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Network Italiano
Neurologia Autoimmune

AINI EQAS 2022

FINAL REPORT

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ON BEHALF OF THE AINI SCIENTIFIC BOARD AND RIN SCIENTIFIC BOARD

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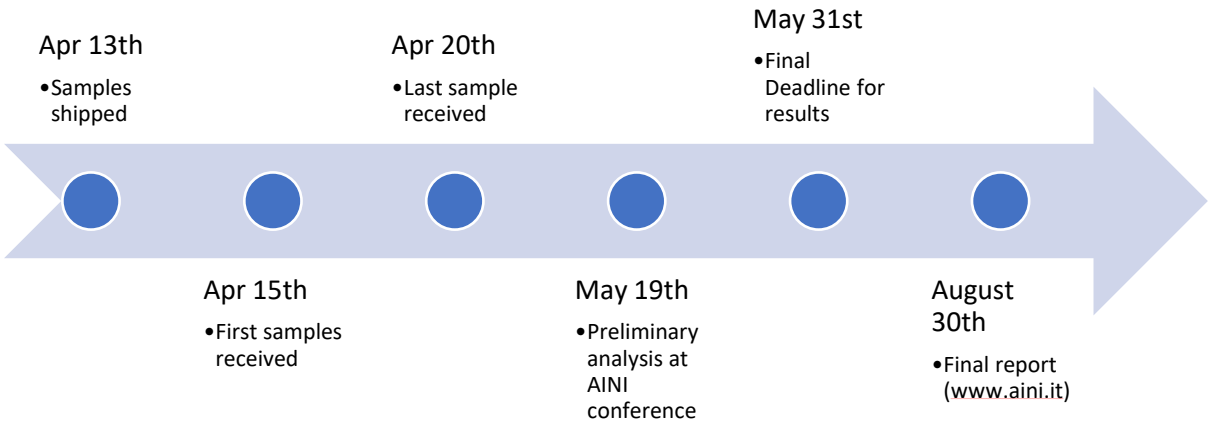
Introduction

As every year, the Italian Association of Neuroimmunology (AINI), in cooperation with the "Rete degli IRCCS di Neuroscienze" (RIN), has organized an External Quality Assessment Scheme (EQAS) to promote standardization in neuroimmunology laboratory diagnostic in Italy. In the evolving scenario of the neuroimmunology diagnostic, these schemes are an essential tool to promote self-evaluation, to highlight critical assays and to identify issues to tackle to improve laboratory diagnostic.

The philosophy of the AINI EQAS so far, has not been to provide a certification of accuracy to the participating labs, but mostly to work as a community to improve our diagnostic level. Considering the relevant clinical implications of the testing we perform, we must be able to provide reliable feedback to clinicians, even if that means recognizing the weakness of specific assays. The comparison with the reference result (the one codified as "sent as.." in the following report) should always be interpreted cautiously, and not necessarily looked at as a gold standard. Indeed, the definition of "true positive", in the absence of a proper gold standard is extremely tricky. In addition, mistakes are always possible, both from the participating and coordinating labs. As all the people involved in lab diagnostics know very well, the jungle of pre-analytical, analytical and post-analytical error is deep and full of traps. We hope that the results presented in this report will be of help to the participating labs, as well as the AINI community.

General Data of AINI EQAS 2022

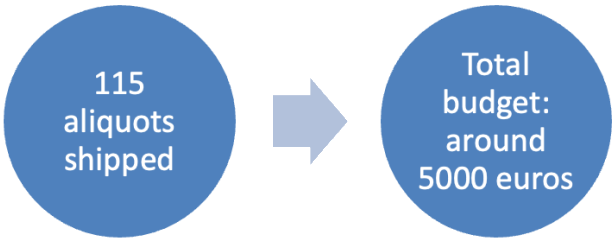
The timeline of the 2022 AINI EQAS



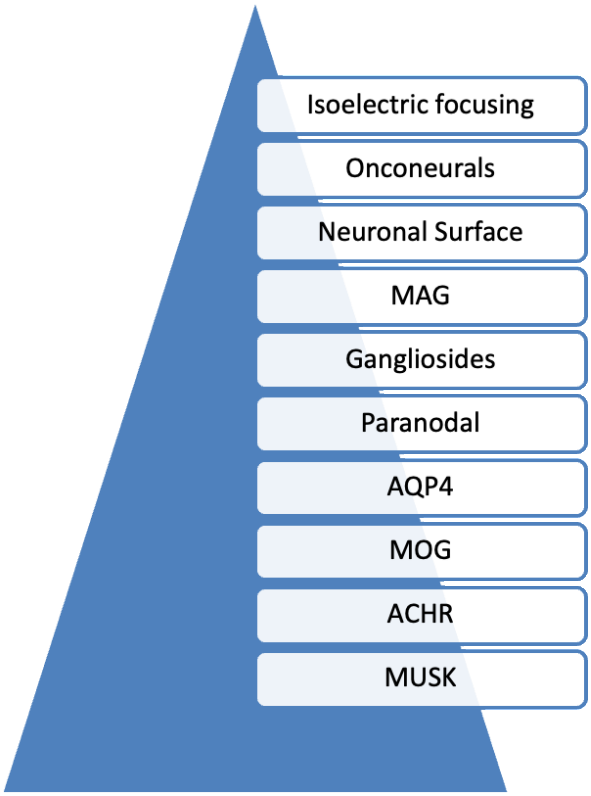
This year the samples shipment has been delayed due to issues in the organization. This led to an unfortunate choice, as samples were shipped right before the Easter weekend, with obvious delays, and we apologize for this inconvenience. Many of the labs participating pointed to us that samples had been shipped at room temperature. This has been standard procedure for the AINI EQAS for many years, and the choice is mainly due to the limited funding available for the EQAS. Next year, we will ship all samples in wintertime, when the outdoor temperatures are more favorable to this purpose. To address potential issues with samples testing after being exposed to room temperature for several days, we prepared an additional aliquot that was retested at the coordinating lab after 10 days exposure at room temperature, ensuring that the reactivity was not altered. Despite this modest proof of concept, however, we cannot exclude that some of the results obtained in this EQAS might have been influenced by storage conditions.

The numbers of the 2022 AINI EQUAS

43	• Samples
31	• Centres
10	• Schemes



The schemes included in the 2022 AINI EQAS



This year we included all the schemes of our previous EQAS, with 2 important modifications. First, as a novelty, we included the paranodal antibody scheme. Paranodal antibodies (NF155, NF186, CNTN, CASPR1) are becoming increasingly important in the laboratory diagnostic of peripheral neuropathies. However, paranodal antibody testing is only available in a few specialized labs, and little data are available on assay standardization. Secondly, we potentiated the AQP4, MOG, ACHR and MUSK schemes, increasing the number of samples to 5. All these antibodies have entered the clinical practice several years ago, but the diagnostic field is now modifying due to the introduction of novel assays, such as the commercial cell based assay for MOG, ACHR and MUSK. Considering the epidemiological relevance of Neuromyelitis Optica spectrum Disorders (NMOSD), MOG associated disorders (MOGAD) and Myasthenia Gravis, we decided to investigate this area more deeply, to understand whether corrective measures are needed.

Participants to the AINI EQAS 2022

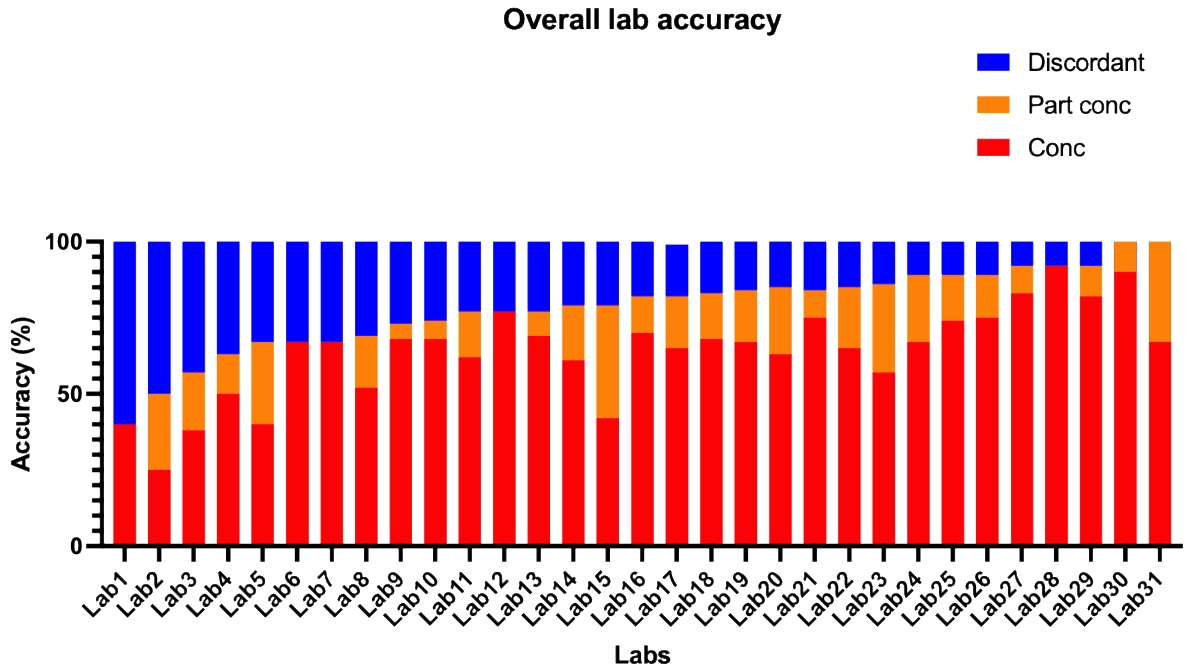
As in the previous editions, along with a long list of Italian collaborators that have participated to the EQAS for several years, we invited to contribute several labs from all around Europe. The contribution of this labs is extremely valuable, as allows our community to compare with some of the most relevant laboratories in the field.

