Pilot UK NEQAS for N-methyl-D-aspartate receptor (NMDAR) antibodies

S. BEX, H. WILKINSON, D. PATEL, R. SARGUR

UK NEQAS for Immunology, Immunochemistry & Allergy (UK NEQAS IIA)
Northern General Hospital, Sheffield, United Kingdom
ukneqas@immqas.org.uk

INTRODUCTION

N-methyl-D-aspartate receptors (NMDAR) are ligand-gated cation channels, which are crucial in synaptic transmission and plasticity[1]. In the Central Nervous System, NMDARs are one of the main excitatory receptors on synapses of neurons, which regulate balance between neuronal inhibition and excitation. In the brain, NMDARs have involvement in neuroplasticity, neurotoxicity, and excitatory neurotransmission[2]. Structurally, NMDAR is mostly triheteromeric, comprising GluN1, GluN2A and GluN2B subunits (Figure1).

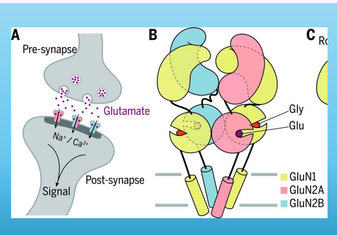


Figure1

A) NMDARs are localized in the post synapse. (B) The ion channel opens when glycine binds to the GluN1 subunits, and glutamate binds to the GluN2 subunits, and the Ligand Binding Domain (LBD) 'clamshells' close [3]

NMDAR antibodies are associated with autoimmune diseases such as anti-NMDAR encephalitis and systemic lupus erythematous (SLE). In Autoimmune Encephalitis (AE), NMDAR antibodies against NR1, NR2A and NR2B subunits are overproduced, causing neurotransmitter dysregulation, resulting in seizures and other symptoms including psychosis, headaches, hallucinations, changes in mental status, dysautonomia, and orofacial dyskinesia[2]. NMDAR antibodies are suspected where AE has no known infectious cause (viruses such as herpes simplex or varicella zoster are main cause of encephalitis). Commercially available Indirect Immunofluorescence (IIF) assays use either brain tissue (hippocampus or cerebellum) or monospecific cells (the HEK cell line is transfected with the NMDAR NR1 receptor). In the cerebellum, the stratum granulosum fluoresces. In the hippocampus, fluorescence of the stratum moleculare (neuropil staining) is observed in NMDAR antibody positive samples. [4]

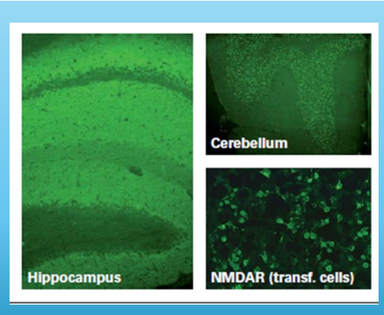


Figure 2

Commercially available IIF assays and their appearance with NMDAR positive samples under immunofluorescence microscopy[4]

AIMS AND OBJECTIVES

- To determine the level of interest and feasibility of developing a pilot UK NEQAS EQA scheme for NMDAR antibodies.
- To gather information from participants currently providing NMDAR antibody testing to aid scheme design.
- To set up an EQA scheme for NMDAR antibodies, in addition to the existing neuroimmunology EQA schemes offered by the Centre (Paraneoplastic Antibodies, Ganglioside Antibodies, and Myelin Associated Glycoprotein IgM Antibodies (MAG).

METHOD

UK NEQAS IIA sent out a survey to participants in February 2020 to gather information from laboratories who were providing testing for NMDAR antibodies. This was to assess the level of interest in a new NMDAR antibody scheme, and to determine key scheme requirements (such as sample volume and frequency of testing). Positive and negative samples were also sourced. Unfortunately the COVID-19 pandemic meant we had to delay the development of this scheme to 2022.

RESULTS

86 participants responded to the survey.

66 (77%) respondents stated that they offered a service for NMDAR antibody testing. 50/63 labs (79%) preferred to measure NMDAR antibodies in serum (Figure 3). 61/63 (97%) of laboratories surveyed also measure NMDAR antibodies in Cerebrospinal Fluid (CSF) samples.

