EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay

DECISION SUMMARY

A. DEN Number:

DEN150030

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation of the KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay

C. Measurand:

Aquaporin-4 Autoantibody (AQP4Ab)

D. Type of Test:

Manual enzyme-linked immunosorbent assay, semi-quantitative

E. Applicant:

KRONUS, Inc.

F. Proprietary and Established Names:

KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.5665, Aquaporin-4 autoantibody immunological test system

2. Classification:

   Class II (special controls)

3. Product code:

   PNI – Aquaporin-4 Autoantibody

4. Panel:

   Immunology (82)

H. Indications for use:

The KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay is for the semi-quantitative determination of autoantibodies to Aquaporin-4 in human serum. The KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay may be useful as an aid in the diagnosis of Neuromyelitis Optica (NMO) and Neuromyelitis Optica Spectrum Disorders (NMOSD). The KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay is not to be used alone and is to be used in conjunction with other clinical, laboratory, and radiological (e.g., MRI) findings.

Special conditions for use statement(s):

For prescription use only

Special instrument requirements:

ELISA Plate Reader suitable for 96-well format and capable of measuring at 405 nm and ELISA plate shaker capable of 500 shakes/minute.

I. Device Description:

The KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay consists of ELISA strip wells coated with human recombinant AQP4 protein (amino acid position: 23-323) (96 wells in total) and supplied as 12 strips of 8 wells in a frame and sealed in a foil pouch with desiccant; ready-to-use 5 levels of calibrators (1.5, 5, 20, 40, and 80 U/mL), 5 x 0.7 mL; a ready-to-use positive (2 x .7 mL) and a negative control serum (1 x 0.7 mL); AQP4-Biotin (lyophilized), 3 x 1.5 mL (reconstituted); ready-to-use reconstitution buffer for AQP4-Biotin, 1 x 10 mL; Streptavidin Peroxidase (SA-POD dilute before use) 1 x 0.8 mL; ready-to-use diluent for SA-POD, 1 x 15 mL; ready-to-use peroxidase substrate (TMB), 1 x 15 mL; concentrated wash solution (dilute 1:10 with deionized water before use), 1 x 120 mL and ready-to-use stop solution, 1 x 14 mL.

J. Standard/Guidance Document Referenced (if applicable):

CLSI EP05-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition

CLSI EP6-A: Evaluation of Linearity of Quantitative Measurement Procedures; A Statistical Approach; Approved Guideline

CSLI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline- Second Edition

CLSI C28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline- Third Edition

K. Test Principle:

The KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay depends on the divalent properties of the AQP4 autoantibodies (AQP4Ab) to form a bridge between AQP4 coated on ELISA plate wells and liquid phase AQP4-biotin. The level of bound AQP4Ab is then quantitated by the sequential addition of streptavidin peroxidase and a chromogenic substrate with reading of final absorbance at b(4) Trad nm. The absorbance of each well is directly proportional to the amount of autoantibody present in the patient samples. Calibrator values are plotted on semi-log graph paper and the antibody concentrations of the controls and patient samples are interpolated from the curve.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision: Precision measurements were conducted according to CLSI EP05-A2. A panel consisting of ten human sera samples with levels of AQP4Ab that cover the measuring range was assayed in duplicate, twice a day, for 20 days with one reagent lot (b(4) Trade Secret). All %CV values were within the manufacturer's pre-determined acceptance limit. The results are summarized in the table below:

Precision								
Mean value (U/mL)	Within-Run		Between-Run		Between-day		Total	
	SD	%CV	SD	%CV	SD	%CV	SD	%CV
2.1	0.2	8.2	0.2	10.2	0.2	7.3	0.3	14.5
2.8	0.2	6.1	0.2	8.2	0.2	6.1	0.3	11.8
3.5	0.2	6.5	0.2	4.5	0.2	6.0	0.4	9.9
4.5	0.3	5.7	0.5	10.8	0.4	8.8	0.7	15.0
4.4	0.2	4.5	0.4	9.5	0.4	8.2	0.6	13.4
7.8	0.4	5.2	0.8	10.6	0.6	8.2	1.1	14.3
9.2	0.5	5.2	0.9	9.4	0.7	7.4	1.2	13.0
32.0	1.1	3.4	3.5	10.9	2.5	7.8	4.4	13.8
70.2	2.9	4.1	4.6	6.6	3.3	4.6	6.7	9.6
72.1	3.6	4.9	2.0	2.8	3.9	5.5	5.3	6.7

