

Background and aims of NMDA testing guidance document in the Oxford Diagnostic Immunology service

Since July 2015, the Oxford Diagnostic Immunology service has been performing the measurement of NMDA receptor antibodies using a commercially available kit supplied by Euroimmun. At this time it was decided that a parallel testing study should be performed on all samples sent to the Oxford Diagnostic immunology service for NMDA testing so that all samples were tested using the Euroimmun Fixed Cell assay and the Oxford University Academic **research** Neuroimmunology Live cell assay, the original NMDA receptor antibody assay available in Oxford pre July 2015.

There is no internationally accepted “GOLD STANDARD” for testing antibodies against NDMA

The aim of this study was to try to determine the performance of each assay in relation to each other with regards to being able to detect ‘True positive’ and ‘True negative’ patients when testing for NMDA receptor antibodies in a prospective **new** patient cohort. The intention of these findings was to help create this guidance document to help users understand the changes to the Oxford service, the performance characteristics of the two assays and how they should request NMDA receptor antibody assays in the future from Oxford.

Summary of recommendations for NMDA receptor antibody testing

Results generated from the Oxford study (detailed below) highlight that it is **not obvious that one sole assay should be offered over the other for testing of NMDA receptor antibodies**. To confront this, the Oxford Diagnostic Immunology service will continue to make both assays available for users to request.

So that Oxford can offer requesting clinicians confidence of safe quick testing as well as empowerment to investigate each patient they see as appropriate. Based on the results from our study. The testing process offered is as follows:

- 1) All samples unless explicitly stating otherwise be tested for NMDA receptor antibodies using the Fixed cell assay as a first line test.

This test shows a 99.2% Negative Predictive Value (NPV) when analysing consecutive **new** patient samples – **A negative result produced by the Fixed cell assay has a 99.2% chance of being a ‘True Negative’.**

The Fixed cell assay has a significantly higher Positive Predictive Value (aprox 100% PPV)– than the live cell assay. - **A positive result produced by the Fixed cell assay is likely to have a 100% chance that a Positive result is a ‘True Positive’ - when compared to the Live cell assay.**

Important:- for patients, using the fixed cell assay means the Oxford Diagnostic Immunology service will offer a **7 day turnaround** for testing using this assay and a price of **£35**, a reduction from £50 traditionally charged by Oxford for all NMDA assays performed. In addition, the **Fixed cell assay will be UKAS accredited** in May 2016.

- 2) In our study, the Live cell assay has a greater NPV (approx 100%) compared to the Fixed cell assay. – **A negative result produced by the Live cell assay is likely to have a 100% chance of being a 'True Negative'.**

The Live cell assay has a significantly lower Positive Predictive Value (50%) when detecting **'True Positive' prospective new patients** -**A positive result on the Live cell assay has a 50% chance of being 'True Positive'.**

Important: The live cell assay has a longer turnaround time than the fixed cell assay (**turnaround of 14 days**) and is **more expensive at £50**. The **Live Cell assay will NOT be UKAS accredited** in May 2016

- 3) Due to the increase (0.8%) in NPV seen in the Live cell assay when compared to the Fixed cell assay, all reports for the Fixed cell assay will carry a statement instructing users that should the result be negative despite their patient clinically still showing a high suspicion of autoimmune encephalitis, that they are encouraged to contact the Oxford Diagnostic Immunology laboratory to request a Live cell assay on the sample. All samples are stored for 4 months to help facilitate this option.
- 4) Should a user of the service deem it appropriate, they may initially request a Live cell assay on any patient sample that they feel it necessary, this must be stated clearly on all request cards that this is required.

General statistics of the study

The data set was collected on all samples that were sent to the Oxford Diagnostic Immunology Laboratory for NMDA testing.

The data set was collected between 1st July 2015 – 18th September 2015 and included in total 2171 samples. This could be broken down into the following groups:

- 1992 samples that were concordant negative (1818 unique patients)
- 62 samples that were concordant positive (36 unique patients)
- 117 samples that were discordant (106 unique patients)

Due to the desire to understand how these assays perform on **new prospective** investigations for NMDA receptor antibody specific autoimmune encephalitis, all previous positive patients, likely to have undergone significant immunosuppressive therapy prior to the parallel study beginning, who were tested in the study period were omitted from the final analysis. This changed the numbers of the above groupings as follows:

- **Concordant negatives = 1818**
- **Concordant positives = 18**
- **Discordant results = 73**