Here is a list of the 31 centres participating to the EQAS

Foggia	Azienda Ospedaliero Universitaria OO RR di Foggia, Laboratorio Analisi Centrale,	Michele Falcone
Innsbruck	Neurological Research Laboratory, Dept. of Neurology**,** Medical University of Innsbruck	Markus Reindl
Gallarate	Laboratorio Analisi P.O. Gallarate. ASST Valle Olona	Pettini Paola/ Sferrazzo Annarita
Milano	SSD medicina di Laboratorio SMEL 122, **Isituto Besta**	Elena Corsini
Pisa	Laboratorio di Neurobiologia Clinica e diagnostica Liquor**, Ospedale Santa Chiara**	Andrea Bacci
Taranto	Patologia Clinica PO SS. Annunziata, via F. Bruno n.1	Tampoia Marilina, Notaristefano Norma
Firenze	laboratorio generale AOU careggi	Tiziana Biagioli
Padova	UOC Medicina di Laboratorio, DIDAS Servizi di Diagnostica Integrata, Azienda Ospedale-Università Padova	Giulia Musso, Nicoletta Gallo
Trento	Laboratorio di Diagnostica Molecolare Avanzata (CIBIO-DMA)	Valentina Greco
Modena	**Laboratorio di neuroimunologia, Ospedale Baggiovara**	Roberta Bedin
Monza	Laboratory analysis ASST Monza San Gerardo	Cappellani Adele
Bologna	IRCCS Istituto delle Scienze Neurologiche	Maria Pia Giannoccaro
Prato	Laboratorio Analisi**, Ospedale di Prato**	Annalisa Azzurri
Roma	UOC Laboratorio Analisi e Biochimica Clinica Ospedale Sant'andrea di Roma	Vittoria Polidori
Bari	Neurochemistry Lab -University of Bari	Maddalena Ruggieri -Claudia Palazzo
Genova	Autoimmunology Laboratory, San Martino Hospital, Genoa, Italy	Diego Franciotta
Lione	Centre de Recherche en Neurosciences de Lyon	Romain Marignier / Anne Ruiz
Vicenza	Laboratorio di Neurobiologia **, Ospedale san Bortolo**	Luigi Zuliani
Milano	Laboratorio Neuroimmunologia**,** Ist. Neurologico Besta	Francesca Andreetta
Vienna	Koneczny lab, Division of Neuropathology and Neurochemistry, Department of Neurology**,** , Medical University of Vienna	Inga Koneczny
Milano	Laboratorio analisi**, Ospedale San Raffaele**	Stefania Del Rosso
Gallarate	LABORATORIO ANALISI- **,** ASST VALLE OLONA- P.O. GALLARATE	Dott.ssa Paola Pettini - Dott.ssa Annarita Sferrazzo
Vienna	Division of Neuropathology and Neurochemistry, Department of Neurology**,** , Medical University of Vienna	Romana Höftberger
Milano	**Laboratorio autoimmunità, Isituto Humanitas**	Claudia Giannotta
Catania	Laboratorio Analisi**, ARNAS Garibaldi**	DI PROSSIMO MARIA ELENA
Barcellona	Unità di Neurologia Autoimmune**, Hospital San Pau**	Cinta Lleixà / Luis Querol
Bologna	LUM AUSL BOLOGNA	TANIA SILVESTRI
Orbassano	SCDO NEUROLOGIA-CENTRO SM- ORBASSANO	SALA ARIANNA
Verona	Neurology and Neuropathology Unit, University of Verona	Sara Mariotto
Oxford	Neuroimmunology laboratory**, John Radcliffe Hospital**	Paddy Waters
Marseille	Centre de Recherche en Neurobiologie et Neurophysiologie de **Marseille**	Jerome Devaux

Results summary

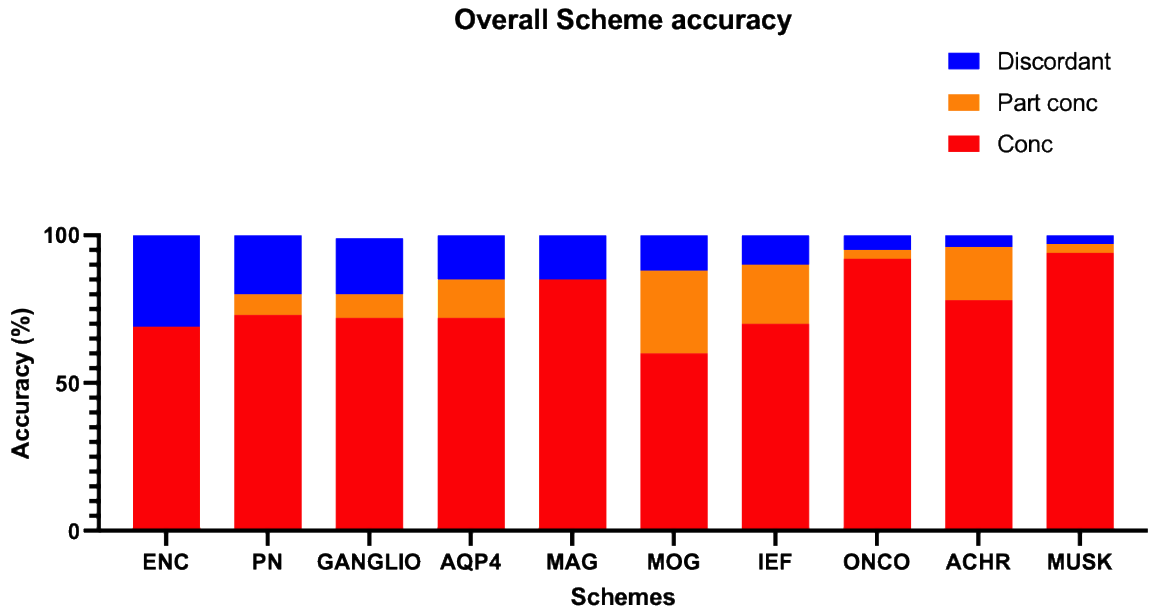
Overall accuracy of the laboratories



Overall accuracy can be estimated according to the % of samples tested that were concordant or at least partially concordant with the reference result established according to the coordinating lab results. These results are reported in detail for each scheme. The term “partially concordant” was applied to samples providing a weak positive with samples sent as strong positive or vice versa. Such differences have clearly lower practical impact, and therefore should not be considered as crucial.

Half of the laboratories had an accuracy over 50%, and only 4 above 90%. However, we have to bear in mind that these results do not take into account the number of schemes to which each lab participated.

Overall accuracy of the schemes



In the graph are represented the performances in the 10 schemes of the EQAS. ENC= Neuronal surface antibodies; PN= paranodal antibodies; GANGLIO= ganglioside antibodies; IEF= isoelectric focusing; ONCO= intracellular neuronal antibodies.

There is no clear threshold to define a "critical" scheme. In tests that have huge clinical implications, such as the AQP4 antibodies, an accuracy below 90% will have worrying implications for patient management and should be considered relevant in our opinion.

At least 6 of the schemes evaluated were somehow critical. The neuronal surface scheme (ENC) has a dramatically low accuracy mainly due to a mistake in sample selection by the coordinating lab and should therefore considered as "not determined" (see the dedicated section). Ganglioside antibodies, paranodal antibodies, MAG antibodies, AQP4 antibodies and MOG antibodies were highly or moderately critical. Onconeural antibodies, ACHR and MUSK antibodies has satisfactory performances.

