

Gold Dot NR2 Antibody ELISA
Catalog Number GD1-001 REF

**ELISA for In Vitro Quantitative Determination of Antibodies
to NR2 Subunit of NMDA Receptor in Serum**

For *In Vitro* Diagnostic Use in CE Marking Countries IVD

For Research Use Only in USA

Store all test components at 2°C – 8°C

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Read this package insert completely before using the product. Follow the instructions carefully when performing tests. Failure to follow the instructions may result in inaccurate results.



1. Intended Use

The Gold Dot NR2 Antibody assay is a serological enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of antibodies to the NR2 subunit of human NMDA glutamate receptor in serum. The assay is intended to be used in conjunction with clinical evaluation and radiological methods for diagnosis of transient ischemic attack (TIA) and ischemic stroke vs stroke-like disorders. The Gold Dot NR2 Antibody assay correlates with prior isolated or multiple ischemic stroke and increased risk of near-term cerebral ischemic events in patients with pre-existing conditions (diabetes, hypertension, and atherosclerosis, history of alcohol and nicotine consumption, open heart surgery).¹⁻⁶ The test allows ischemic stroke to be differentiated from hemorrhagic stroke.²

2. Summary and Explanation

Thrombotic or embolic processes restrict neuronal oxygen and glucose supply, leading to cerebral ischemia.⁶ Excessive release of glutamate due to cerebral ischemia causes over-activation of NMDA receptors. These major excitatory neuroreceptors regulate neuronal electrical signals and microvessel function. Abundant amounts of NMDA receptors (especially NR2 subunit) are cleaved by serine proteases, resulting in NR2 peptide fragments. These fragments pass through the compromised blood brain barrier and enter the bloodstream, activating the immune system and generating antibodies. The antibodies to NR2 are detectable 3-6 months after prior isolated or multiple ischemic stroke and their presence predicts an increased risk for near-term TIA and ischemic stroke. Monitoring blood samples within 3-8 hours of a TIA/stroke can help differentiate ischemic cerebrovascular events from hemorrhagic and non-stroke events.

The connection between neurotoxicity and cerebral ischemia has been described in recent studies.^{2,4} NR2 antibody concentrations in healthy adults are <1.7 ng/mL. Elevated concentrations of NR2 antibodies (ie, above 2.0 ng/mL) correlate with the presence of prior isolated or multiple cerebral ischemia and increased risk of near-term ischemic stroke. Results of several studies have shown that NR2 antibodies are strong predictors of TIA/stroke.

Reference values for the NR2 antibody assay are presented in the table below. Preexisting conditions include hypertension (controlled: systolic pressure above 140/diastolic pressure above 90), diabetes (Type 1 and 2, HbA1C >8) atherosclerosis (cholesterol >240 mg/dL, LDL >160 mg/dL), arterial fibrillation, open heart surgery, and history of alcohol and nicotine addiction without psychiatric illness.

Reference Values of Gold Dot NR2 Antibody Assay

Normal	Disease
≤ 2.0 ng/mL	> 2.0 ng/mL

3. Principle of the Test

Concentrations of NR2 antibodies are determined immunochemically in a serological assay. NR2 peptide, the fragment of NMDA receptors, is coated on the solid phase of a microtiter



plate (MTP). In a first incubation step, antibodies in the sample react with the solid phase bound NR2 peptide. After intensive washing, the antibodies captured on the MTP react with horseradish peroxidase labeled Protein A (Protein A, HRP).

//-NR2 Peptide ← Serum Antibodies ← Protein A-HRP

The immunocomplex that is formed is quantitatively determined via HRP/TMB-detection reaction. An acidic stopping solution is then added. The color converts from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of NR2 antibodies in the sample. A dose response curve of the absorbance measured at 450 nm or using dual wave measurement at 450 nm and 630 nm vs concentration is generated. NR2 antibody concentrations in the diluted serum samples are determined directly from this calibration curve.