Reproducibility: A panel of 50 serum samples with AQP4Ab concentration at various levels across the measuring range was tested in duplicate in one run at three different laboratories (two in the U.K. and one located in the U.S.) with one reagent lot to evaluate the site-to-site reproducibility. The correlation between sites was $R^2 > 0.99$. The results are summarized in the table below:

Site-to-site reproducibility				
Sample	**Regression**	**Slope** (95% CI)	**Y- Intercept** (95% CI)	**R^2**
Laboratory 1 vs. Laboratory 2	y= 0.96x–0.23	0.96 b(4) Trade Secret	-0.23 b(4) Trade Secret)	0.99
Laborator 1 vs. Laboratory 3	y= 1.02x–0.39	1.02 b(4) Trade Secret)	0.39 b(4) Trade Secret)	1.00
Laboratory 2 vs. Laboratory 3	y= 1.02x–0.16	1.0188 b(4) Trade Secret	-0.16 b(4) Trade Secret)	0.99

To evaluate lot-to-lot reproducibility, a panel of 15 samples with AQP4Ab concentration at various levels across the measuring range was assayed with b(4) Trade Secret production lots of the KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay. Mean and %CV for each sample were calculated and %CV values were from 3.22–14.13% for all samples. The results are summarized in the table below:

Lot-to-lot reproducibility				
Sample	**Number of lots**	**Mean** (U/mL)	**SD** (U/mL)	**%CV**
1	b(4) Trade Secret	2.49	0.35	13.98
2		3.48	0.36	10.44
3		4.68	0.21	4.52
4		5.60	0.54	9.70
5		7.04	0.72	10.20
6		13.25	1.52	11.46
7		14.89	1.45	9.72
8		15.49	0.50	3.22
9		27.28	2.90	10.62
10		32.29	3.65	11.29
11		35.52	4.75	13.45
12		53.89	5.98	11.09
13		60.35	8.08	13.38
14		60.44	7.22	11.75
15		63.27	8.94	14.13

b. *Linearity/assay reportable range:*

Linearity: Linearity and recovery characteristics of the KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay were evaluated according to the CLSI EP6-A. b(4) Trade Secret 1.5–79.5 U/mL. Each dilution was tested in duplicate. The observed values were graphed against the calculated values and a linear regression

was performed. Results summarized in the table below were within the manufacturer's pre-determined acceptance criteria of recovery.

Sample pool	Test Range (U/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	% Recovery
1	2.0–10.0	1.00 b(4) Trade Secret)	0.38 b(4) Trade Secret)	0.99	101.67–118.38
2	1.5–20.0	0.95 b(4) Trade Secret	0.05 b(4) Trade Secret	0.99	90.77–106.74
3	8.0–72.0	0.97 b(4) Trade Secret	-1.42 b(4) Trade Secret	0.99	85.31–112.29
4	10.0–79.5	0.97 b(4) Trade Secret)	-3.13 b(4) Trade Secret	0.98	80.76–101.40

The assay is linear from 1.5–79.5 U/mL. The Package Insert Calculation Section states: *"For samples that result in values greater than the 80 U/mL (greater than the highest calibrator) KRONUS recommends reporting the value as "greater than 80 U/mL".*

Hook effect: Three native serum samples having AQP4Ab concentration above assay measuring range (> 80 U/mL) were serially diluted in assay negative control. No hook effect was observed up to 14000 U/mL.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

i) *Traceability and value assignment:*
There is no recognized standard or reference material for AQP4Ab. The calibrator and control values are directly traceable to a panel of Master Control samples prepared from AQP4Ab positive patient sera that are used to create the linear calibration curves for the KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay.

The calibrators and positive controls are made by b(4) Trade Secret There are five levels with assigned values from 1.5–80.0 U/mL. Newly manufactured AQP4Ab calibrators are checked against a previous lot of calibrators (as a reference) and a panel of Master Control samples. Each calibrator and positive control level must fall within their previously established OD range.

ii) *Assay stability:*

Closed assay stability– An ongoing real-time stability study performed on b(4) Trad b(4) Trade n lots of the KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay supports a shelf-life claim of 4.5 months at 2–8°C.