Isoelectric focusing (IEF) scheme

Partecipants: 18
Samples: 4 sera+4 cerebrospinal fluids (pairs)
Judgment: mildly critical

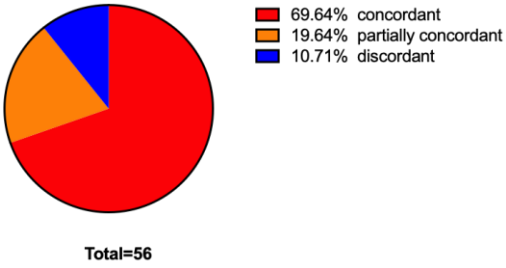
Samples			
Code	**Sent as**	**Detailed lab results**	**Clinical vignette**
S1L1	PATTERN 2 (unique-to-CSF OCB)	-	Pt with MS
S2L2	PATTERN 1 (polyclonal)	-	Pt with normal pressure hydocephalus
S3L3	PATTERN 5 (monoclonal gammopathy)	-	Pt with MGUS
S4L4	PATTERN 3 (mixed)	-	Sample was obtained by mixing a pattern 2 with a pattern 4. Unique to-CSF OCB were extremely faint, therefore both PATTERN 3 and PATTERN 4 were considered as acceptable answers

Methods		
Assay	N of centres	Description
Sebia kit	5/14 (36%)	Agarose gel IEF (Sebia)
Home made	4/14 (29%)	Agarose gel IEF (Home made)
Helena Biosciences Kit	3/14 (21%)	Agarose gel IEF (Helena)
Not specified	2/14 (14%)	-

Results

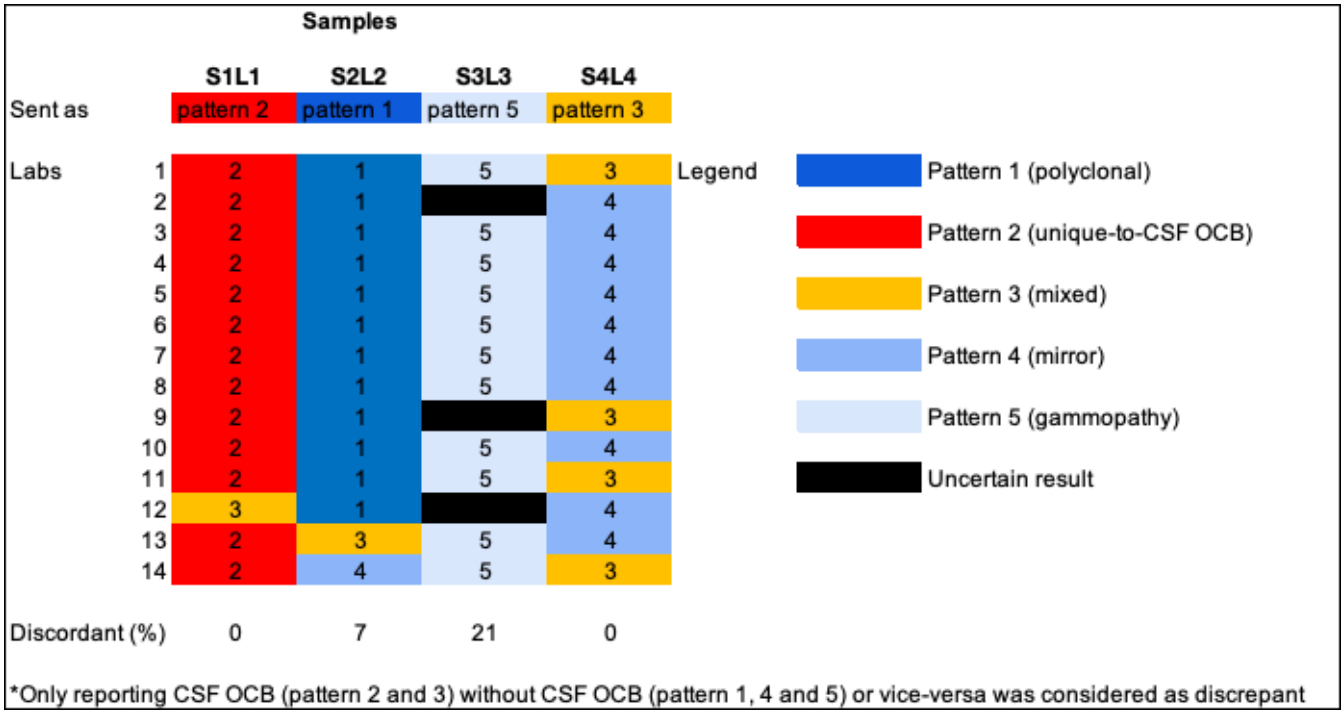
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

The overall accuracy was 89.3%. Only one lab reported CSF OCB in a negative sample (S2L2). Sample S4L4 was highly critical, likely due to the fact that it was prepared by pooling together a pattern 2 and 4. The final appearance of this artificial pattern was the presence of extremely faint unique-to-CSF OCB. For this reason, both “pattern 4” and “3” were considered acceptable as results

AQP4 antibodies scheme

Partecipants: 19
Samples: 5
Judgment: highly critical

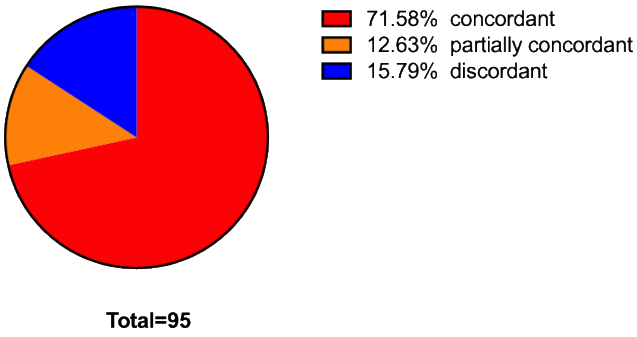
Samples			
Code	**Sent as**	**Detailed lab results**	**Clinical vignette**
AQP1	STRONG POS	LCBA pos (tit 1:40960) FBCA pos (score 4)	Pt with LES with ANA antibodies; optic neuritis, single episode; brain and spinal cord MRI negative; CSF analysis:
AQP2	WEAK POS	LCBA pos (1:160) FCBA pos (score 1)	
AQP3	NEG	LCBA neg FCBA neg	Pt with AD
AQP4	STRONG POS	LCBA pos (1:10240) FCBA pos (score 2)	Patient with NMOSD (2 episodes of TM, one classified as LETM); brain MRI neg; CSF analysis: no OCB
AQP5	STRONG POS	LCBA pos (1:20480) FCBA pos (score 3)	Patient with NMOSD (1 episode of ON and one of TM); brain MRI neg; CSF analysis: no OCB

Methods		
Assay	N of centres	Description
LCBA	2/18 (11%)	Live cell based assay with M23 AQP4 isoform; assessment with fluorescent microscope (in-house)
FACS-LCBA	1/18 (6%)	Live cell based assay with M23 AQP4 isoform; assessment with flow-cytometry (in-house)
FCBA	15/18 (83%)	Fixed cell based assay with M23 AQP4 isoform; assessment with fluorescent microscope (commercial, Euroimmun, Lubeck)
IIF on brain tissue	1/18 (6%)	Indirect immunofluorescence on monkey brain; assessment with fluorescent microscope (commercial, Euroimmun, Lubeck)*used in combination with