4. Reagents and Materials

Materials supplied with the kit

The reagents in one kit are sufficient for 41 determinations in duplicate. Each test kit contains:

Product Number	Kit Components	Quantity
GD1-C01	① Microtiter plate (MTP) with strips precoated with NR2 synthetic peptide	1 (96 wells)
UP-003	② Working buffer	1 tablet
GD1-C02 - C06	③ Calibrators, ready to use (C1 – 0 ng/mL, C2 – 1.5 ng/mL, C3 – 3.0 ng/mL, C4 – 6.0 ng/mL, C5 – 12.0 ng/mL)	5 x 1.3 mL
GD1-C07	④ Negative control (N: 1.56 ng/mL), ready-to-use	1.3 mL
GD1-C08	⑤ Positive control (P: 8.4 ng/mL), ready-to-use	1.3 mL
UP-004	⑥ Protein A-HRP reagent (Protein A labeled with horseradish peroxidase 1000X)	150 µL
GD1-C09	⑦ Ancillary buffer 10X	1.5 mL
UP-001	⑧ 3,3',5,5'-Tetramethylbenzidine (TMB) liquid substrate system for ELISA, ready-to-use solution	15 mL
UP-002	⑨ Stop reagent for TMB substrate	0.6 g
N/A	⑩ Package insert	1

Materials required but not supplied

- Distilled or deionized water
- Precision pipettes calibrated to deliver 10-1000 µL
- A multi-channel dispenser or repeating dispenser
- Absorbent paper
- Vortex-mixer
- Plate shaker with temperature control
- Microtiter plate (MTP) reader



5. Warnings and Precautions

- For in vitro diagnostic use in CE marking countries.
- For research use only in USA
- Treat all blood samples as potentially biohazardous material.
- ③ Calibrators and ④ ⑤ controls contain monoclonal antibodies as rodent source material.
- ⑧ 3,3',5,5'-Tetramethylbenzidine (TMB) liquid substrate system for ELISA. If exposed to skin, immediately flush area with soap and abundant amounts of water. If eyes are exposed, flush with abundant amounts of water. Assure adequate flushing by separating the eyelids with fingers. If swallowed, wash out mouth with water. Seek immediate medical attention. If inhaled, get access to fresh air. If breathing becomes difficult, call a physician.
- ⑧ 3,3',5,5'-Tetramethylbenzidine (TMB) liquid substrate system for ELISA is light sensitive and should be protected from light.
- ⑨ Stop reagent for TMB substrate. If swallowed, wash out mouth with water. In case of skin contact, flush with abundant amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. In case of contact with eyes, flush with abundant amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. If inhaled, get access to fresh air. If experiencing breathing difficulties, give oxygen. Call a physician in all cases.
- Dispose of test kits and reagents in a manner consistent with relevant regulations.
- Do not use reagents past their expiration dates.
- Do not mix components from different lots of Gold Dot NR2 Antibody test kits.
- Exposure of samples to room temperature should be minimized to less than 3 hours (including blood draw, processing and transport time). This does not include incubation on the ELISA plate.
- It is strongly recommended that the “positive” and “negative” controls (provided with the kit) be included in each patient run. If control values are not within accepted limits, repeat the assay. Additional quality control testing may be necessary according to local requirements.

6. Reagent Preparation and Storage

Upon receipt, store unopened test kits at 2°C–8°C. Once opened, keep the microwell strips sealed in the foil pouch with desiccant to minimize exposure to moisture. Once opened, test kits will remain stable until the indicated expiration date, provided they are stored as indicated.

Dissolve ② the working buffer tablet in 500 mL of distilled or deionized water. Mix thoroughly on magnetic stirrer to reach complete solubility. Once prepared, the working buffer may be stored refrigerated for up to 30 days.



7. Specimen Collection and Preparation

- Collect blood samples in any serum collection tube (eg, gel separation).
- Centrifuge and separate samples within 3 hours of venipuncture.
- Prepare all samples by removing or separating serum from red blood cells using standard separation procedures.
- Store all serum samples refrigerated (2°C–8°C) immediately upon collection; however, NR2 antibodies in samples are stable for up to 3 hours at room temperature.
- Test serum within 3 days of collection when stored at 2°C–8°C.
- For longer storage up to 3 months, freeze processed aliquoted samples at or below –20°C. For longer storage, freeze samples at –80°C.
- Processed serum samples may be frozen and thawed up to two times without significantly affecting the amount of NR2 antibodies.
- When transporting samples, ship samples at 2°C–8°C on ice packs.

