries regarding the results addressed in this document, or to exchange samples for double-checking. We are also extremely happy to receive your complaints and suggestions to improve our EQAS, including potential additional assays that you would like to be evaluated.

See you next year!

Matteo Gastaldi
Diego Franciotta
Roberto Furlan

The NINA scientific Board
The AINI scientific Board

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Appendix: abbreviations

AINI: associazione italiana di neuroimmunologia
CBA: cell based assay
FN: false negative
FP: false positive
IIF: indirect immunofluorescence
NINA: Network Italiano Neurologia Autoimmune
TBA: tissue based assay
TN: true negative
TP: true positive