Results

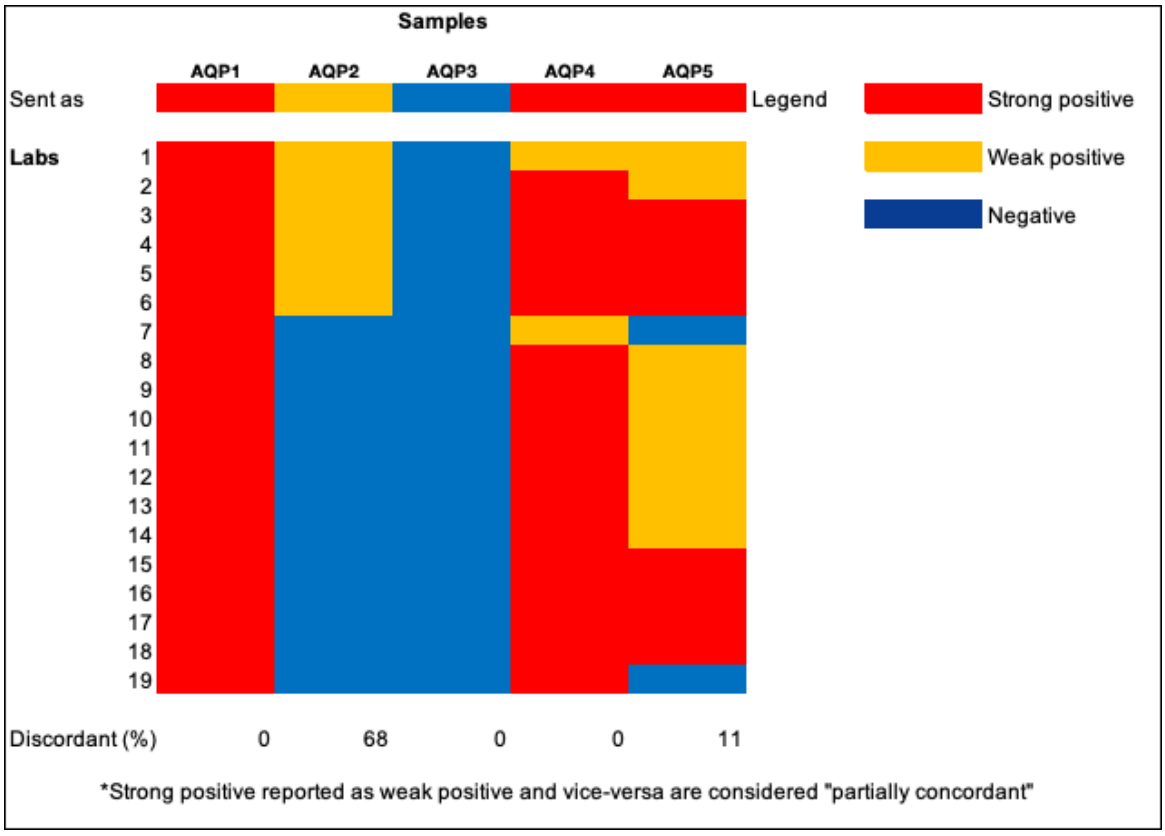
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

The overall accuracy is 83% which, considering the diagnostic relevance of AQP4 antibodies is sufficient to consider the scheme as problematic. Sample AQP2 was highly critical, as up to 72% of the laboratories did not identify a weak positive. Sample AQP5 was moderately critical, as 11% of the laboratories did not identify a strong positive.Since AQP2 and AQP5 samples were positive on both LCBA and FCBA, the critical results are likely associated with issues in the final evaluation of the results, rather than the assay itself.

MOG antibodies scheme

Partecipants: 18
Samples: 5
Judgment: moderately critical

Samples			
Code	**Sent as**	**Detailed lab results**	**Clinical vignette**
MOG1	STRONG POS	LCBA IgGtot pos (titre 1:2560) LCBA IgG1 pos F-CBA pos (score 3)	Isolated optic neuritis; brain MRI and spinal cord negative; CSF analysis: IEF polyclonal
MOG2	WEAK POS	LCBA IgGtot pos (titre 1:1280) LCBA IgG1 pos F-CBA pos (score 2)	Isolated optic neuritis; brain MRI and spinal cord negative; CSF analysis: IEF polyclonal
MOG3	NEG	LCBA IgGtot: neg LCBA IgG1: neg F-CBA: neg	Pt with Multiple Sclerosis
MOG4	WEAK POS	LCBA IgGtot pos (titre 1:640 LCBA IgG1: pos F-CBA pos (score 1.5)	Isolated optic neuritis; brain MRI and spinal cord negative; CSF analysis: IEF polyclonal
MOG5	STRONG POS	LCBA IgGtot pos (titre 1:2560) LCBA IgG1 pos FCBA pos (score 3)	Relapsing optic neuritis; brain MRI and spinal cord negative; CSF analysis: IEF polyclonal

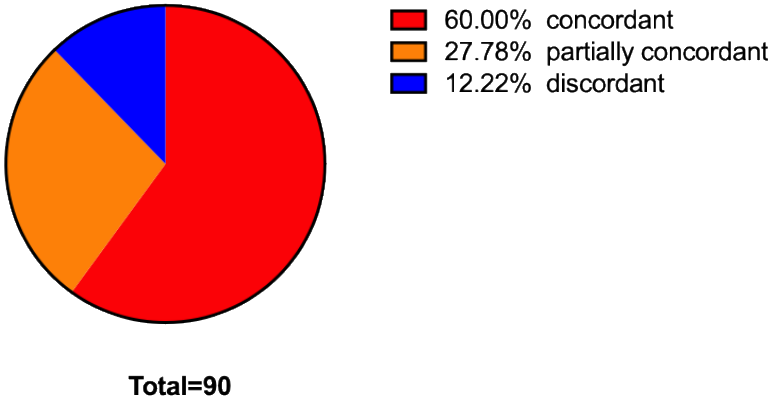
Methods		
Assay	N of centres	Description
LCBA IgGtot	5/18 (28%)*	Live cell based assay with full length human MOG isoform; anti Fc total IgG human secondary ab; assessment with fluorescent microscope (in-house)
LCBA IgG1	2/18 (11%)*	Live cell based assay with full length human MOG isoform; anti IgG1 human secondary ab; assessment with fluorescent microscope (in-house)
LCBA FACS	1/18 (5%)	Live cell based assay with full length human MOG isoform on a stabile transfected cell line; anti Fc total IgG human secondary ab; assessment with flow cytometry (in-house)
F-CBA	11/18 (61%)	Fixed cell based assay with full length human MOG isoform; anti Fc total IgG human secondary ab; assessment with fluorescent microscope (commercial, Euroimmun, Lubeck)

*one lab performed both methods

Results

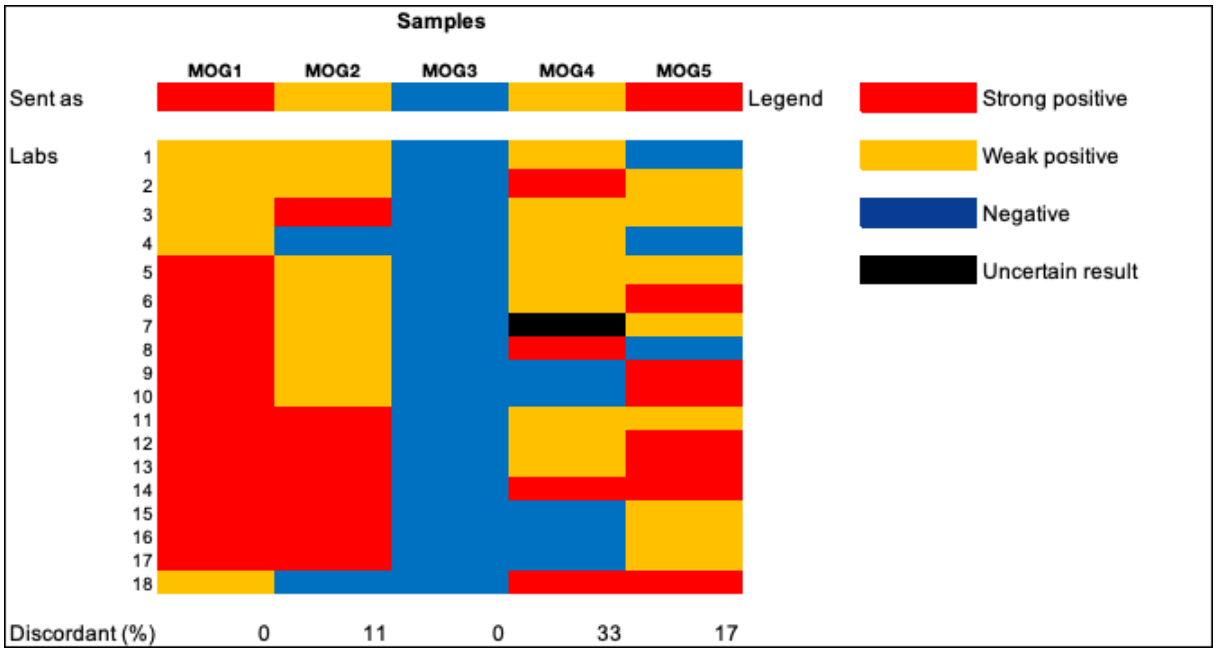
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

This scheme included a high proportion of labs using in house assays (7/18, 39%)
Sample MOG4 was highly critical, as 33% of labs did not identify a strong positive
Both MOG2 and MOG5 were moderately critical as respectively 11% and 17% of labs did not identify low positive and strong positive samples
Overall, this scheme proved to be challenging as 3/4 positive samples were somehow critical. All positive samples included in the scheme were positive for both LCBA IgGtot, LCBA IgG1 and FCBA, suggesting that the discrepancies should not only be attributed to the type of assay used. Moreover, discrepancies were present both in labs using LCBA and FCBA. These results point towards a major issue in the standardization of the assay, with relevant clinical implications