8. Assay Procedure

Preparatory Steps

1. Working buffer ②.
 - Dissolve ② the working buffer tablet in 500 mL of distilled or deionized water. Mix thoroughly on magnetic stirrer to reach complete solubility. Once prepared, the working buffer may be stored refrigerated for up to 30 days.
2. Serum samples.
 - Thaw the serum samples at 2°C–8°C. Do not thaw the samples at room temperature or in a warm water bath.
 - Note: it takes about 4 hours to thaw 1 mL of serum sample from –80°C to +4°C.
 - Dilute serum samples 1:50 in the working buffer. Pipette 980 µL of working buffer into 1.5 to 3 mL volume tubes and add exactly 20 µL of each serum sample. Mix thoroughly. Avoid foaming.
3. Microtiter plate.
 - Bring the MTP to room temperature.
 - Calculate the number of MTP strips required. It is recommended that each calibrator, control, and serum sample be run in duplicate.
 - Remove the MTP frame with the strips from the foil pouch.
 - Leave the required number of microwell strips in the frame and place the remaining strips back in the pouch. Ensure that the foil pouch is completely resealed with the desiccant. Store at 2°C–8°C.
4. Stop reagent ⑨. Add 15 mL deionized or distilled water to the white bottle. Mix well for at least 10 minutes. Once dissolved, the stop reagent may be refrigerated for up to 30 days.
5. Ancillary buffer. Dilute the entire amount (1.5 mL) of 10X ancillary buffer ⑦ in 13.5 mL of working buffer for a final concentration of 1X.
6. The minimal volume per one strip is 800 µL of each of the Protein A-HRP, TMB, and stop solution reagents plus at least 200 µL of dead volume.

Note: It is recommended but not essential to use any standard mechanical 8 or 12 channel pipetter.



Number of strips	Minimal volume (required + dead volume)
1	1000 µL
2	1800 µL
3	2600 µL
4	3400 µL
5	4200 µL
6	5000 µL
7	5800 µL
8	6600 µL
9	7400 µL
10	8200 µL
11	9000 µL
12	9800 µL

Procedure

1. Wash the MTP with 200 µL of working buffer once for 5 minutes at room temperature on a shaker. Discard content and tap the inverted plate against a paper towel placed on a flat surface.
2. Calibrators, controls, and sample incubation
 - Vortex calibrators and controls vials gently for 2 seconds.
 - Pipette 100 µL calibrators, negative (N), positive (P) controls, and prepared serum samples (S1-S41) into each well according to the following scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	C1	C1	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
B	C2	C2	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
C	C3	C3	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
D	C4	C4	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
E	C5	C5	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
F	N	N	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
G	P	P	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
H	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41

- If using only one sample, use this scheme:

	1	2
A	C1	C1
B	C2	C2
C	C3	C3
D	C4	C4
E	C5	C5
F	N	N
G	P	P
H	S1	S1

- Incubate MTP at 37°C for 30 minutes on a shaker.
 - During the MTP incubation, prepare 1:1000 (v:v) dilution of Protein A-HRP ⑥ in 1X ancillary buffer.
 - For example, to prepare a sufficient amount of Protein A-HRP working solution for 12 strips, mix 15 µL Protein A-HRP ⑥ with 15 mL of 1X ancillary buffer.
 - Mix thoroughly using a vortex-mixer. The Protein A-HRP working solution is stable for only 1 hour.
 - The Protein A-HRP working solution cannot be stored.
 - After 30 minutes of MTP incubation at 37°C, discard the content and tap the plate. Wash with 200 µL of working buffer 3 times for 5 minutes on a shaker. Discard the content and tap inverted plate against the paper towel placed on a flat surface.
 - Add 100 µL Protein A-HRP working solution (from Step 4) into each well.
 - Incubate the plate at 37°C for 30 minutes on a plate shaker. Discard the content and tap the plate as mentioned above in Step 5.
 - Wash 3 times with 200 µL working buffer at 37°C for 3 minutes on a shaker. Discard content and tap the plate each time as in Step 5.
 - Wash 1 time with 200 µL deionized or distilled water at 37°C for 1 minute on a shaker. Discard content and tap the plate. **DO NOT USE TAP WATER.**
 - Add 100 µL TMB substrate into each well. Gently shake the plate to ensure mixing and incubate at room temperature for 10 minutes in darkness to achieve color development. Do not use the shaker. DO NOT discard the contents.
 - Add 100 µL Stop Reagent ⑨ per each well to stop the reaction. The blue color should change to yellow. Gently shake the plate on a flat surface for 20-30 seconds to ensure mixing.
- NOTE: Make sure that the blue color completely changes to yellow.
- Wipe moisture from the bottom of the plate using a paper towel.
 - Read the optical density (O.D.) within 10 minutes at 450 nm/630 nm dual wave using a microplate reader. Subtract the optical density at 630 nm from OD at 450 nm.



