EQAS
EXTERNAL QUALITY ASSESSMENT SCHEME

AINI EQAS 2025

FINAL REPORT

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On behalf of the AINI Scientific Board
and NINA Scientific Board

ASSOCIAZIONE ITALIANA NEUROIMMUNOLOGIA

NINA Network Italiano Neurologia Autoimmune

Con il contributo incondizionato di

ALEXION AstraZeneca Rare Disease

argenx

ASSOCIAZIONE ITALIANA NEUROIMMUNOLOGIA

NINA Network Italiano Neurologia Autoimmune

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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”.

The results of the current EQAS have been presented during the annual AINI conference in Torino, 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.

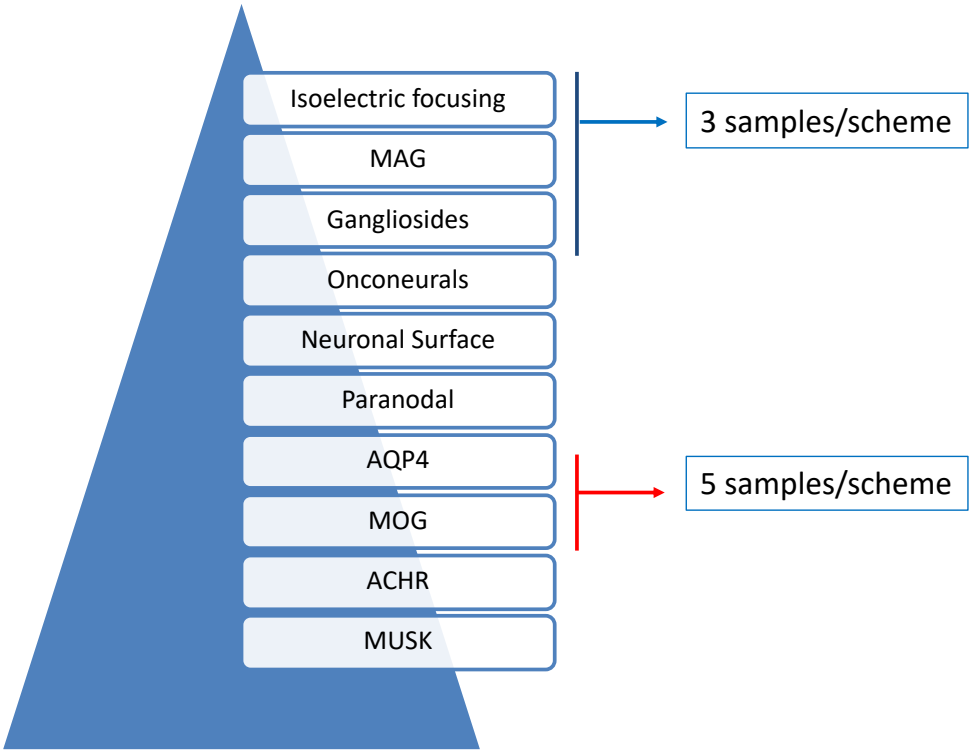
General Data of the 2025 AINI EQAS

The numbers of the 2025 AINI EQUAS

2022	2023	2024	2025
43 • Samples	44 • Samples	39 • Samples	39 • Samples
31 • Centres	41 • Centres	47 • Centres	41 • Centres
10 • Schemes	10 • Schemes	10 • Schemes	10 • Schemes

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 reduction in the number of participating laboratories from 47 to 41.

The schemes included in the 2025 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.