Open assay stability– A real-time stability study supports the stability of the ELISA strip wells after first opening of the foil pouch at four months at 2–8°C. The package insert recommends that unused wells be placed in the self-seal plastic bag provided. Opened and diluted SA-POD and Concentrated Wash Solution are stable at 2–8°C for up to 16 weeks and 11 months, respectively. The AQP4-Biotin is single-use and to be reconstituted immediately before use. Opened calibrators and controls are stable for three months at 2–8°C.

iii) Sample stability and storage:

The package insert recommends that sera be assayed soon after separation or stored in aliquots at or below -20°C.

d. Detection limit:

The analytical sensitivity was determined in accordance with CLSI EP17-A2.
The limit of blank (LoB) was determined by testing b(4) Trade Secret . The higher LoB calculated from the b(4) lots is b(4) U/mL.
The limit of detection (LoD) was determined by testing b(4) Trade Secret The higher LoD calculated from the b(4) lots is b(4) U/mL.
The limit of quantitation (LoQ) was calculated using within-laboratory precision as the acceptance goal. Using an accuracy goal of 20%CV, the higher LoQ calculated from the b(4) lots is b(4) L.

e. Analytical specificity:

i) Endogenous interference:

Interferences were assessed by testing 5–11 samples with AQP4Ab concentration that covers the entire assay measuring range. Each sample was spiked with known quantities of the interfering substances and analyzed in triplicate, in one assay run, with one assay lot. The recovery was calculated by comparing to control samples spiked with the same volume of diluents. No interference was detected in the samples up to the test concentrations listed in the table below:

Potential Interfering Substances	Test Concentration
Bilirubin	20 mg/dL
Intralipid	3000 mg/dL
Hemoglobin	150 mg/dL
Rheumatoid factor	50 IU/mL

The Package Insert 'Specimen Collection and Handling Section' lists rheumatoid factor (at > 50 IU/mL) and hemoglobin (at > 250 mg/dL) as interfering substances and states *"Do not use lipemic or hemolysed serum samples"*.

ii) Cross-reactivity:

Refer to test results for 483 serum samples from patients in the Non-Target Disease Group in the table presented in the section on Clinical studies.

f. Assay cut-off:

The assay cut-off (greater than or equal to 3.0 U/mL is positive) for the KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay was determined by testing specimens from 509 healthy blood donors in the U.S. Using the 97.5th percentile value plus 2 SD (1.4) for samples > LoD, a cut-off value of 3.0 U/mL was deemed appropriate. Utilizing the 3.0 U/mL cut-off value, 1.86% (9/483) of the samples from patients in the Non-Target Disease Group were positive for AQP4Ab. Given these results and taking into account the analytical sensitivity of the assay, values less than 3 U/mL are considered negative for AQP4Ab, and values greater than or equal to 3 U/mL are considered positive for AQP4Ab.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. Matrix comparison:

Not applicable. Serum is the only recommended matrix. The Package Insert 'Specimen Collection and Handling Section' states "*Do not use plasma in the assay*".

3. Clinical studies:

a. Clinical sensitivity and specificity:

A total of 620 serum samples were included in the clinical validation for the KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay. The validation set of samples includes 85 patients diagnosed with NMO[1], 52 patients diagnosed with NMOSD[1], and 483 samples from patients with multiple sclerosis[2] and other non-target diseases listed in the table below.

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[1]Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG. 2006. Revised diagnostic criteriafor neuromyelitis optica. *Neurology* 66:1485-1489.

[2] Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, Lublin FD, Metz LM, McFarland HF, O'Connor PW, Sandberg-Wollheim M, Thompson AJ, Weinshenker BG, Wolinsky JS. 2005. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Ann Neurol.* 58:840-846.