9. Procedural Notes

- Store all test reagents at 2°C–8°C. Allow the reagents to equilibrate to room temperature prior to use (about 40 min).
- Bring the MTP to room temperature. Store the unused strips in the re-sealable foil pouch with desiccant to minimize exposure to moisture.
- Optional: Use MTP lid (not supplied) to reduce evaporation.
- Calibrator set ③ is used to generate a standard curve with 5 points, ranging from 0 ng/mL –12.0 ng/mL.
- The volume of working buffer required during the washing procedure is 200 µL per well.
- The standard volume of all other solutions added to each well is 100 µL.
- Discard the contents from the MTP. Then, with the MTP inverted, tap it 3 times on a paper towel placed on a flat surface.
- **DO NOT ALLOW THE MICROWELL STRIP TO DRY DURING THE PROCEDURE.** This affects the accuracy of the results.
- For accurate measurement, dispensing of samples, calibrators, and controls must be precise. Pipette carefully using only calibrated equipment.
- Always have the next reagent ready 2–3 minutes prior each next step.

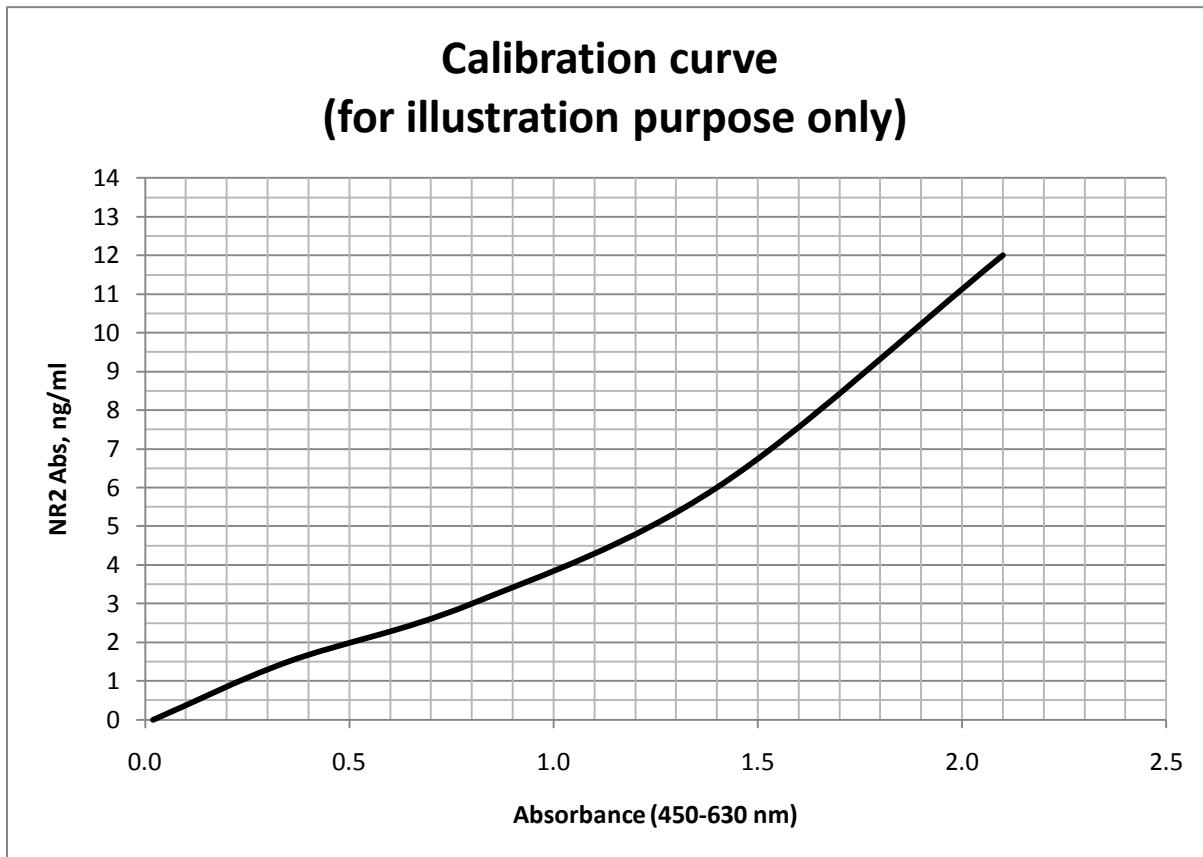
10. Calculation of Results

1. Draw a standard calibration curve by plotting the mean absorbance obtained for each calibrator on the x-axis versus the NR2 antibody concentration in ng/mL on the y-axis. Use a point-to-point curve fit with appropriate computer software to construct the standard calibration curve.
2. Using the mean absorbance value for each sample, determine the corresponding concentration of NR2 antibodies in ng/mL from the calibration curve.