ASSOCIAZIONE ITALIANA NEUROIMMUNOLOGIA

NINA Network Italiano Neurologia Autoimmune

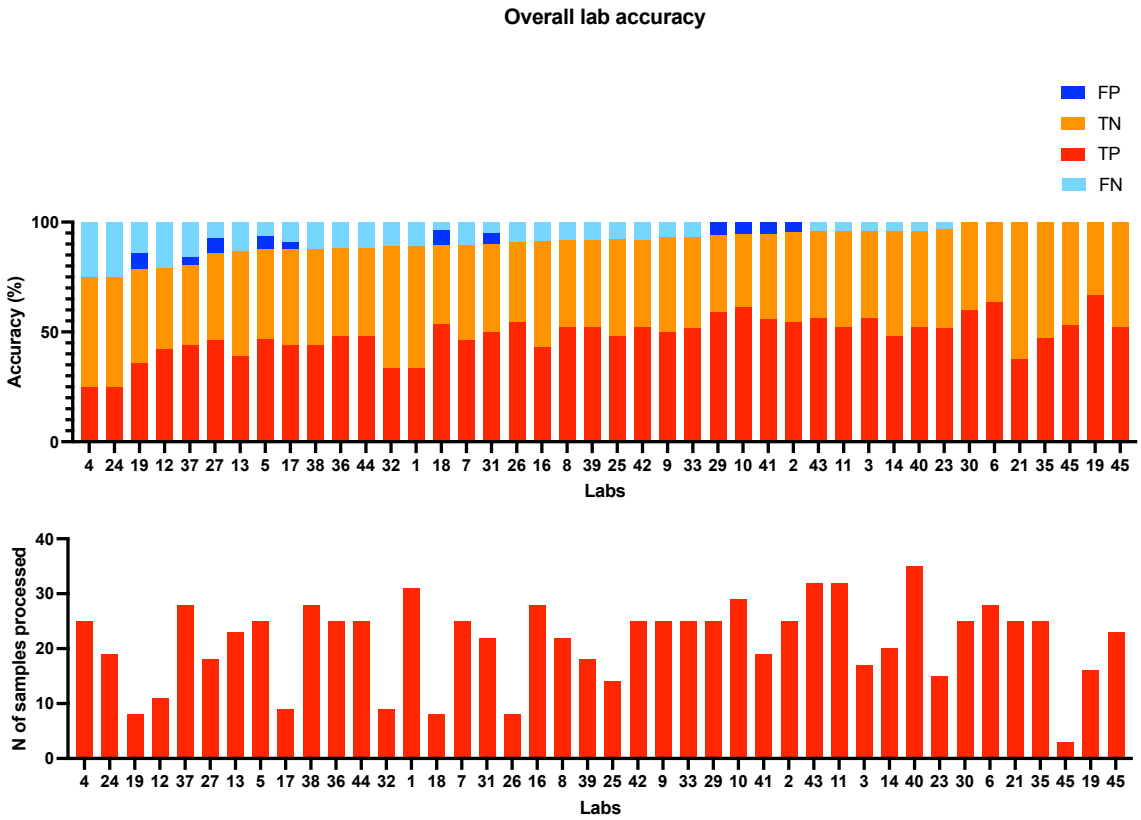
Participants to the AINI EQAS 2025

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***
SOS Patologia Clinica Santo Stefano Prato	Prato
Laboratorio patologia clinica	Merano
Institut de génomique fonctionnelle	Marseille
Hospital Clinic de Barcelona (Immunology Lab)	Barcelona
Laboratorio di Autoimmunità, Allergologia e Biotecnologie Innovative - Santa Maria Nuova AUSL IRCCS	Reggio Emilia
Laboratorio analisi e biochimica clinica Ospedale Sant'Andrea Roma	Roma
Laboratorio di assistenza e ricerca traslazionale. Azienda ospedaliero-universitaria Senese	Siena
Centro Sclerosi Multipla asl 8 Cagliari	Cagliari
Laboratorio analisi	Modena
Laboratorio Diagnostica Neuroimmunologica-Ist. Neurologico Besta	Milano
Laboratorio Autoimmunità SC Analisi AOU Alessandria	Alessandria
Laboratorio di Neuropatologia, AOUI e Università di Verona	Verona
Lab diagnostica liquorale-Clinica neurologica-IRCCS Policlinico San Martino	Genova
Division of Neuropathology and Neurochemistry, Department of Neurology, Medical University of Vienna	Vienna
S.C. Laboratorio Analisi San Giovanni Bosco - ASL Città di Torino	Torino
Laboratory of Autoimmunity, ASU FC	Udine
UOC Medicina di Laboratorio Azienda Ospedale-Università Padova	Padova
Laboratorio Analisi Chimico Cliniche ed Ematologiche AOVR	Vicenza
Programma patologia neuromuscolare e neuroimmunologia- IRCCS istituto scienze neurologiche di Bologna	Bologna
Laboratorio Neurobiochimica Clinica e Biobanca, IRCCS Fondazione Santa Lucia	Roma
neurobiologia san luigi	Orbassano
SC Laboratorio di Patologia Clinica ASST Papa Giovanni XXIII	Bergamo
Laboratorio Autoimmunologia - S.C. Laboratorio Analisi ASL1 Imperiese	Imperia
Laboratorio EUROIMMUN Italia	Padova
DOMP-LABORATORIO DI NEUROBIOLOGIA-SC NEUROLOGIA-OMV	Torino
Laboratorio Diagnostico di Autoimmunologia, IRCCS Ospedale Policlinico San Martino	Genova
UOC Patologia Clinica-Dipartimento di Igiene e Medicina Valutativa-AOU San Giovanni di Dio e Ruggi d'Aragona	Salerno
UOC Patologia Clinica - Azienda Ospedaliero Universitaria di Ferrara	Ferrara
Laboratorio di Immunopatologia, AOR San Carlo	Potenza
Lab. Autoimmunità Ospedale San Raffaele	Milano
LABORATORIO PATOLOGIA CLINICA AOU SAN GIOVANNI DI DIO E RUGGI D'ARAGONA	Salerno
U.O.C. Medicina di Laboratorio aulss 8	Vicenza
Ospedale Humanitas	Milano
laboratorio generale AOU careggi	Firenza
Laboratorio Patologia Clinica, Azienda Ospedaliero Universitaria Pisana	Pisa
U.O.C.PATOLOGIA CLINICA - OSPEDALE SAN FILIPPO NERI - ASLROMA1	Roma
U.O.S. Immunologia Clinica, LUM AUSL BO	Bologna
Laboratorio di Patologia Clinica "Santissima Annunziata"	Taranto
CISMed-DMA University of Trento	Trento
Laboratorio di Patologia clinica	Sassari

Results' summary

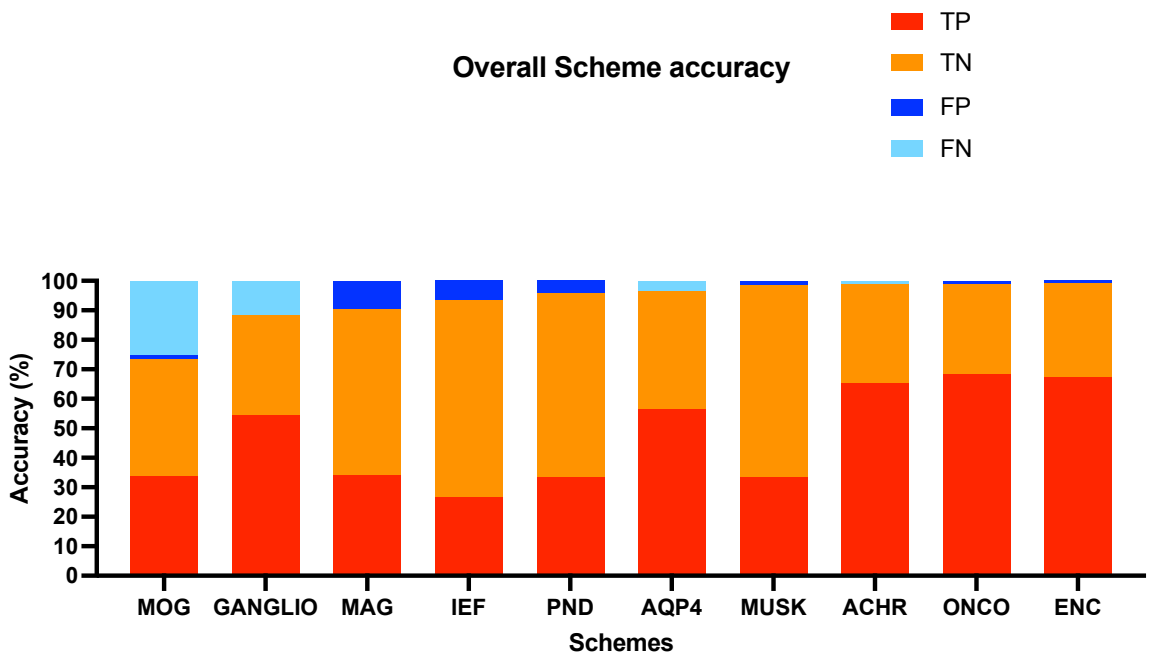
Overall accuracy of the laboratories



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 comaprable to that of the last years' EQAS, as only 41% of the laboratories has shown values ≥90%. 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. Two schemes (MOG and GANGLIO) had an accuracy lower than 90% and were considered critical. In addition, AQP4 was considered critical as well (see detailed scheme report). 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.

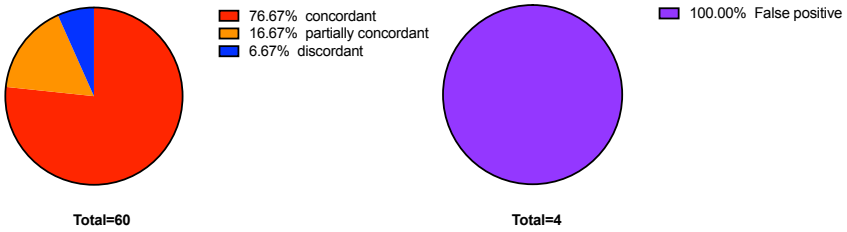
Isoelectric focusing (IEF) scheme

Participants: 16
Samples: 4 sera+4 cerebrospinal fluids (pairs)
Judgment: satisfactory

Results

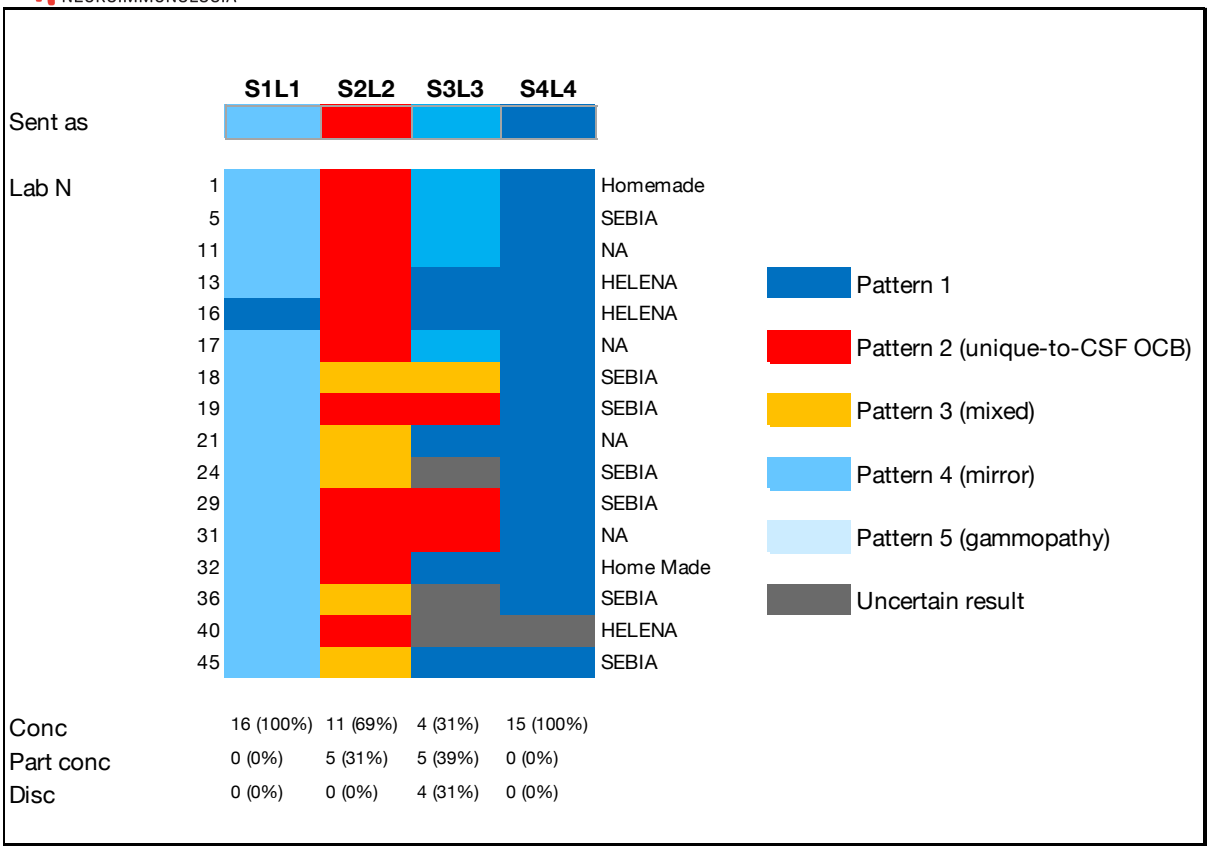
Overall concordance of all tests performed