Intracellular neuronal antibodies scheme

Partecipants: 12
Samples: 3
Judgment: satisfactory

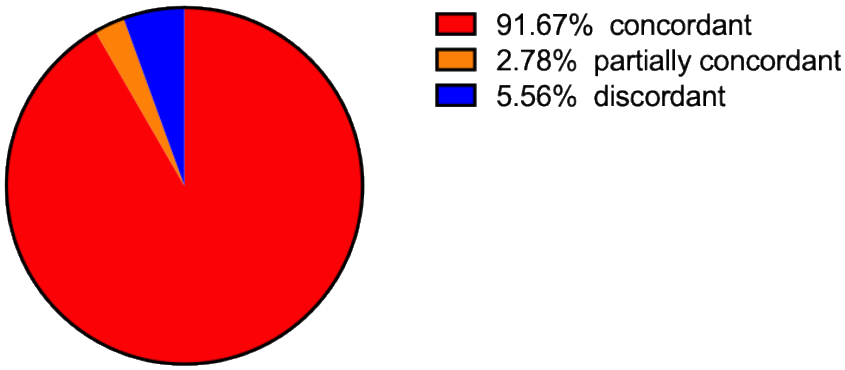
Samples			
Code	**Sent as**	**Detailed lab results**	**Clinical vignette**
ONCO1	STRONG POS	GAD Abs on TBA, line Blot and FCBA (home made)	Pt with brain MRI bilateral temporal lobe abnormalities and drug resistant epilpesy
ONCO2	NEG	Neg on TBA, CBA and blot	Pt with dementia
ONCO3	STRONG POS	Hu Abs on IIF, line blot (RAVO and Euroblot)	Pt with sensory ganglionopathy and SCLC

Methods		
Assay	N of centres	Description
IIF+Line blot	8/12 (67%)	IIF on monkey or rat brain, followed by confirmation on line blot; most labs did not specify further
Line blot only	3/12 (25%)*	Only line blot * one lab additionally used an in-house F-CBA
IIF only	1/12 (8%)	Only IIF

Results

Overall concordance of all tests performed

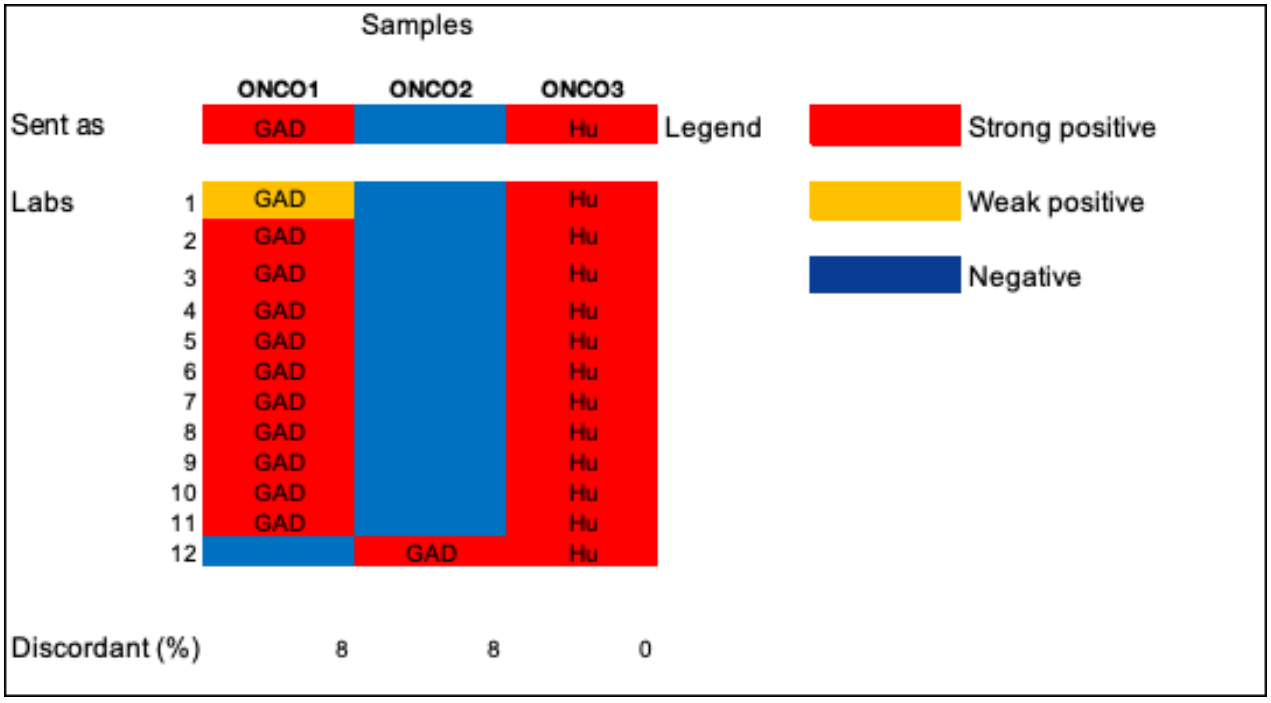
The graph represents all tests performed within the scheme



Total=36

Heatmap

The graph represents the detailed results for each sample



Comments

According to AINI recommendation, the suitable assay for this scheme is the combination of IIF followed by confirmation blot. However, 4/12 labs used either blot or IIF alone

Only one lab provided discrepant results (lab 12), likely due to a misclassification of samples ONCO1 and ONCO2. Overall, the performance of the scheme was satisfactory

Neuronal surface antibodies scheme

Partecipants: 14
Samples:
Judgment: Not determined

Samples			
Code	**Sent as**	**Detailed lab results**	**Clinical vignette**
ENC1	WEAK POS	LGI1 weak pos* FCBA: neg IHC: weak pos LCBA: pos (titre 1:80)	Limbic encephalitis with FBDS in remission phase
ENC2	STRONG POS	FCBA: pos GABAB IHC: strong pos LCBA: strong pos	Pt with limbic encephalitis
ENC3	NEG	FCBA:neg IHC: neg	Pt with AD

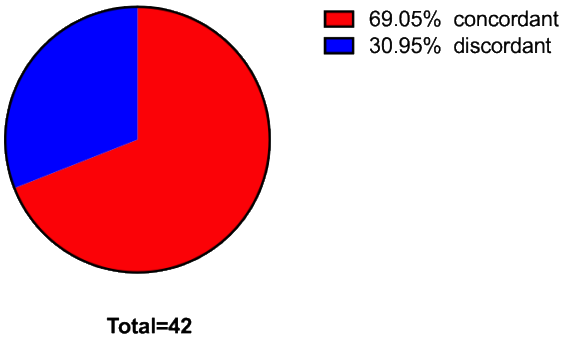
Methods		
Assay	N of centres	Description
FCBA	13/14 (93%)	Fixed CBA mosaic (commercial, Euroimmun)
LCBA	1/14 (7%)	Live cell based assay (in house)
TBA*	2/14 (14%)	Tissue based assay on lightly fixed rat brain tissue

*both labs used it in addition to FCBA

Results

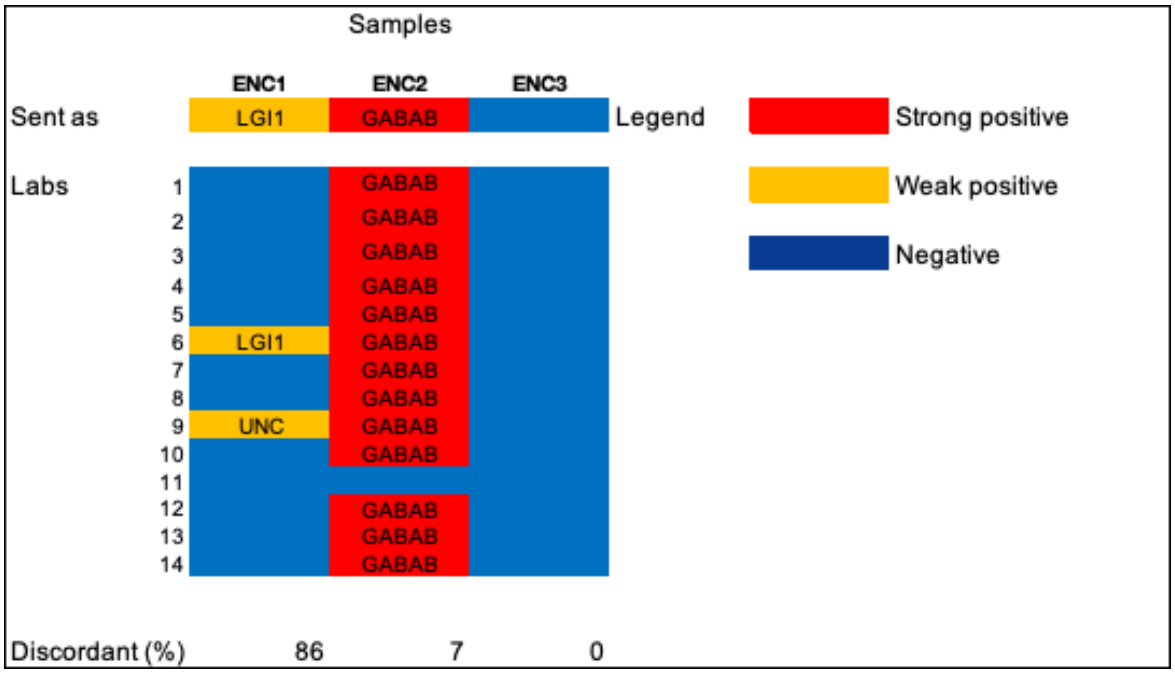
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