Example of calibration curve

Results of a typical standard calibration curve with O.D. readings at 450–630 nm are shown below (see page 9). A standard calibration curve should be generated by the user for each assay performed.

	Range of Absorbance (O.D. at 450 - 630 nm)	NR2 antibodies, ng/mL
Calibrator #1	< 0.05	0
Calibrator #2	0.2 – 0.5	1.5
Calibrator #3	0.6 – 1.0	3.0
Calibrator #4	1.2 – 1.6	6.0
Calibrator #5	1.9 – 2.3	12.0



11. Limitations

Procedure

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
- The washing procedures are critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
- Samples with NR2 antibodies concentrations greater than the highest calibrator should be diluted with the working buffer and retested.

Clinical Interpretation

- NR2 antibodies levels should be interpreted in conjunction with clinical findings and other diagnostic tests (MRI/DWI).

12. Expected Values

Samples from apparently healthy males (n=89) and apparently healthy females (n=88) in the clinically relevant age range of 48–80 years, were evaluated with the Gold Dot NR2 Antibody test. The reference population was represented by the following ethnic backgrounds: African-American (n=51), Caucasian (n=122), Hispanic (n=1), and Asian (n=3). All participants in the study were recruited between January 2006 and January 2007. The protocol was approved by DeKalb Medical Center (Atlanta, GA, USA) Institutional Review Board. Informed consent was obtained from each subject. Blood samples were drawn from participants on enrollment.

The distribution of healthy controls according to NR2 antibody cutoffs

NR2 antibody cutoffs, ng/mL	Percentile %	Number of patients in each distribution
<1.35	45.20	80
[1.35, 1.6]	67.80	40
[1.6, 2]	92.09	43
>2	100	14
Total	-	177

The reference interval calculated from the samples (92%) was found to be 0.87–2.0 ng/mL. These ranges are provided as guidelines only and are not intended to address “critical values” or medical decision limits. Each laboratory should establish its own reference intervals. Guidance for establishing reference intervals can be found in NCCLS Standard C28-A2 (*How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline—Second Edition*).

Recent studies¹⁻³ have demonstrated an increased risk of cerebrovascular disease (TIA and ischemic stroke) associated with NR2 antibody values >2.0 ng/mL (upper 90% of populations with stroke-like symptoms) vs <2.0 ng/mL (lower 90% of the studied populations with non-stroke and non-TIA). Therefore, a more conservative approach for identifying individuals with a significantly increased risk for TIA and ischemic stroke attributable to NR2 antibodies may be the 90th percentile value of the population. In the current Gold Dot NR2 Antibody test, this threshold value corresponds to 2.0 ng/mL.

13. Quality Control

The manufacturer recommends use of control samples for quality control purposes. Control samples should be analyzed with each run of the calibrators and patient samples. The O.D. mean value for negative control sample should be ≤ 0.4 (≤ 1.67 ng/mL) and, for the positive control sample, 1.5–1.9 (between 6.7 ng/mL and 10.1 ng/mL). In assays in which one or more of the quality control sample values lie outside the acceptable limits, the results for the patient sample may not be valid.

14. Performance Characteristics

Sensitivity

The minimum detection limit is 0.6 ng/mL, as calculated by interpolation of the mean plus 2 standard deviations of 20 replicates of the 1.5 ng/mL Gold Dot NR2 Antibody Calibrator #2.

Assay Precision

Total and within-run precision was determined by testing the matrix control, containing monoclonal antibodies against NR2 peptide Calibrator #5, 12 ng/mL, and two human serum controls with normal and high NR2 antibody concentrations, according to NCCLS Guidelines EP5-A. The samples were assayed in quadruplicate, using a single lot of reagents for 20 days. A five-point calibration curve was run in duplicate.