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 93.3%, and the only inaccurate results were four false positives. All laboratories were able to detect CSF OCBS in the only positive sample (S2L2), even though distinction between pattern 2 and pattern3 was difficult, as observed in previous EQAS. The False positives derive from the misinterpretation of a pattern 4 (mirror), rather common in the population and not indicative of a CNS compartimentalized immune response, with a pattern 2, indicative of intrathecal IgG synthesis. The overall results represent an improvement compared to those critical reported in the past years.

AQP4 antibody scheme

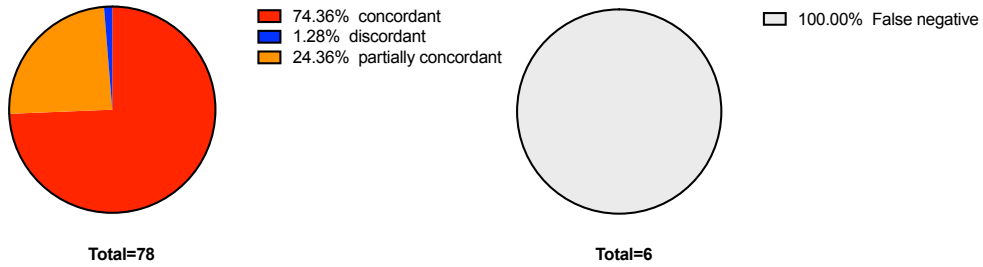
Partecipants: 34
Samples: 5 (3 strong positive, 2 negative; all positive samples were positive on both in-house LCBA and commercial FCBA in the reference laboratory)
Judgment: critical

Methods		
Assay	N of centres	Description
LCBA	2/34(5.8%)	Live cell based assay with M23 AQP4 isoform; assessment with fluorescent microscope or flow cytometry (in-house)
FCBA	32/34 (94.2%)	commercial fixed CBA

Results

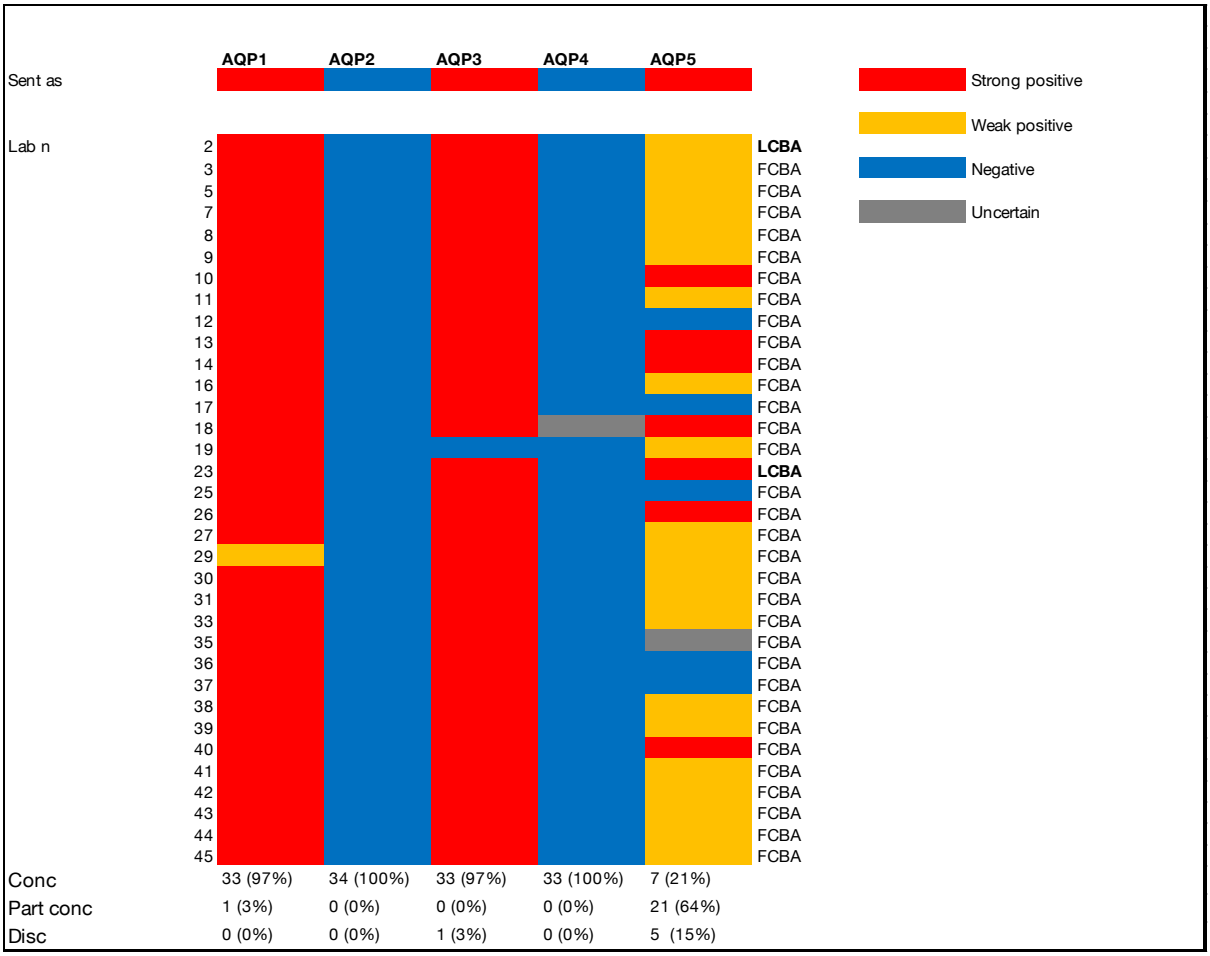
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

The overall accuracy was very high (98.72%). However, this scheme was still considered critical due to the presence of 6 false negative results. There were obtained by 6 different laboratories with samples #2 and #5, that were sent as clear positive. Notably, the two laboratories performing live CBAs correctly identified the positive samples. However, since most laboratories used a commercial fixed CBA and correctly identified the positive samples, it is unlikely that the inaccurate results depend on issues related to the assay.