The low accuracy shown in this scheme is mainly due to a mistake made by the coordinating lab in sample selection. Sample ENC1 was an extremely low positive sample selected by mistake, that provided a faint reactivity only on live CBA and lightly fixed TBA, but not on FCBA. Therefore, was impossible to identify by most labs that used FCBA. Indeed, the only 2 labs that identified the sample used home-made techniques. Nonetheless, these unexpected results confirm a higher sensitivity of LCBA and TBA compared to FCBA.

We apologize for our mistake, that led to the judgment “not determined” for the whole scheme. As far as the other results, only one lab did not identify a strong GABAB positive sample.

Ganglioside antibodies scheme

Partecipants: 12
Samples: 3
Judgment: highly critical

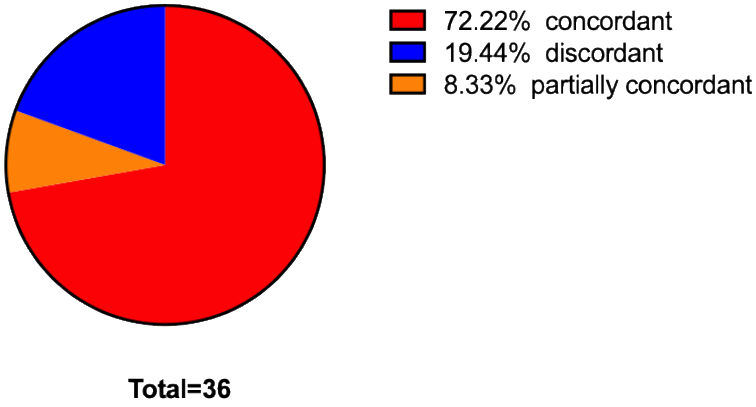
Samples			
Code	**Sent as**	**Detailed lab results**	**Clinical vignette**
GANGLIO1	STRONG POS	Gq1b IgG with Buhlmann ELISA (200%)	Pt with Miller Fisher
GANGLIO2	NEG	Neg on Buhlmann ELISA (<50%)	Diabetic neuropathy
GANGLIO3	NEG	Neg on Buhlmann ELISA (<50%)	Diabetic neuropathy

Methods		
Assay	N of centres	Description
Immunoblot	6/12 (50%)	Generic Assays immunoblot in 2 labs, Euroimmun blot in 1, not specified in the others
ELISA Home made	2/12 (17%)	ELISA home made
ELISA	3/12 (25%)	ELISA Buhlmann in 2 labs, not specified in the remaining one
Immunenzimatic	1/12 (8%)	Not specified

Results

Overall concordance of all tests performed

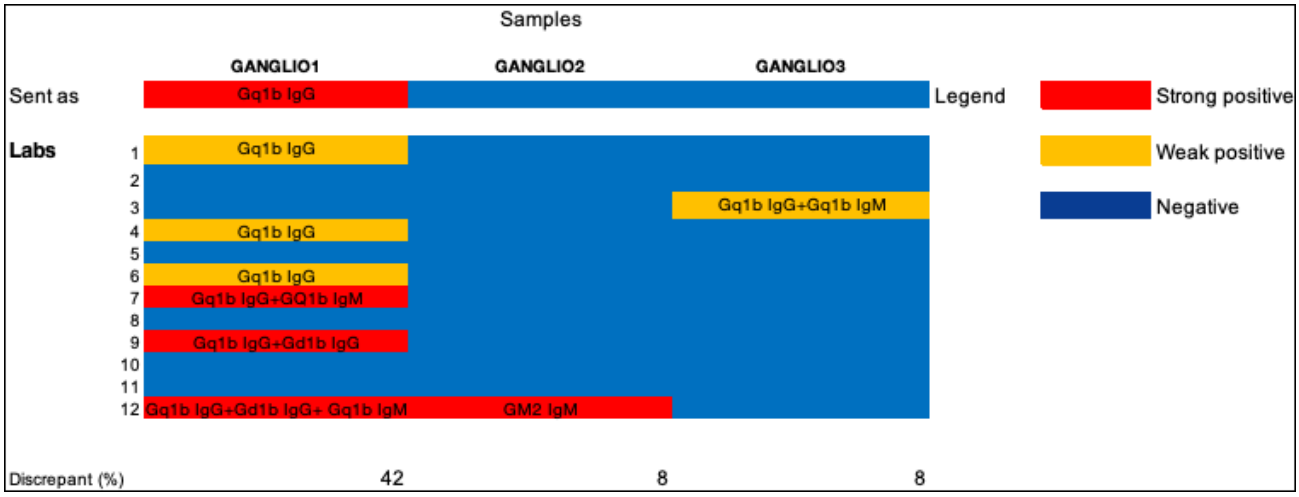
The graph represents all tests performed within the scheme



Total=36

Heatmap

The graph represents the detailed results for each sample



Comments

The methods for assessing ganglioside antibodies remain extremely heterogeneous. Sample GANGLIO1 was highly critical, as 42% of labs did not identify high titre Gq1b IgG. Three labs identified additional reactivities. Sample GANGLIO2 was sent as a "tricky control", as it gave a borderline result for GM1 IgM in a patients without a compatible phenotype. Indeed, only one lab identified a reactivity in this sample pertaining to a different ganglioside. One lab identified Gq1b antibodies in a negative sample, lilkely due to a misclassification of sample GANGLIO1 and GANGLIO3.

MAG antibodies scheme

Partecipants: 9
Samples: 3
Judgment: satisfactory

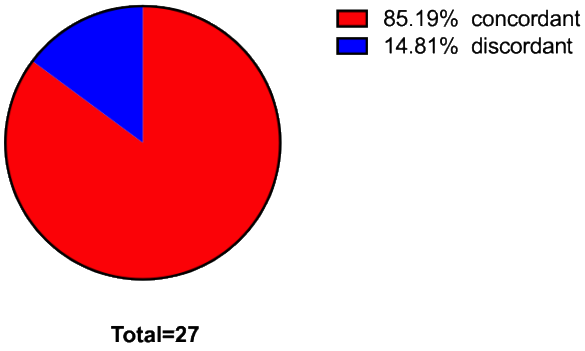
Samples			
Code	**Sent as**	**Detailed lab results**	**Clinical vignette**
MAG1	NEG	4800 BTU using Buhlmann ELISA	Pt with diabetic neuropathy, no MGUS
MAG2	NEG	<1000 BTU using Buhlmann ELISA	Pt with diabetic neuropathy, no MGUS
MAG3	STRONG POS	58300 BTU using Buhlmann ELISA	Pt with DADS and IgM monoclonal gammopathy

Methods		
Assay	N of centres	Description
ELISA	7/9 (78%)	ELISA Buhlmann
IIF	2/9 (22%)	Indirect immunofluorescence on sciatic nerve (in one lab confirmed with immunoblot)

Results

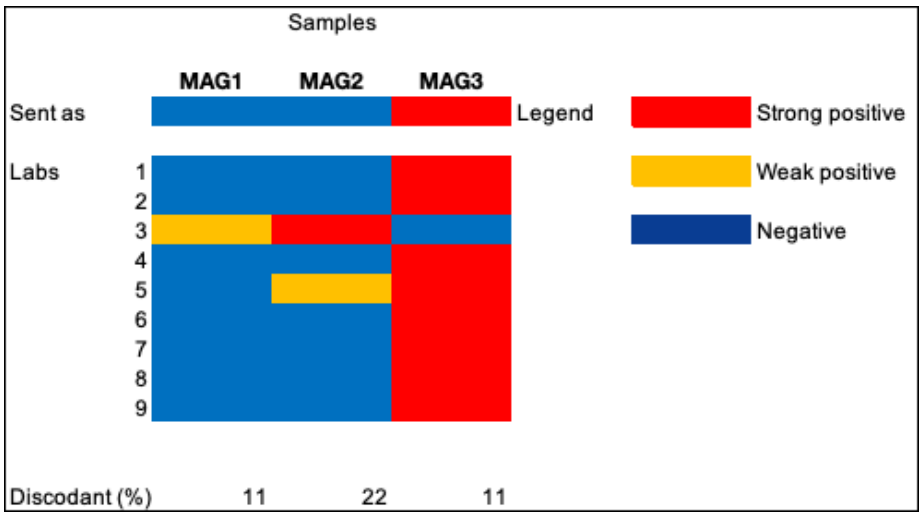
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

Methods used were quite homogeneous, as 78% of labs used similar ELISA kits. Sampe MAG1 was sent as a “tricky” negative sample, as it provided a reactivity that is above the cutoff defined by the kit producer, but comes from a patient without a compatible clinical phenotype. AINI guidelines previously suggested that, in order to increase the specificity of the assay, a cut-off of 10000 BTU is ideal. Despite the difficulties related to the sample, most laboratories appropriately identified it as negative. A few false positive and negatives were detected also with MAG2 and MAG3 samples. The overall performance of the scheme was satisfactory.