Limitations of the study

NMDA receptor antibody specific autoimmune encephalitis has no clear 'gold standard' for diagnosis and in addition, full clinical follow up of this entire cohort of patients was an unrealistic goal due to the worldwide origin of these samples. In order to be able to produce statistics that although not absolutely accurate are able to give an indication of the difference in the performance between assays, the following assumptions had to be made based on already known performance comparisons of the two assays:

- The Live cell assay shows greater sensitivity than the Fixed cell assay and so the Live cell assay was assumed to have a sensitivity of 100% and a negative predictive value (NPV) of 100% for the purposes of these calculations.
- The Fixed cell assay shows greater specificity than the Live cell assay and so the Fixed cell assay was assumed to have a specificity of 100% and a positive predictive value (PPV) of 100% for the purposes of these calculations.
- Since only a subset of the discordant patients could be clinically followed up and assigned as either 'True positive', 'True negative' or undetermined, the ratio of these three groups was calculated and used to extrapolate numbers from the total discordant cohort gathered to ensure that these values complemented other groupings such as concordant positives for calculation purposes.

Discordant patient cohort clinical findings

Out of the 73 patients that were new to the service and gave discordant values between the two assays, information for 16 patients was able to be gathered (22%). These were grouped as follows:

<u>Diagnosis</u>	Number	% of 16 patients	Extrapolated value in total discordant cohort (n=73)
Definite	3	18.8%	14
Possible	6	37.5%	27
Unlikely	7	43.8%	32

Patients grouped into the 'Possible' category were removed from any further analysis.

Live cell assay profile

Using the patient groupings defined above the Live cell assay shows the following characteristics

		True Result			
		POSITIVE	NEGATIVE		
Live cell assay	POSITIVE	32	32	PPV%	50.0%
	NEGATIVE	0	1818	NPV%	100%
		Sensitivity	Specificity		
		100%	98.3%		

This shows a specificity of over 98% and a PPV of 50% - **If a sample result was positive, on the Live cell assay, when investigating a new patient, there is a 50% chance that this result is a 'True positive'.**

Important: UKAS status of the Live cell assay (ISO15189)

The Live cell assay is performed **at the Academic Neuroimmunology Research laboratory** under the cover of the Oxford Diagnostic Immunology laboratories CPA accreditation. This accreditation will change in May 2016 to the stricter UKAS ISO15189 standard. Although the Academic Neuroimmunology Research laboratory provides a valuable service which needs to continue, the structure of this laboratory, its staff and its operation are not currently meeting the new ISO15189 standards. The Live Cell assay will **not be assessed** for UKAS accreditation in May 2016.

Turnaround time and cost of test

The Live cell assay is a very labour intensive assay that requires up to 4 days of cell culture prior to assay commencement. Assays are required to be batched due to the logistical challenges of making samples available to the Academic Neuroimmunology Research laboratory, situated at a completely different hospital site to the Oxford Diagnostic Immunology laboratory. The current 95th percentile turnaround time for this assay when at full capacity (11.5k tests/year) is 14 days and the price is £50.

Fixed cell assay profile

Using the patient groupings defined above the Fixed cell assay shows the following characteristics

		True Result			
		POSITIVE	NEGATIVE		
Fixed cell assay	POSITIVE	18	0	PPV%	100%
	NEGATIVE	14	1850	NPV%	99.2%
		Sensitivity	Specificity		
		56.3%	100%		

This shows the Fixed cell assay has a sensitivity of over 56% and a NPV% of over 99% - **If a sample result was negative, on the Fixed cell assay, when investigating a new patient, there is a 99.2% chance that this result is a 'True negative'.**

Quality status of the Fixed cell assay (ISO15189)

The Fixed cell assay is an assay that is supplied as a CE marked, FDA approved kit. This has been verified to ISO 15189 standards and is performed in the Oxford Diagnostic Immunology Laboratory by HCPC registered staff and is anticipated to meet the required ISO standards without incident. In May 2016. The clinical governance procedures in the Diagnostic laboratory is UKAS ready.

Turnaround time and cost of test

This assay can be performed in 4 hours from start to finish and is performed at the same site as where samples arrive. This allows a turnaround time of 7 days to be offered for this test when at full capacity (11.5k tests/yr). To further benefit users, particularly within the NHS, the savings made in labour time will be passed on to the user by reducing the traditional test price of NMDA receptor antibodies in the Oxford Diagnostic Immunology service of £50 to £35.