Non-Target Disease Group			
	n	**#Positive**	**%Positive**
Multiple sclerosis (MS)	**75**	**1**	**1.33**
Relapsing-Remitting (RRMS)	53	1	1.9
Secondary- Progressive (SPMS)	7	0	0
Primary-Progressive (PPMS)	5	0	0
Progressive-Relapsing (PRMS)	10	0	0
Stroke	**21**	**0**	**0**
Infectious diseases	**119**	**5**	**4.20**
Lyme disease	30	1	3.33
Shingles	10	0	0
Syphilis	30	4	13.33
Human Immunodeficiency Virus (HIV)	10	0	0
Hepatitis B	31	0	0
Tuberculosis	8	0	0
Autoimmune diseases	**268**	**3**	**1.12**
Sjogren's Syndrome	30	2	6.67
Systemic Lupus Erythematosus (SLE)	30	0	0
Systemic Vasculitis	4	0	0
Sarcoidosis	6	0	0
Graves' disease	110	0	0
Type 1 Diabetes	33	0	0
Rheumatoid Arthritis	17	1	5.9
Hashimoto's Disease	16	0	0
Addison's Disease	12	0	0
Myasthenia Gravis	10	0	0
Combined total non-target diseases	**483**	**9**	**1.86**

Using a cut-off of 3 U/mL, 69 (81%) of the 85 NMO patient samples and 25 (48%) of the 52 NMOSD tested positive. When combined, 94 (68.6%) of the 137 NMO/NMOSD samples tested positive.

One of 75 MS samples (1.3%) was positive for AQP4Ab. None of the 21 samples from patients with stroke were positive for AQP4Ab. Eight of 387 samples (2.1%) from patients with either infectious or autoimmune diseases tested positive for AQP4 Ab. Four of the 30 samples (13.33%) from patients with syphilis were positive. The package insert states that "*a current or past syphilis diagnosis may increase risk of obtaining a false positive result.*"

Clinical sensitivity, specificity and overall agreement in this sample cohort are summarized in the following tables:

	Diagnosis		
KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay	**Positive** NMO	**Negative** (Non-Target Diseases)	**Total**
Positive	69	9	78
Negative	16	474	490
Total	85	483	568
Sensitivity: 81.2% (69/85) 95% CI: 71.6%–88.1% Specificity: 98.1% (474/483) 95% CI: 96.5%–99.0% Overall agreement: 95.6 % (543/568) 95% CI: 93.6%–97.0%			

	Diagnosis		
KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay	**Positive** NMOSD	**Negative** (Non-Target Diseases)	**Total**
Positive	25	9	34
Negative	27	474	501
Total	52	483	535
Sensitivity: 48.1% (25/52) 95% CI: 35.1%–61.3% Specificity: 98.1% (474/483) 95% CI: 96.5%–99.0% Overall agreement: 93.2% (499/535) 95% CI: 90.8%–95.1%			

	Diagnosis		
KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay	**Positive** NMO or NMOSD	**Negative** (Non-Target Diseases)	**Total**
Positive	94	9	103
Negative	43	474	517
Total	137	483	620
Sensitivity: 68.6% (94/137) 95% CI: 66.4%–75.8% Specificity: 98.1% (474/483) 95% CI: 96.5%–99.0% Overall agreement: 91.6% (568/620) 95% CI: 89.2%–93.6%			

b. Other clinical supportive data (when a. is not applicable):

Not applicable.

4. Clinical cut-off:

   Refer to assay cut-off.

5. Expected values/Reference range:

   The expected value from 509 healthy individual blood donors was determined in accordance with CSLI C28-A3c. The AQP4Ab values in normal healthy sera (NHS) ranged from <1.04 - 4.20 U/mL. Two NHS samples (0.4%) were positive for AQP4Ab. The 97.5th percentile value for all samples was 1.55 U/mL. Similar values were obtained when evaluations were based on gender, age and sex. It is the responsibility of each laboratory to establish its own reference ranges for the population of patients it serves, as expected values are affected by many different factors.