MOG antibody scheme

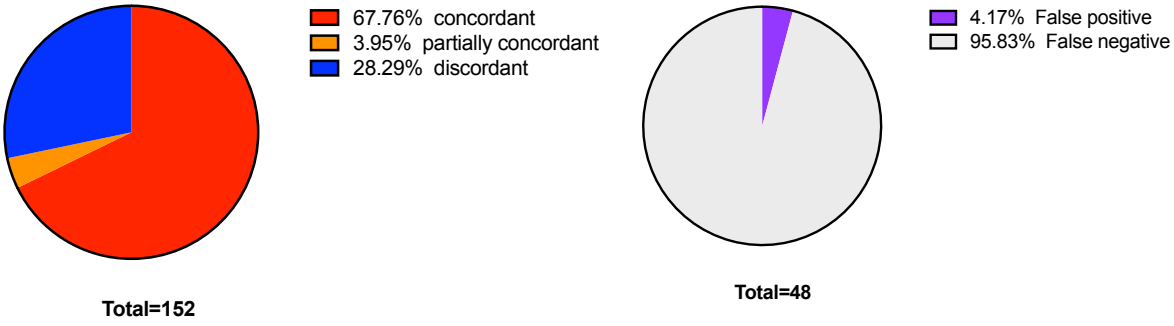
Partecipants: 31
Samples: 5 (2 strong positives, 1 weak positive, 2 negative; all positive samples were positive on both LCBA for total IgG, LCBA for IgG1, and FCBA in the referral laboratory; all patients fulfilled the current diagnostic criteria for MOGAD)
Judgment: highly critical

Methods		
Assay	N of centres	Description
LCBA	4/31 (12.9%)	Live cell-based assay with human full length MOG isoform (in-house).
FCBA	27/31 (81.1%)	Commercial fixed cell-based assay with full length human MOG isoform; human anti-Fc total IgG secondary ab; assessment with fluorescence microscopy

Results

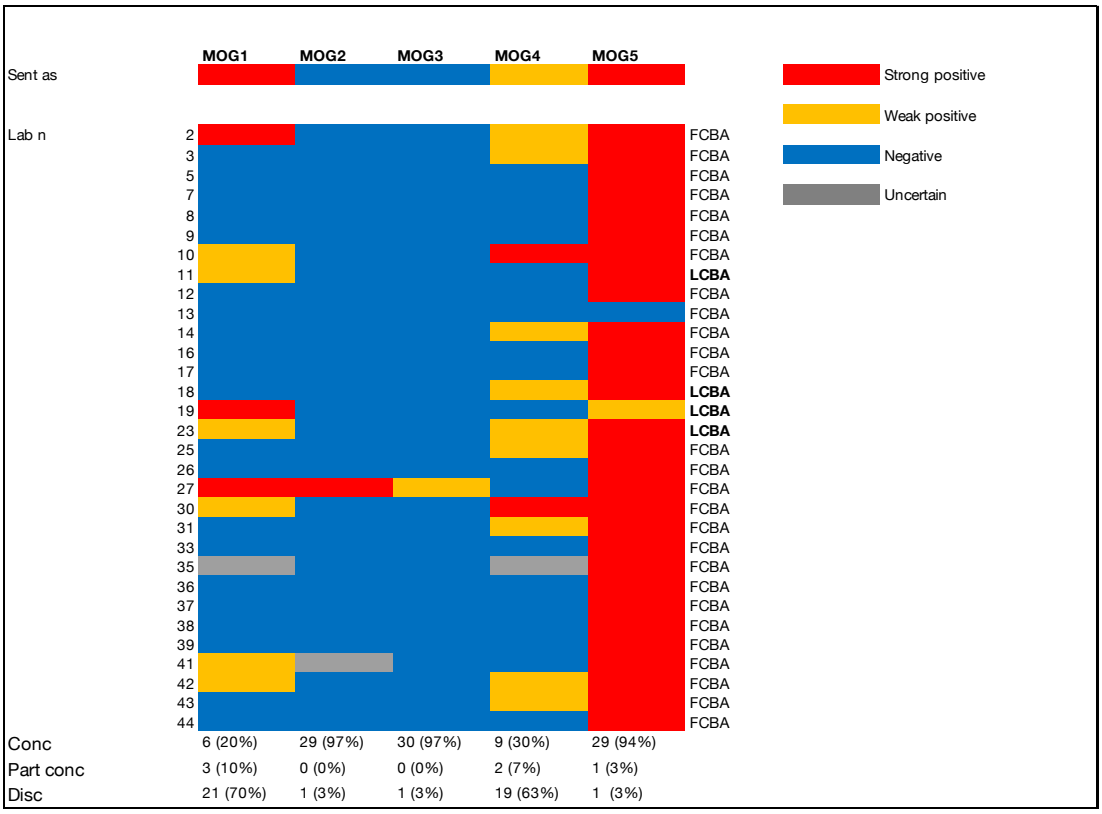
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

The overall accuracy was 71.6%, making this scheme the one with the worst performance in the entire EQAS. Most critical results were false negatives related to the difficulty in identifying sample#1 (a clear positive) and sample #4 (a weak positive). Similarly to the AQP4 scheme, several laboratories using commercial fixed CBA correctly identified all of the positive samples, confirming that the performance was not only affected by the type of assay used. Notably, ¾ laboratories using a live CBA did not identify at least one of the positive samples, highlighting how, despite being the gold standard for MOG-IgG detection, the standardization of live CBAs used in the routine diagnostic is still critical.

Intracellular neuronal antibody scheme

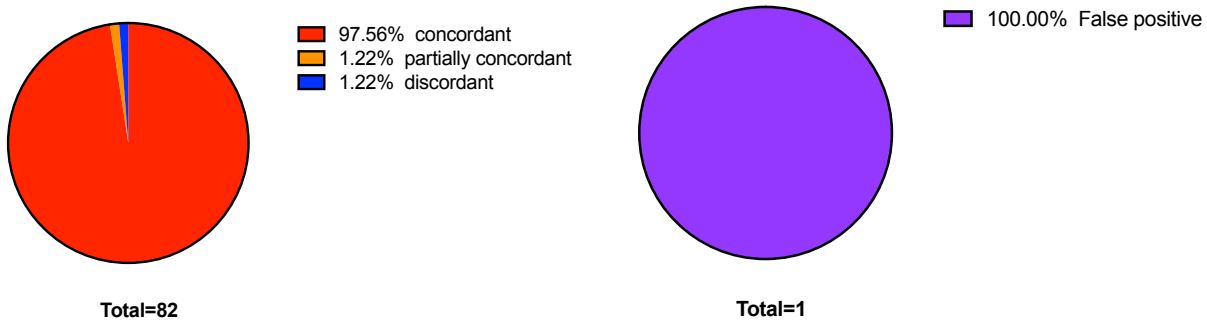
Partecipants: 28
Samples: 3 (1 positive for GAD, 1 positive for Yo, and 1 negative)
Judgment: satisfactory

Methods		
Assay	N of centres	Description
TBA+line blot	20/28 (71.4%)	Included different type of commercial or in-house TBA
Line blot only	7/26 (26.9%)	Included different commercial line blots
Unknown	1/28 (3.6%)	-