Sample Name	ng/mL	OD	Total % CV	Within-run % CV
Calibrator #5, 12 ng/mL	N/A	2.17 ± 0.091	6.9	1.5
Serum control low anti-NR2 activity	1.64 ± 0.03	N/A	8.9	5.0
Serum control high anti-NR2 activity	20.28 ± 0.41	N/A	9.2	5.6

Linearity

Six serum samples with known high NR2 antibody levels were intermixed with six serum samples with known low NR2 antibody levels. Percent recovery was determined as the measured value divided by the expected value, multiplied by 100. The average recovery was 100%, demonstrating linearity of the diluted samples over a range of 1.2–10.56 ng/mL NR2 antibodies.

Interfering Substances

Five endogenous blood substances were evaluated for potential assay interference. Five individual serum samples with NR2 antibody values ranging from 1.44–1.80 ng/mL were spiked, each with one of the substances. No appreciable interference was observed at spiked levels of 500 mg/dL hemoglobin, 3000 mg/dL triglycerides, 500 mg/dL cholesterol, 20 mg/dL bilirubin, and a total of ~6 g/dL albumin.

Cross-Reactivity

Cross-reactivity with other antigens was determined by assessing samples both with NR2-coated MTPs and with MTPs coated with other brain antigens. The results show no interference with other brain antigens.

Antibodies against receptor fragments:

μ-opioid peptide	0 %
δ-opioid peptide	0 %
GluR4-peptide	0 %
NR1-peptide	0 %
D2-peptide	0 %
D3-peptide	0 %
D4-peptide	0 %

15. Clinical Studies

Evaluation of TIA and ischemic stroke in the Emergency Room

The purpose of this study was to compare NR2 antibody concentrations in patients presenting with a history of TIAs or strokes and in control group to determine whether NR2 antibody level could be considered to be the main predictor variable for TIA and stroke.³ We observed patients presenting with symptoms of recent TIA, acute stroke and a discharge diagnosis of stroke at DeKalb Medical Center (DMC), Decatur, GA, USA. We included historical and imaging measures of recurrent stroke and compared these to antibody levels as a part of ongoing acute stroke research using the Gold Dot NR2 Antibody test. For controls, we used healthy subjects without a history of stroke. Of the 100 (54 female, 46 males) patients with cerebrovascular events, 89 were determined to have acute or prior ischemic stroke (45 female, 44 males), 8 had TIA, and 1 had intracerebral hemorrhage (ICH). The control group included age/gender matched 48 healthy subjects including 15 and 6 individuals with controlled hypertension and diabetes mellitus, respectively. All participants in the study were recruited between January 2006 and January 2007. The protocol was approved by DMC IRB. Informed consent was obtained from each subject or from a family member as appropriate.

Some patients from the stroke group had extremely large areas of damage (n=4), with volumes >70 cc, and two patients had small volumes of 2 cc that corresponded to NR2 antibody concentrations <1.5 ng/mL (norm). In a previous clinical study,² analysis of the lesion volumes defined by multimodal MRI scans and NR2 antibody concentrations in stroke patients showed significant correlations only for lesion volumes of 10-30 cc. The detailed analysis of five different cutoff values for NR2 antibody concentrations detected in stroke patients with lesion volumes within 5-70 cc is presented in Table 1. Calculations demonstrated the best test sensitivity (95.9%) at a cutoff value of 2.0 ng/mL, while the risk ratio was highest (3.45) at the same cutoff value as reported above (1.6 ng/mL).

Table 1. NR2 antibody results subdivided by different cutoffs for stroke group (lesion volumes >5 cc and <70 cc)

NR2 antibody ng/mL cutoffs	Stroke n/N (%)	No stroke n/N (%)	Risk Ratio
≥1.5	81/99 (81.8%)	18/99 (18.2%)	2.93
<1.5	12/43 (27.9%)	31/43 (72%)	
≥1.6	80/91 (87.9%)	11/91 (12%)	3.45
<1.6	13/51 (25.5%)	38/51 (74.5%)	
≥1.7	77/84 (91.7%)	7/84 (8.3%)	3.32
<1.7	16/58 (27.6%)	42/58 (72.4%)	
≥1.9	73/80 (91%)	7/80 (8.8%)	2.83
<1.9	20/62 (32.3%)	42/62 (67.7%)	
≥2.0	71/74 (95.9%)	3/74 (4%)	2.97
<2.0	22/68 (32.4%)	46/68 (67.6%)	

The preliminary analysis of Gold Dot NR2 Antibody test performance for TIA assessment had the highest risk ratio of 32.9 at a cut-off value of 2.0 ng/mL (Table 2).