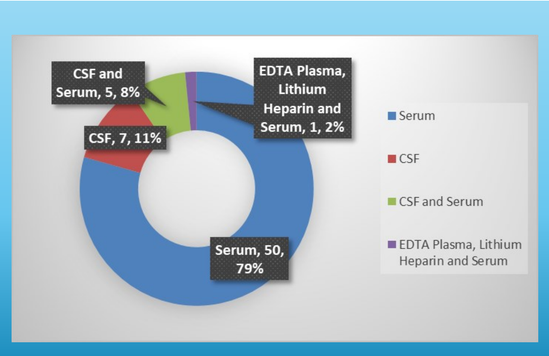


Figure 3

Preferred matrix for NMDAR measurement

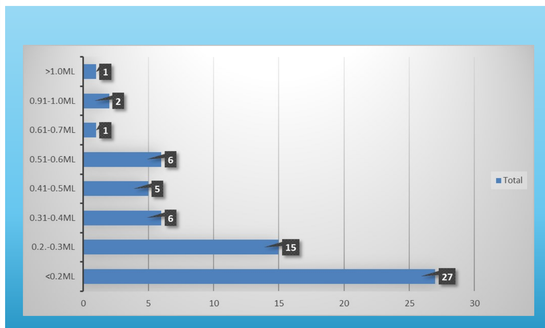


Figure 4

How much sample volume (mL) do you require for your NMDAR test?

Most respondents (42/63; 67%) required a sample volume of 0.3 mL or less (Figure 4). 37/63 labs (59%) were not participating in a sample exchange scheme for NMDAR antibodies. 25/57 (44%) of laboratories surveyed would be willing to provide samples for use within the pilot EQA scheme, to help ensure EQA scheme continuity.

RESULTS

Methodology

65/66 (98%) participants stated they use a commercial kit (Euroimmun). The most popular assay type is fixed cell, which is used by 63/66 labs(95%), with 2/66 (3%) using transfected cells, and only 1 lab (2%) using Indirect Immunofluorescence Test (IIFT) (Figure 5). The majority of labs (51/66: 77%) use an automated assay platform.

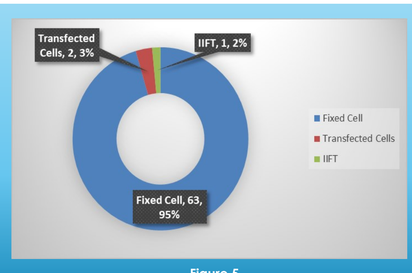


Figure 5

NMDAR antibody methodology

Reporting of Results

43/66 (65%) participants report only qualitative NMDAR antibody results and 5/66 (8%) report only quantitative results, but 18/66 (27%) report both qualitative and quantitative results (Figure 6). Labs returning quantitative results report using 3 different measuring units: intensity, titre, or ratio (Figure 7). 41/63 (65%) participants provide interpretative comments as part of the results which they report.

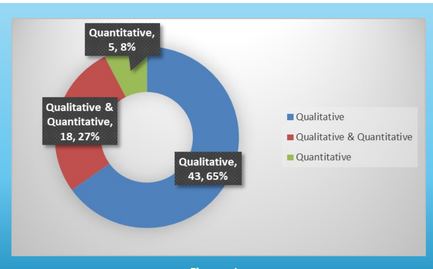


Figure 6

Format of NMDAR Antibody results reported

Frequency of Testing

10/63 (16%) participants analyse <10 samples per month, with 26/63 (41%) labs analysing 40 or more samples per month (Figure 8). Most laboratories perform NMDAR antibody testing either weekly or bi-weekly (Figure 9). A three week timeframe would therefore be suitable for participants to return data for an NMDAR antibodies EQA scheme.