Paranodal antibodies scheme

Partecipants: 5
Samples: 3
Judgment: highly critical

Samples			
Code	**Sent as**	**Detailed lab results**	**Clinical vignette**
PN1	STRONG POS	CASPR1 pos on home made ELISA, LCBA (1:320) TBA on lightly fixed brain (score 2.5)	Pt with CIDP
PN2	STRONG POS	NF155 pos on home made ELISA, LCBA (score 4) TBA on lightly fixed brain (score 1.5)	Pt with CIDP
PN3	NEG	NEG using home made ELISA, LCBA and TBA on lightly fixed brain	Pt with DADS and IgM monoclonal gammopathy

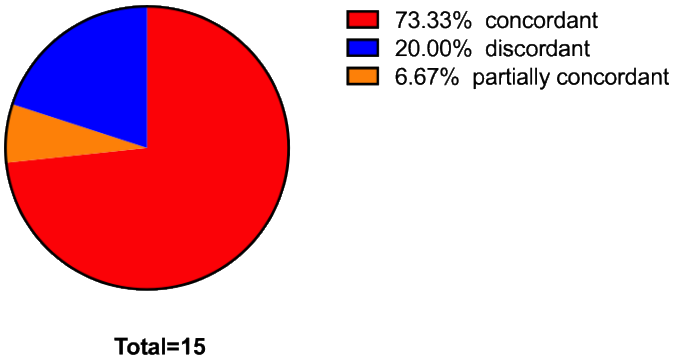
Methods		
Assay	N of centres	Description
ELISA*	4/5 (80%)	Home made ELISA for CASPR1, CNTN, NF155 and 186
LCBA*	3/5 (60%)	Home made live cell based assay CASPR1, CNTN, NF155 and 186
TBA*	2/5 (40%)	Tissue based assay on lightly fixed rat brain (1 lab) or sciatic nerve (1 lab)

*two labs used a combination of ELISA, CBA and TBA

Results

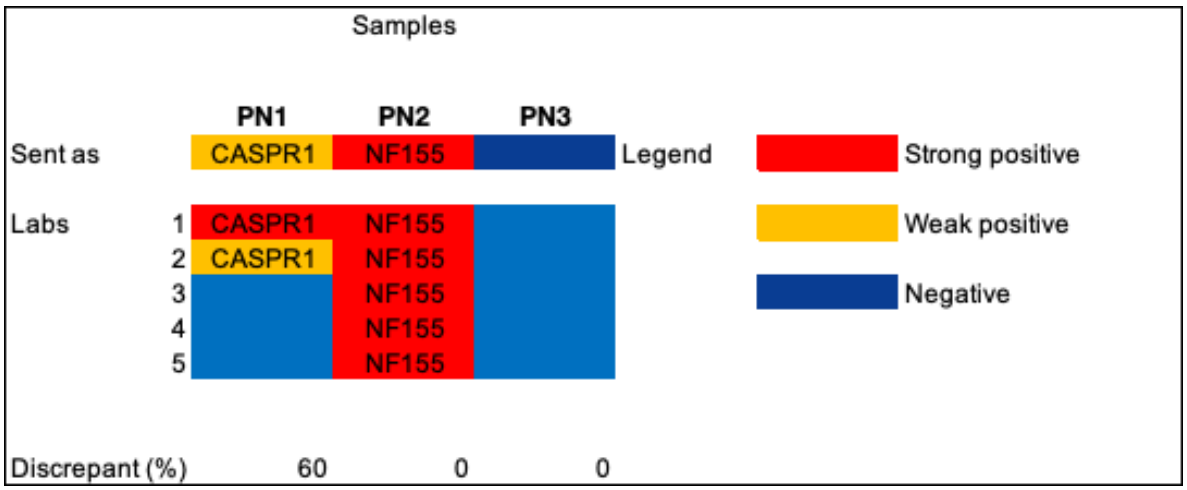
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

This is a novel scheme introduced this year in the AINI EQAS. Paranodal antibody testing is becoming more and more relevant in clinical practice, but testing is limited to a few specialized labs. Insufficient data are available on assay standardization. Sample PN1 was highly critical, as only 2/5 labs detected CASPR1 antibodies that resulted positive using three different techniques (CBA, ELISA and TBA). On the other hand, no discrepancies were detected with the remaining samples, suggesting that the discrepancies might be specific to the CASPR1 assay. Larger standardization schemes are required to explore this hypothesis.

Nicotinic acethylcholine receptor scheme

Partecipants: 8
Samples: 5
Judgment: satisfactory

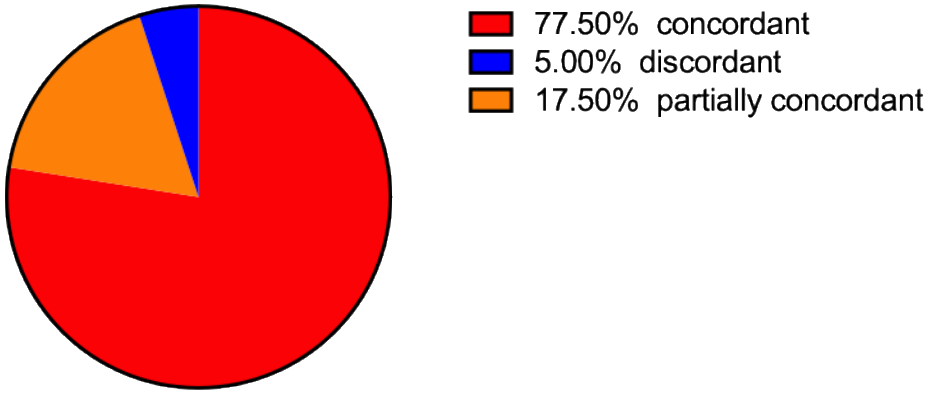
Samples			
Code	**Sent as**	**Detailed lab results**	**Clinical vignette**
ACHR1	STRONG POS	ELISA pos; LCBA pos (score 3) FCBA pos	Pt with generalized Myasthenia Gravis at onset
ACHR2	STRONG POS	ELISA pos; LCBA pos (2.5) FCBA pos	Pt with generalized Myasthenia Gravis at onset
ACHR3	WEAK POS	ELISA pos; LCBA pos (2) FCBA pos	Pt with generalized Myasthenia Gravis in remission phase
ACHR4	NEG	ELISA neg LCBA neg; FCBA neg	Diabetic polineuropathy
ACHR5	STRONG POS	ELISA pos; LCBA pos (2) FCBA pos	Pt with generalized Myasthenia Gravis in remission phase

Methods		
Assay	N of centres	Description
ELISA	3/8 (37.5%)	Commercial ELISA
RIA	3/8 (37.5%)	Commercial RIA
LCBA	2/8 (25%)	Home made live cell based assay for foetal and adult ACHR isoforms