Results

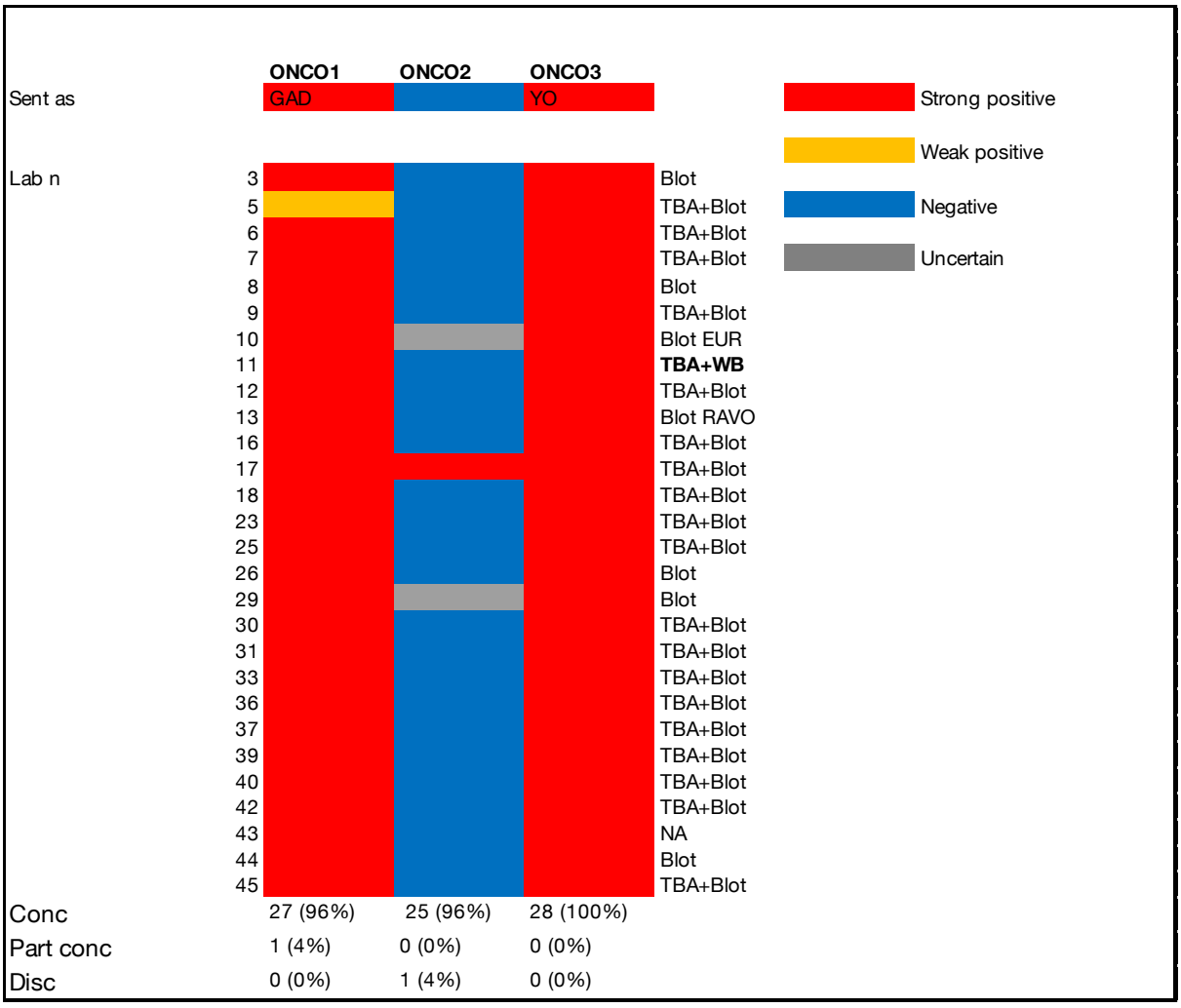
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

The performance was excellent, as the overall accuracy was 98.8%. Only one laboratory identified a single false positive result. Importantly, even though the AINI guidelines recommend the use of line blots in association with a TBA, 26.9% of the laboratories still relies on line blots alone, therefore increasing the risk of false positive. We strongly recommend the use of TBA in association to line blots.

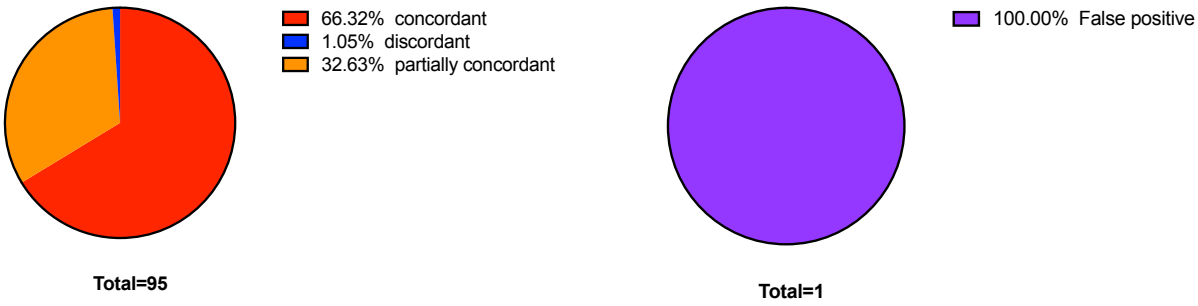
Neuronal surface antibody scheme

Partecipants: 32
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	31/32 (3.1%)	Commercial panel; two labs performed TBA in association
LCBA	1/32 (96.9%)	CBA performed for each antigen separately

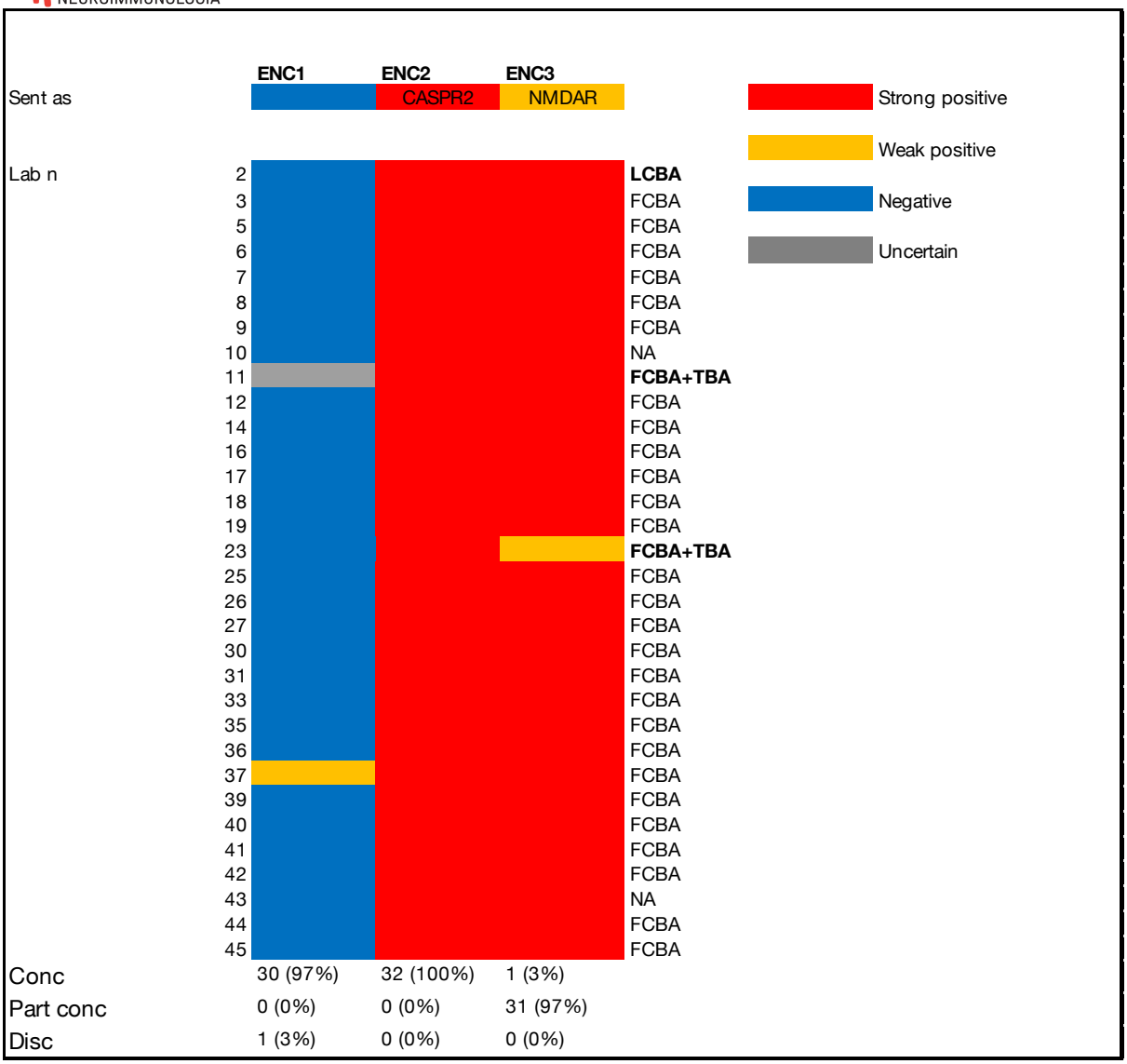
Results

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 positive”. Overall the scheme was considered satisfactory.