Table 2. NR2 antibody results subdivided by different cutoffs for TIA group

NR2 antibody ng/mL cutoffs	Stroke n/N (%)	No stroke n/N (%)	Risk Ratio
≥1.5	8/26 (30.8%)	18/26 (69%)	N/A
<1.5	0/00	31/31 (100%)	
≥1.6	7/18 (39%)	11/18 (61%)	15.17
<1.6	1/39 (2.6%)	38/39 (97.4%)	
≥1.9	7/14 (50%)	7/14 (50%)	21.50
<1.9	1/43 (2.3%)	42/43 (97.7%)	
≥2.0	7/10 (70%)	3/10 (30%)	32.90
<2.0	1/47 (2%)	46/47 (98%)	

Prediction of Neurological Adverse Events Before Cardiac Surgery

To determine the efficacy of the Gold Dot NR2 Antibody test as a predictor of risk of TIA/stroke, NR2 antibody levels were measured in 1129 banked serum samples from a retrospective, masked, multicenter observational study involving 33 centers in the United States.⁵ Approval was obtained from the Institutional Review Boards of all centers and written informed consent was obtained from all patients. Inclusion criteria included high-risk adult patients (aged >18 years) undergoing cardiac surgery on CPB, such as combined CABG and valve surgery, or urgent and emergent CABG or valve surgery.

Samples used for the Gold Dot NR2 Antibody test were from participants aged 48–80 years. This was a case-cohort study, where samples from all the cases (373) were tested together with 211 appropriately matched healthy participants (controls).

Table 3 presents the preoperative NR2 antibodies at different cutoffs depending on postoperative adverse event. Neurological adverse events group included patients with confusion, TIA and stroke (neurocode of 9 score). Patients who had no neurological event were assigned to group of “No Neuro Event.” Anxiety (neurocode of 1 score) was assigned to the “No Event” category.

Table 3. Neurological complications (confusion, TIA/ Stroke) preoperatively or within 48 hours vs preoperative NR2 antibodies

Preoperative NR2 antibodies, ng/mL	Neuro Event n/N (%)	No Neuro Event n/N (%)
< 1.5	7/213 (3.3%)	206/213 (96.7%)
1.5 to < 2.0	12/159 (7.6%)	147/159 (92.5%)
≥ 2.0	25/26 (96.2%)	1/26 (3.9%)

Table 4 shows a detailed analysis of six different cutoffs for NR2 antibody concentrations, from 1.5 to 2.0 ng/mL. Although the event rate increases in the cutoffs from 1.5 to 2.0 for both groups, it increases faster in the “Neuro Event” group. Therefore, the risk ratio increases significantly over the analyzed range, with the best risk ratio corresponding to 2.0 ng/mL.

Table 4. NR2 antibodies results subdivided by different cutoffs

Pre-Op NR2 antibodies, ng/mL	Neuro Event n/N (%)	No Neuro Event n/N (%)	Risk Ratio ¹ (Lower 95% Bound) ²
< 1.5	8/214 (3.7%)	206/214 (96.3%)	5.2
≥ 1.5	36/184 (19.6%)	148/184 (80.0%)	(2.8)
< 1.6	10/288 (3.5%)	278/288 (96.5%)	8.9
≥ 1.6	34/110 (30.9%)	76/110 (69.1%)	(5.1)
< 1.7	12/319 (3.8%)	307/319 (96.2%)	10.8
≥ 1.7	32/79 (40.5%)	47/79 (59.5%)	(6.4)
< 1.8	17/351 (4.8%)	334/351 (95.2%)	11.9
≥ 1.8	27/47 (57.5%)	20/47 (42.6%)	(7.6)
< 1.9	19/364 (5.2%)	345/364 (94.8%)	14.1
≥ 1.9	25/34 (73.5%)	9/34 (26.5%)	(9.4)
< 2.0	20/373 (5.4%)	353/373 (94.6%)	17.9
≥ 2.0	24/25 (96.0%)	1/25 (4.0%)	(12.4)

¹Ratio of event rate among patients with positive pre-op NR2 antibodies divided by event rate among patients with negative pre-op NR2 antibodies; ²Lower one-sided 95% confidence bound on the risk ratio

A total of 96.0% (24/25) of patients with NR2 antibodies concentrations ≥ 2.0 ng/mL preoperatively had neurological complications within 48 hours post-CPB vs only 5.4% of patients with NR2 antibodies concentrations ≤ 2.0 ng/mL, resulting in a 17.9-fold increase (95% CI, 11.6–27.6) in the predictive ability of a postoperative neurological adverse event.