Frequency of NHS AQP4Ab Values by Gender								
Gender	< 1.04 U/mL	1.04–1.49 U/mL	1.50–1.99 U/mL	2.00–2.49 U/mL	2.50–2.99 U/mL	3.00–3.49 U/mL	3.5–3.99 U/mL	> 3.99 U/mL
Male	0.96	0.02	0.01	0	0	0	0	0.01
Female	0.91	0.02	0.06	0.01	0	0	0	0

Frequency of NHS AQP4Ab Values by Age								
Age	< 1.04 U/mL	1.04–1.49 U/mL	1.50–1.99 U/mL	2.00–2.49 U/mL	2.50–2.99 U/mL	3.00–3.49 U/mL	3.5–3.99 U/mL	> 3.99 U/mL
< 20	0.93	0.04	0.04	0	0	0	0	0
20-39	0.95	0.02	0.02	0.01	0	0	0	0.01
40-59	0.95	0.05	0.03	0	0.01	0	0	0
> 59	0.95	0.03	0.03	0	0	0	0	0

Frequency of NHS AQP4Ab Values by Race								
Race	< 1.04 U/mL	1.04–1.49 U/mL	1.50–1.99 U/mL	2.00–2.49 U/mL	2.50–2.99 U/mL	3.00–3.49 U/mL	3.5–3.99 U/mL	> 3.99 U/mL
Black	0.98	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Hispanic	0.91	0.04	0.02	0.00	0.02	0.00	0.00	0.02
Caucasian	0.91	0.04	0.04	0.01	0.00	0.00	0.00	0.01
Unknown	0.88	0.13	0	0	0	0	0	0

M. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809 and the special controls for this device type.

N. Identified Risks and Required Mitigations:

Identified Risks to Health	Required Mitigations
Inaccurate test results that provide false positive or false negative results can lead to improper patient management	Special controls (1), (2), and (3)
Failure to correctly interpret test results can lead to false positive or false negative results	Special controls (1) (iii), (2), and (3)

O. Benefit/Risk Analysis:

Summary	
Summary of the Benefit(s)	This is the first serological test to the aid in the diagnosis of NMO/NMSD to be commercially available in the U.S., providing a clinically important unmet medical need. This is of benefit to patients by providing the possibility of earlier diagnosis of NMO/NMOSD and earlier initiation of appropriate therapy. The presence of the antibody is useful in helping to distinguish NMO/NMOSD from MS, which is important because the drug treatment for NMO/NMOSD and MS differ.

Summary	
Summary of the Risk(s)	The primary risks to patients are related to the consequences of clinical decisions based on false negative and false positive results for a patient due to inaccurate test results or failure to correctly interpret test results. For a false positive result, the risks could include unnecessary testing or inappropriate treatment related to an inaccurate result. For a false negative result, the risk could include a missed or delayed diagnosis. The results from this test would be used with results from other clinical, laboratory, and radiological (e.g., MRI) findings, which would mitigate these risks. The risk to patients of false negative and false positive results are also mitigated by statements in the indications for use statement which states that the assay is not to be used alone and is to be used in conjunction with other clinical, laboratory, and radiological (e.g. MRI) findings. The risks are further mitigated by the special controls established for this device. The test requires that a blood sample be obtained during routine phlebotomy. This is standard procedure in clinical care, and the risk to patients is minimal. The risk to laboratory workers is no greater than that for the routine collection and handling of blood specimens, given that the test is for use by laboratory professionals in a clinical laboratory setting.
Summary of Other Factors	None
Conclusions Do the probable benefits outweigh the probable risks?	Given the well characterized performance characteristics, statements in the indications for use statement, the combination of required general controls and the special controls established for this device, the performance data, warnings, and precautions and limitations required in the labeling, the probable benefits outweigh the probable risks for this device.

P. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 866.5665. FDA believes that the stated special controls and applicable general controls, including design controls, provide reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code:	PNI
Device Type:	Aquaporin-4 autoantibody immunological test system
Class:	II (special controls)
Regulation:	21 CFR 866.5665

(a) *Identification*. An Aquaporin-4 autoantibody immunological test system is a device that consists of reagents used to measure by immunochemical techniques autoantibodies in human serum samples that react with Aquaporin-4 (AQP4Ab). The measurements aid in the diagnosis of Neuromyelitis Optica (NMO) and Neuromyelitis Optica Spectrum Disorders (NMOSD) in conjunction with other clinical, laboratory, and radiological (e.g., MRI) findings.