Ganglioside antibody scheme

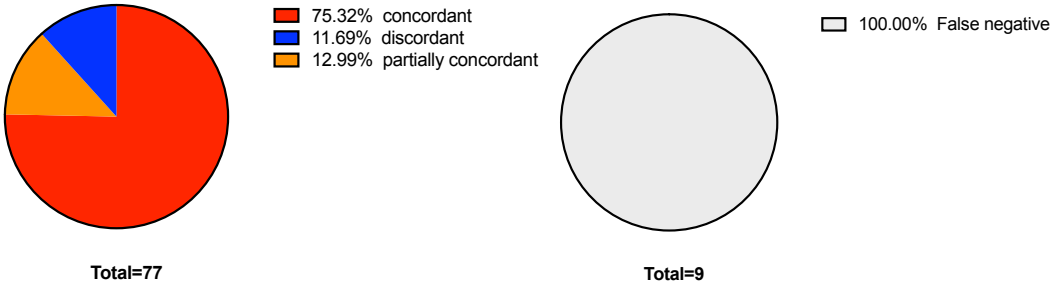
Partecipants: 26
Samples: 3 (1 GM1 IgG positive, 1 GM1 + GD1b IgG positive, 1 negative)
Judgment: highly critical

Methods		
Assay	N of centres	Description
Immunoblot	17/26 (65.4%)	This included different brands of immunoblots
ELISA	6/26 (23.1%)	ELISA: Buhlmann in 5 labs, home made in 1 lab
Unknown	3/26 (1.5%)	-

Results

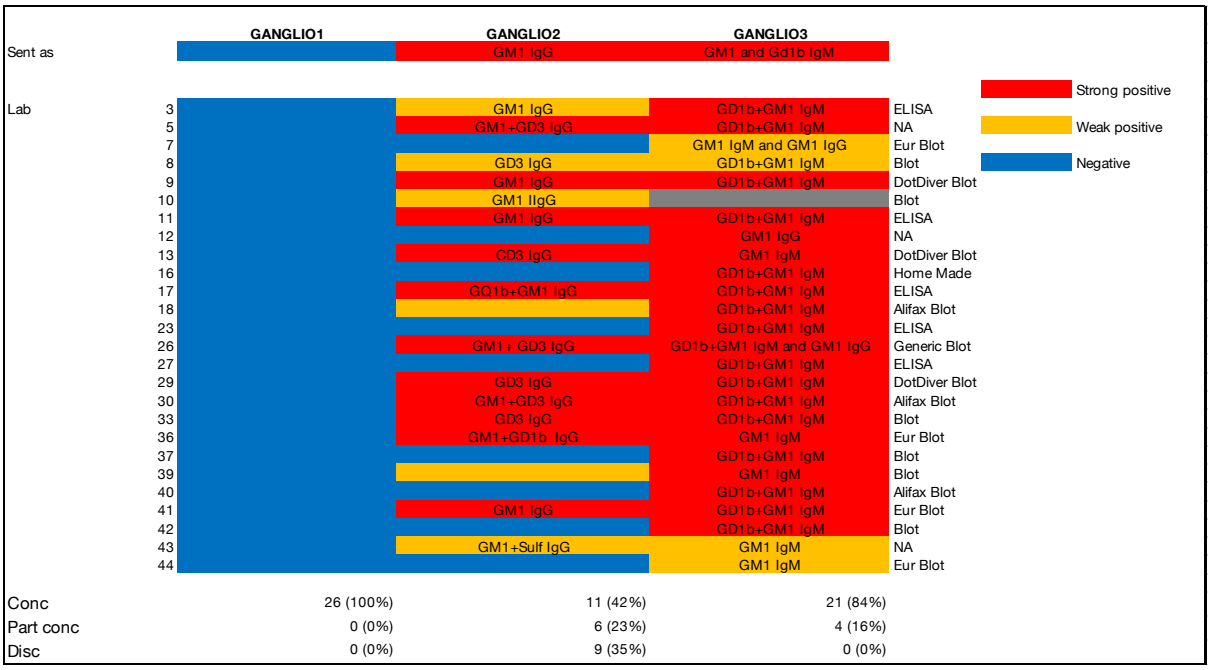
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

The overall accuracy was 88.31%. However, this was only calculated when considering a positive/negative result. When considering the ganglioside reactivity identified, a high heterogeneity was detected among laboratories, suggesting tha the reproducibility of the assay is low. Most of the frankly discrepant results were false negatives obtained with sample#2. Notably, no clear difference was detected in the performance according to the type of assay used. The recommended assay by the AINI guidelined, the ELISA was used only by the 23.1% of laboratories, while the most common assay was the blot.

MAG antibody scheme

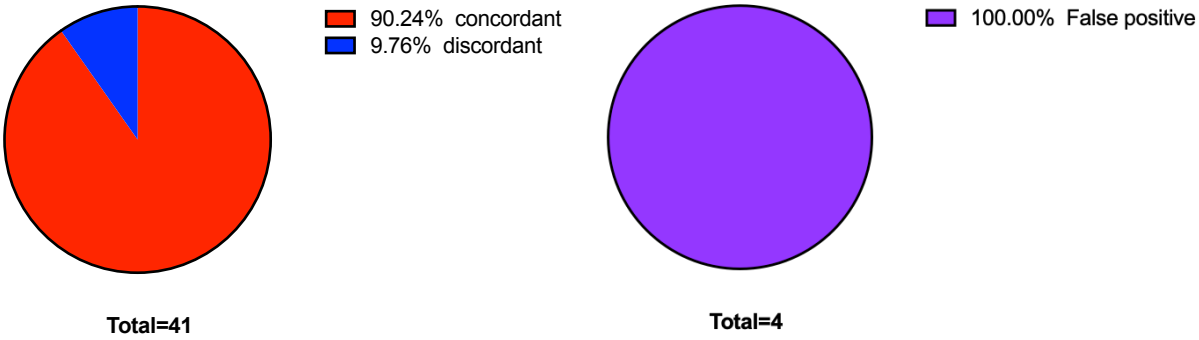
Partecipants: 15
Samples: 3 (1 strong positive, 2 negative)
Judgment: satisfactory

Methods		
Assay	N of centres	Description
ELISA	11/15 (73.3%)	In one case associated with TBA
IIF	4/15 (26.7%)	Indirect immunofluorescence on sciatic nerve

Results

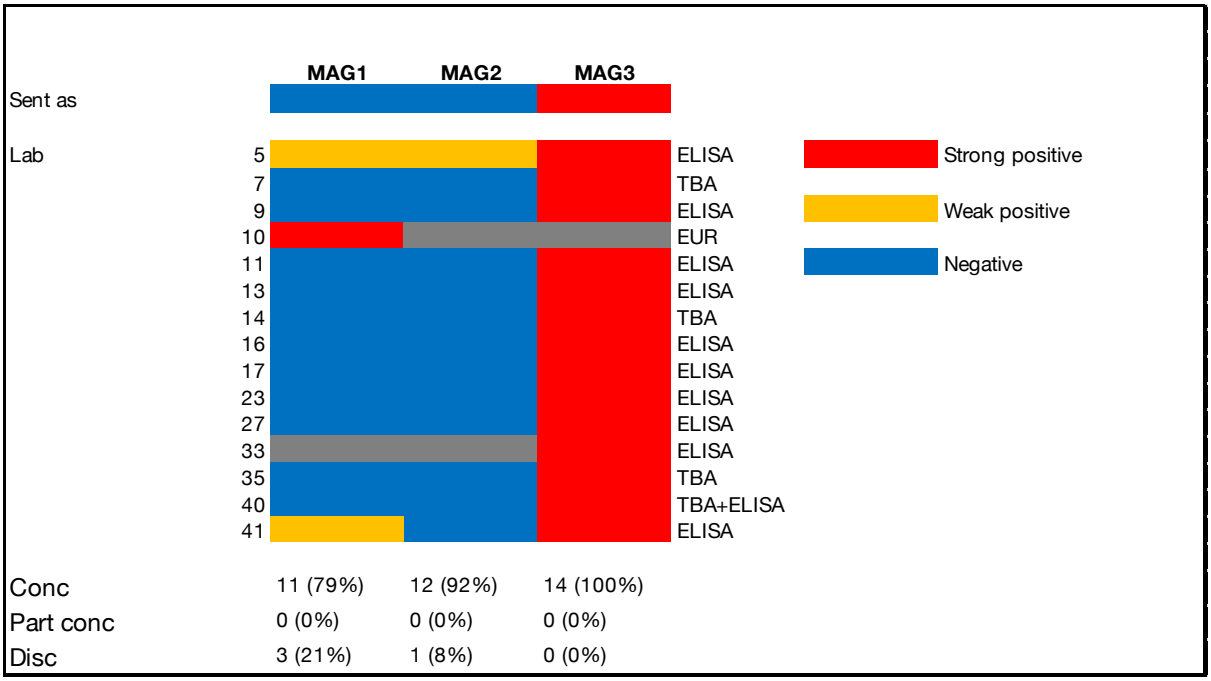
Overall concordance of all tests performed