Results

Overall concordance of all tests performed

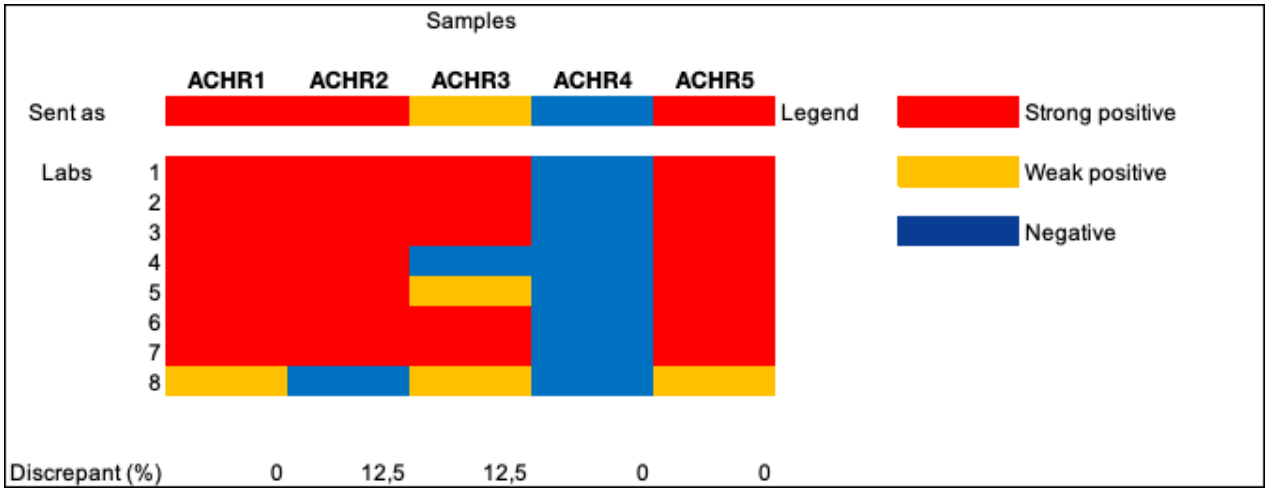
The graph represents all tests performed within the scheme



Total=40

Heatmap

The graph represents the detailed results for each sample



Comments

In this scheme the samples were selected according to the results provided by a LCBA, and not a RIA, that is currently the gold standard for ACHR abs detection. Therefore, inconsistencies in terms of “strong” or “weak” positive are likely irrelevant. In addition, several labs using the RIA were able to identify the positive samples. Despite the heterogeneity of methods used for ACHR abs detection, the results are overall satisfactory, with only two relevant discrepancies with samples ACHR2 and ACHR3. No lab used the recently introduced fixed cell based assay, that could therefore not be evaluated in this scheme.

MUSK antibodies scheme

Partecipants: 7
Samples: 5
Judgment: satisfactory

Samples			
Code	**Sent as**	**Detailed lab results**	**Clinical vignette**
MUSK1	STRONG POS	LCBA pos (score 4) FCBA pos	Pt with generalized Myasthenia Gravis (bulbar involvement)
MUSK 2	STRONG POS	LCBA pos (score 3) FCBA pos	Pt with generalized Myasthenia Gravis (bulbar involvement)
MUSK 3	NEG	LCBA and FCBA neg	Diabetic polineuropathy
MUSK 4	NEG	LCBA and FCBA neg	Diabetic polineuropathy
MUSK 5	WEAK POS	LCBA pos (score 2) FCBA pos	Pt with generalized Myasthenia Gravis (bulbar involvement)

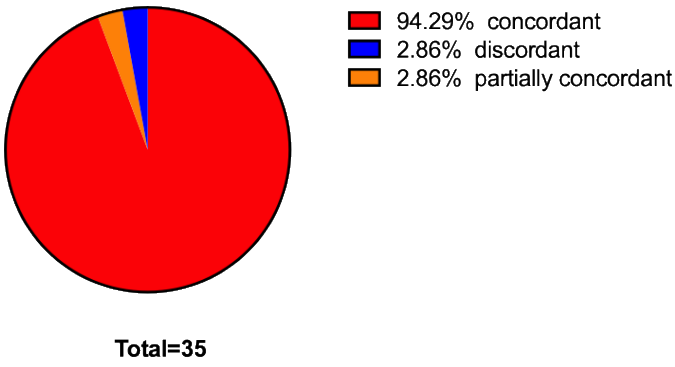
Methods		
Assay	N of centres	Description
ELISA	3/7 (43%)	Commercial ELISA
RIA	2/7 (29%)	Commercial RIA
LCBA	2/7 (29%)	Home made live cell based assay
FCBA	1/7 (14%)*	Commercial fixed cell based assay

*one lab used a combination of ELISA+FCBA

Results

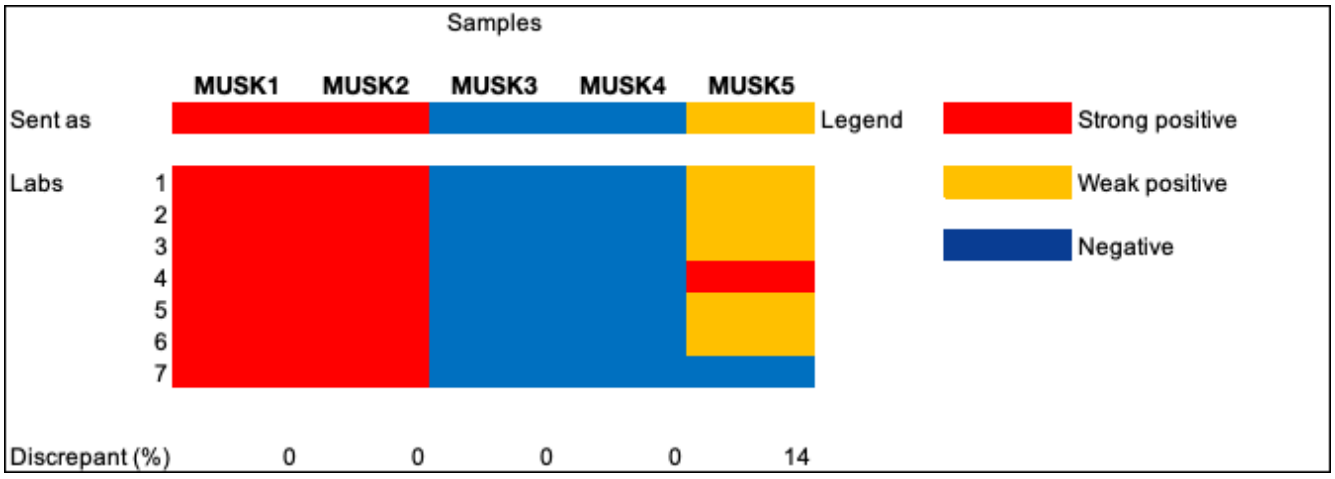
Overall concordance of all tests performed

The graph represents all tests performed within the scheme



Heatmap

The graph represents the detailed results for each sample



Comments

Despite the heterogeneity of methods used for MUSK abs detection, the results are overall satisfactory, with only one relevant discrepancy with sample MUSK3. One lab used the recently introduced commercial FCBA, with good performances. Further data are needed to assess the accuracy of this method.

Conclusions

The results of this EQAS points toward relevant issues in neuroimmunology laboratory diagnostic.

Ganglioside antibody testing has always been a critical topic, and the methods used by different labs remain too heterogeneous.

Paranodal antibody testing provided particularly critical results. However, only 3 samples were explored, and it is unclear whether the issues concern a specific assay (CASPR1). Further studies are needed to understand the reliability of current diagnostic methods for these antibodies.

Particularly worrying are the results with AQP4 and MOG testing. AQP4 are measured in many labs using a commercial kit, whose performances seem to be adequate. However, some issued seem to remain possibly related with the interpretation of the slides.

The issue with MOG antibodies is complex and does not have a clear explanation. According to literature data, the commercial fixed cell based assay seem to be less reliable compared to the live cell based assay, that is considered the gold standard. However, many different techniques and strategies are currently used to perform a live cell based assay. The inaccuracies detected in this scheme cannot simply be explained with the method used and suggest that other factors might be involved.

Starting form the data of the current and previous EQAS, AINI is developing strategies to try and address the issued in neuroimmunology laboratory diagnostic.

Fistly, from December 2021, AINI has been organizing the “Corso di diagnostica di laboratorio in neuroimmunologia”, a two day course where students are faced inttially with the theoretical notions regarding the state of the art of specific diagnostic fields, and the following day they are challenged with practical taks that include direct interpretation of assays slides.

Secondly, AINI is currently promoting the project NINA-Flow, that will provide a tool to sent critical samples for retesting in references labs able to perform the current gold standard method. This will allow to extend the standardization process to problematic samples, and ultimately will provide us sufficient data to analyze and hopefully correct our imprecisions. We hove for the project to be operational within the end of the current year.

More information on both initiatives can be found at www.aini.it.

Finally, I would like to thank all the participants to this EQAS for their valuable contribution. Please feel free to contact us for any queries regarding the results discussed in this document, or to exchange additional samples. We are also extremely happy to receive your complaints and suggestion to improve our EQAS, including potential additional assays that you would like to be evaluated.

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.

See you next year!

Matteo Gastaldi
Diego Franciotta
Roberto Furlan

The AINI scientific Board
The RIN scientific Board