(b) *Classification*. Class II (special controls). An Aquaporin-4 autoantibody immunological test system must comply with the following special controls:

1) Premarket notification submissions must include the following information:

i) A detailed device description including:
   A) A detailed description of all components including all required ancillary reagents in the test.
   B) If applicable, a detailed description of instrumentation and equipment, including illustrations or photographs of non-standard equipment or manuals.
   C) If applicable, detailed documentation of the device software, including, but not limited to, standalone software applications and hardware-based devices that incorporate software.
   D) A detailed description of appropriate internal and external quality controls that are recommended or provided. The description must identify those control elements that are incorporated into the specified testing procedures.
   E) Detailed specifications for sample collection, processing, and storage.
   F) A detailed description of methodology and assay procedure.
   G) A description of how the assay cut-off (the medical decision point between positive and negative) was established and validated as well as supporting data.
   H) Detailed specification of the criteria for test results interpretation and reporting.

ii) Detailed information demonstrating the performance characteristics of the device, including:
   A) Device precision/reproducibility data generated from within-run, between-run, between-day, between-lot, between-site, and total precision for multiple nonconsecutive days, as applicable. A well characterized panel of patient samples or pools from the indicated population that covers the device measuring range must be used.
   B) Device linearity data generated from samples covering the device measuring range, if applicable.
   C) Information on traceability to a reference material and description of value assignment of calibrators and controls, if applicable.
   D) Device analytical sensitivity data, including limit of blank, limit of detection, and limit of quantitation, if applicable.

E) Device analytical specificity data, including interference by endogenous and exogenous substances, as well as cross-reactivity with samples derived from patients with other autoimmune diseases or conditions.
F) Device instrument carryover data, when applicable.
G) Device stability data, including real-time stability under various storage times and temperatures.
H) Specimen stability data, including stability under various storage times, temperatures, freeze-thaw, and transport conditions, where appropriate.
I) Method comparison data generated by comparison of the results obtained with the device to those obtained with a legally marketed predicate device with similar indications of use. A well-characterized panel of patient samples from the indicated population covering the device measuring range must be used.
J) Specimen matrix comparison data, if more than one specimen type or anticoagulant can be tested with the device. Samples used for comparison must be from well-characterized patient samples covering the device measuring range.
K) Clinical performance must be established by comparing data generated by testing samples from the indicated population and the differential diagnosis or non-target disease groups with the device to the clinical diagnostic standard.
    (1) The diagnosis of NMO and NMOSD must be based on clinical findings, laboratory tests (e.g., serological tests), and radiological tests (e.g., Magnetic Resonance Imaging).
    (2) The differential diagnosis or non-target disease group must include the applicable diseases or conditions, including but not be limited to the following: multiple sclerosis, stroke, lyme disease, shingles, syphilis, human immunodeficiency virus, hepatitis B, tuberculosis, Sjörgen's Syndrome, systemic lupus erythematous, systemic vasculitis, sarcoidosis, Graves' disease, Hashimoto's disease, Type I diabetes, rheumatoid arthritis, Addison's disease, and Myasthenia Gravis.
    (3) Diagnosis of diseases or conditions for the differential or non-target disease groups must be based on established diagnostic criteria and clinical evaluation.
    (4) For all samples, the diagnostic clinical criteria and the demographic information must be collected and provided.
    (5) The clinical validation results must demonstrate clinical sensitivity and clinical specificity for the test values based on the presence or absence of NMO and NMOSD.
    (6) The data must be summarized in tabular format comparing the interpretation of results to the disease status.
L) Expected/ reference values generated by testing an adequate number of samples from apparently healthy normal individuals.

iii) Identification of risk mitigation elements used by the device, including

description of all additional procedures, methods, and practices incorporated into the directions for use that mitigate risks associated with testing.

2) The device's 21 CFR 809.10(b) compliant labeling must include warnings relevant to the device including:

   i) A warning statement that reads "The device is for use by laboratory professionals in a clinical laboratory setting."

   ii) A warning statement that reads "The device is not to be used as a stand-alone device but as an adjunct to other clinical information. A diagnosis of Neuromyelitis Optica (NMO) and Neuromyelitis Optica Spectrum Disorders (NMOSD) should not be made on a single test result. The clinical symptoms, results from physical examination, laboratory tests (e.g., serological tests), and radiological tests (e.g. Magnetic Resonance Imaging), when appropriate, should always be taken into account when considering the diagnosis of NMO and NMOSD."

3) The device's 21 CFR 809.10(b) compliant labeling must include a detailed description of the protocol and performance studies performed in accordance with special control (1)(ii) and a summary of the results.