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 90.2%. Two laboratories reported as positive a sample sent as “negative”. Notably, these results occurred in laboratories using both the IIF and the ELISA.

Paranodal antibody scheme

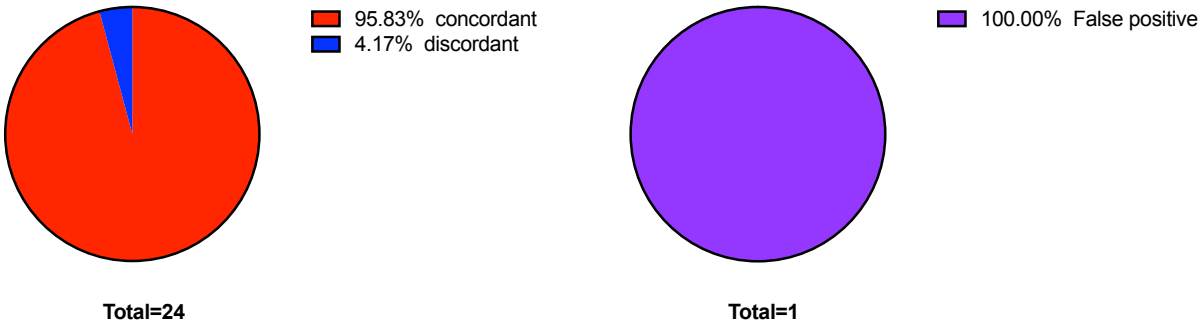
Partecipants: 8
Samples: 3 (1 CNTN1 positive, 2 negative)
Judgment: satisfactory

Methods		
Assay	N of participants	Comments
FCBA	4/8 (50%)	-
Other	4/8 (50%)	3 ELISA+LCBA+TBA; 1 LCBA only

Results

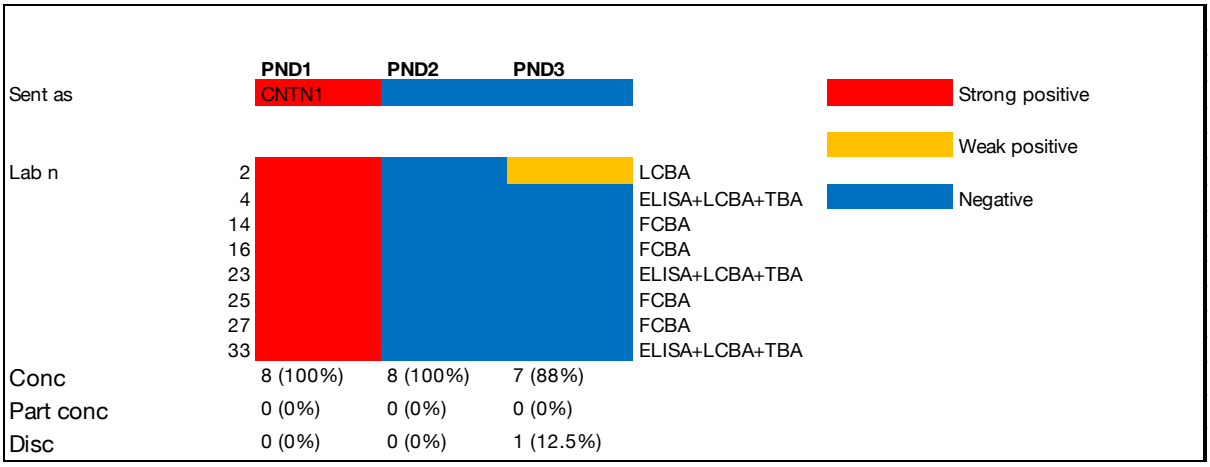
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

The overall accuracy was 95.8%, and only one laboratory performing LCBA only identified a weak positive in a sample sent as negative.

Nicotinic acethylcholine receptor antibody scheme

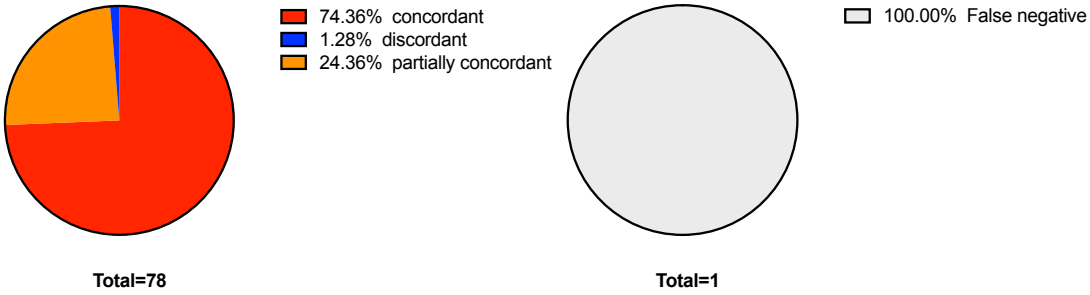
Partecipants: 26
Samples: 3 (1 low positive, 1 strong positive, 1 negative)
Judgment: satisfactory

Methods		
Assay	N of centres	Description
RIA	2/26 (7.7%)	Commercial RIA
FCBA	17/26 (65.4%)	-
ELISA	5/26 (19.2%)	-

Results

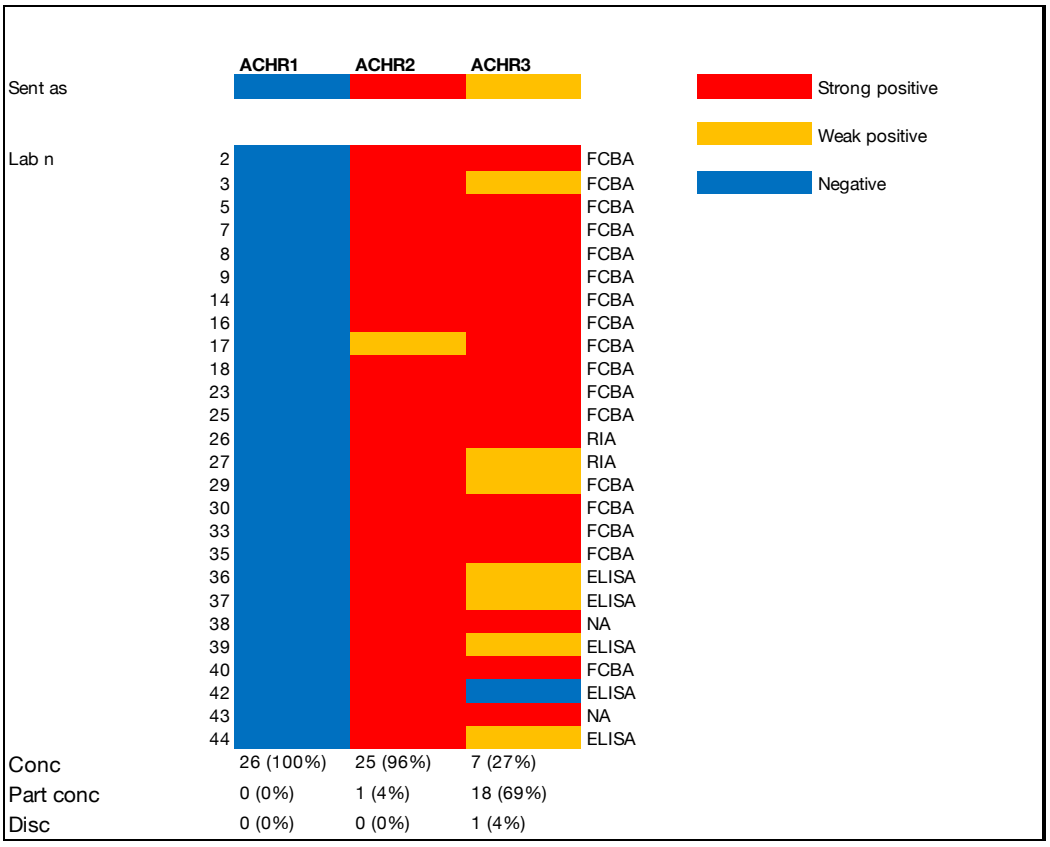
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