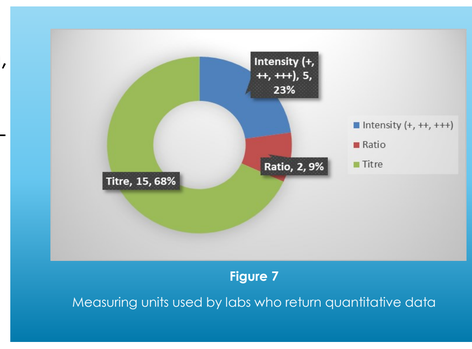


Figure 7

Measuring units used by labs who return quantitative data

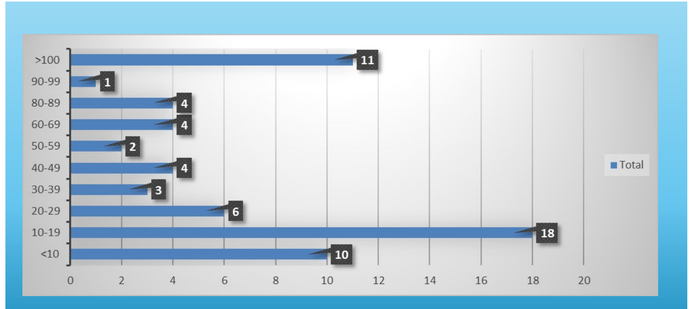


Figure 8

Number of NMDAR antibody tests performed per month

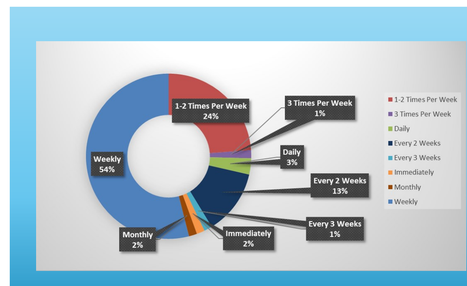
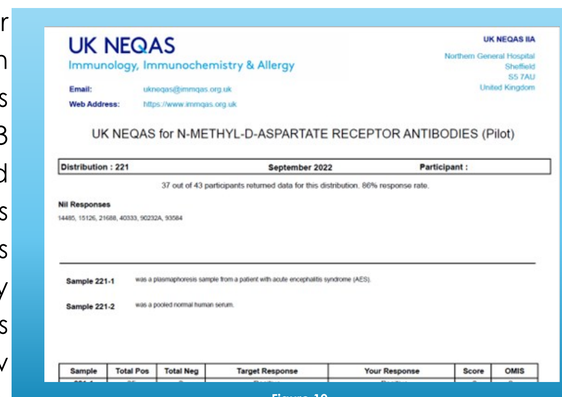


Figure 9

Frequency of NMDAR testing by laboratories

CONCLUSION

The first distribution of the Pilot UK NEQAS for NMDAR Antibodies EQA scheme was sent out in September 2022. The distribution frequency was 2 samples every 8 weeks and participants had 3 weeks to return results. There was a good response rate, with 37/43 (86%) participants returning data in consensus, so both samples were scored (Figure 10). A refreshed web entry screen and report format were used for this scheme; web entry was simplified and the new report format provides additional information. Subsequent distributions showed a good response rate and data consensus. Taken together, these changes benefit participants by allowing them to better monitor EQA performance and so assist with maintaining laboratory accreditation. We look forward to the future development of this pilot EQA scheme.


UK NEQAS
Immunology, Immunochemistry & Allergy

UK NEQAS IIA
Northern General Hospital
Sheffield
S5 7AU
United Kingdom

Email: ukneqas@immqas.org.uk
Web Address: https://www.immqas.org.uk

UK NEQAS for N-METHYL-D-ASPARTATE RECEPTOR ANTIBODIES (Pilot)

Distribution : 221 | September 2022 | Participant :

37 out of 43 participants returned data for this distribution. 86% response rate.

Nil Responses
14485, 15126, 21688, 40333, 902324, 93584

Sample 221-1 was a plasmapheresis sample from a patient with acute encephalitis syndrome (AES).

Sample 221-2 was a pooled normal human serum.

Sample	Total Pos	Total Neg	Target Response	Your Response	Score	OMIS

Figure 10

The first Pilot NMDAR Antibodies report

REFERENCES

1. *Dalmau J., et al. (2008). Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. Lancet Neurol 7 (12), 1091-1098.*
2. *Chen S., et al. (2022). Roles of N-methyl-D-aspartate Receptors (NMDARs) in epilepsy. Frontiers in Molecular Neuroscience doi: 10.3389/fnmol.2021.797253*
3. *Lu W., Du j., Goehring A. and Gouaux E. (2017). Cryo-EM structures of the triheteromeric NMDA receptor and its allosteric modulation. Science 355 doi: 10.1126/science.aal3729*
4. *Euroimmun promotional material. Autoantibodies in neurological diseases. 2016/11.*

REGISTER FOR SCHEMES & iEQA

UK NEQAS
Immunology, Immunochemistry & Allergy

Log on to our web site: www.immqas.org.uk

Fill in the relevant registration form

Send us an email: ukneqas@immqas.org.uk

iEQA Interpretative External Quality Assessment

Sheffield Teaching Hospitals NHS
NHS Foundation Trust