Presently, at least 30% of patients who undergo cardiac surgery have neurocognitive deficit postoperatively. Based on the obtained likelihood ratio of a neuro event of 17.9, NR2 antibodies concentration ≥ 2.0 ng/mL detected preoperatively will predict neurological complications in 89% of patients after surgery.

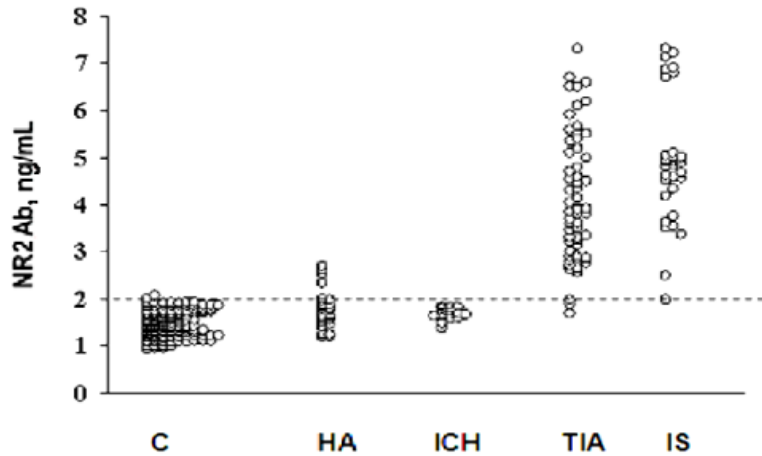
Differentiation of Ischemic Stroke vs Hemorrhagic Stroke and Stroke-like Disorders

Antibodies to NR2 were measured in 360 serum samples: 105 from TIA/stroke patients and 255 from controls, including patients with controlled hypertension/atherosclerosis and gender- and age-matched healthy individuals.²

Figure 1 shows the distribution of serum concentrations of NR2 antibodies in controls (group C) and those with cerebrovascular diseases: hypertension/atherosclerosis (group HA), intracerebral hemorrhage (ICH), transient ischemic attack (TIA), and ischemic stroke as confirmed clinically and/or by MRI/CT. The comparison of mean values of NR2 antibodies in independent age- and gender-matched groups demonstrated that antibody values for the control, hypertension/atherosclerosis, and ICH groups belong to the same distribution. Conversely, significant

differences were observed for patients with cerebral ischemia (TIA/IS) compared with these three groups.

Figure 1. Distribution of serum concentrations of NR2 antibodies in healthy controls (C) and patients with hypertension/atherosclerosis (HA), ICH, TIA and IS



*Dashed line shows cutoff.

NR2 antibodies are independent and sensitive serologic markers capable of detecting TIA with a high posttest probability and, in conjunction with neurologic observation and neuroimaging, ruling out intracerebral hemorrhage.

Conclusions

NR2 antibody strengths

- NR2 antibody is an independent blood-borne marker that rules in cerebral ischemic event and rules out hemorrhage
- NR2 antibody is a marker of neurotoxicity and circulates in the blood 3-6 months after prior isolated or multiple ischemic stroke
- NR2 antibody elevation corresponds to increased risk of near-term ischemic stroke and strongly predicts risk of TIA
- NR2 antibody indicates old ischemic lesions
- NR2 antibody allows follow-up after treatment

NR2 antibody limitations

- NR2 antibody is not sensitive to white matter strokes
- NR2 antibody has low sensitivity to lacunar strokes
- NR2 antibody correlation with size of cortical ischemic stroke limited to areas from 3 cc to approximately 30 cc

16. Product Safety Information

<p>Calibrators, Negative Control, Positive control</p> <p>Xi R36 S24/25-26-46</p>	<p>Stop Reagent for TMB Substrate</p> <p>Xi R36 S24/25-26-46</p>	<p>TMB Liquid Substrate System for ELISA</p> <p>Xi</p>
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S24	Avoid contact with skin
S25	Avoid contact with eyes
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S46	If swallowed, seek medical advice immediately and show this container or label
R36	Irritating to eyes

Symbol Key

Authorized representative	In vitro diagnostic medical device	Batch	Expiry date	Storage conditions	Irritant	Catalog number	European conformity

References

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