The accuracy was very high, with only one laboratory providing a false negative result with a weak positive. Notably, this false positive was provided by a laboratory performing an ELISA, which usually has a lower overall accuracy compared to CBAs. The increased use in this scheme of ELISAs compared to last year (now 19.2% of the laboratories) warrants caution: the labs should be aware of the possibilities of both false negatives and false positives with this assay.

MUSK antibody scheme

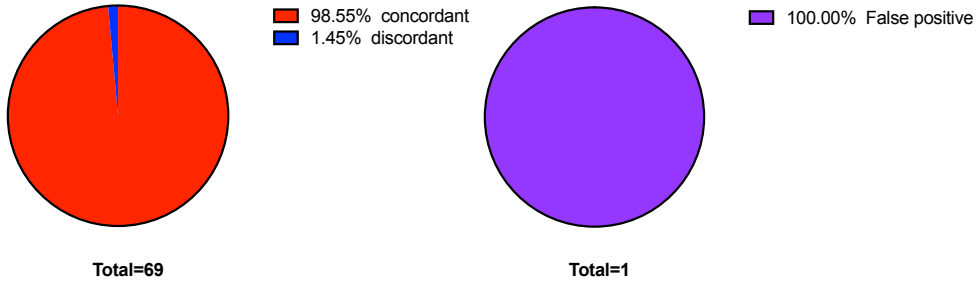
Partecipants: 23
Samples: 3 (1 strong positive, 2 negatives)
Judgment: satisfactory

Methods		
Assay	N of centres	Description
FCBA	16/23 (69.6%)	-
RIA	1/23 (4.3%)	Commercial RIA
LCBA	1/23 (4.3%)	-
ELISA	1/23 (4.3%)	-

Results

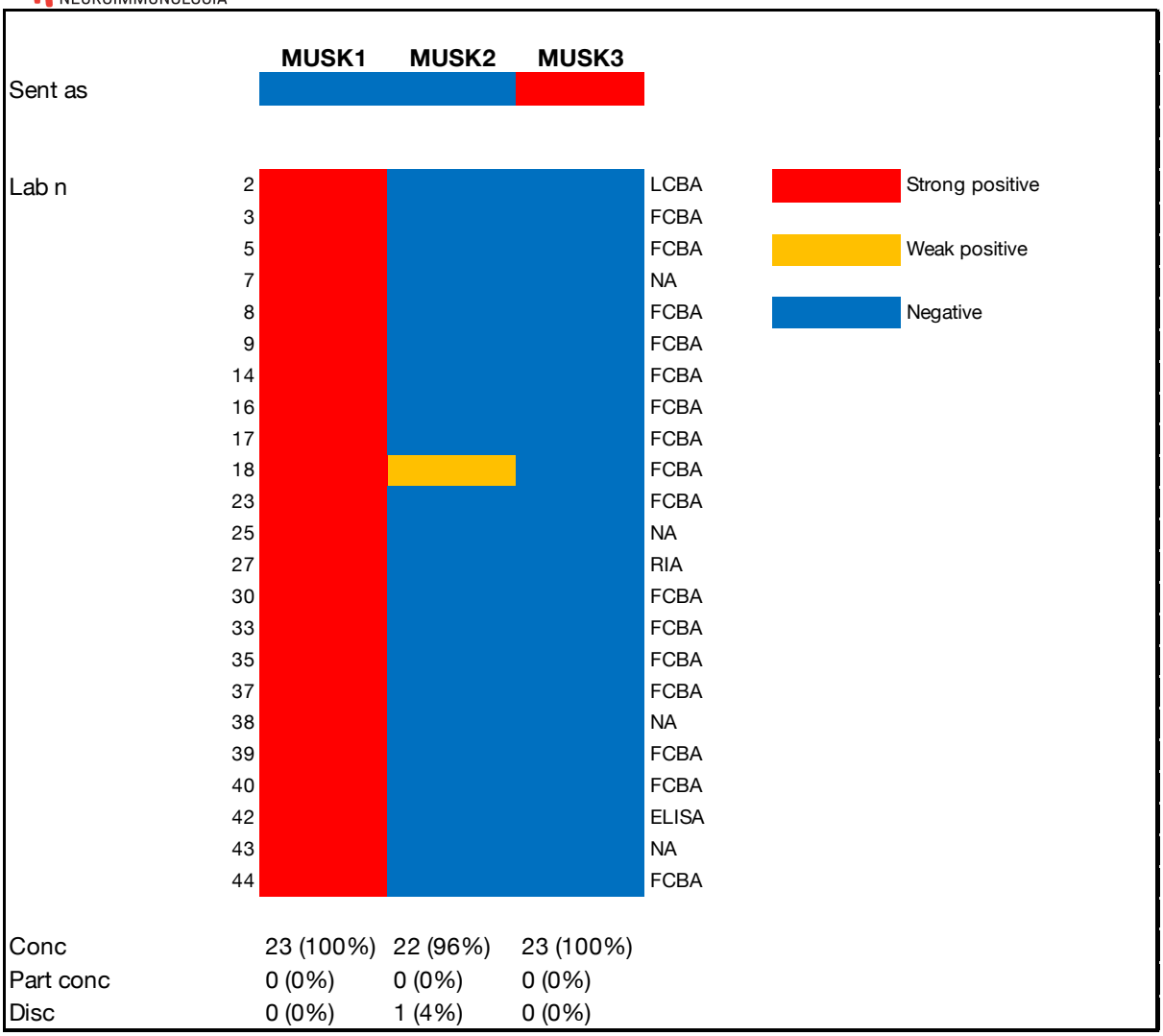
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

The overall accuracy was very high (98.5%). Only one laboratory provided a false positive result.

Conclusions

The results of this EQAS point toward relevant issues in neuroimmunology laboratory diagnostics, especially concerning the MOG, AQP4 and ganglioside antibody schemes. These schemes were judges as critical also in the 2024 EQAS, suggesting that problems especially related to the test interpretation (at least in AQP4 and MOG) are still impacting the routine diagnostic.

To address this, in the past years, AINI implemented two main strategies.

First, AINI organized specific theorico-practical courses focused on the laboratory diagnostics in neuroimmunology. Following this tradition, we are currently organizing the third 3-day course in January 2026 (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. 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. 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

Acknowledgments

A special thanks to Elisabetta Zardini, Silvia Scaranzin, Chiara Morandi and Stine Overdall for all the work and long hours put onto the planning and realization of this EQAS, and to Prof. Paddy Waters of Oxford for the constant support and guidance on standardization issues.
Finally, we thank *Alexion* and *argenx* for the unconditional support to this initiative.

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