ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY NEW YORK SARS-COV MICROSPHERE IMMUNOASSAY FOR ANTIBODY DETECTION (WADSWORTH CENTER AT THE NEW YORK STATE DEPARTMENT OF HEALTH)

For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only

(The New York SARS-CoV Microsphere Immunoassay for Antibody Detection will be performed at the Wadsworth Center at the New York State Department of Health, New York, NY, a laboratory certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a to perform high complexity tests as per Wadsworth Center Diagnostic Immunology Laboratory SOP Dl - 59.0 that was reviewed by the FDA under this EUA.)

INTENDED USE

The New York SARS-CoV Microsphere Immunoassay for Antibody Detection is an immunoassay intended for the qualitative detection of total antibody (IgG, IgM, and IgA) to SARS-CoV-2 in human serum. The New York SARS-CoV Microsphere Immunoassay for Antibody Detection is intended for use as an aid in identifying individuals who may have high levels of SARS-CoV-2-reactive antibodies in their blood that reflect an adaptive immune response to SARS-CoV-2 indicating recent or prior infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. The New York SARS-CoV Microsphere Immunoassay for Antibody Detection should not be used to diagnose acute SARS-CoV-2 infection. Testing is limited to the Wadsworth Center at the New York State Department of Health, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the detection of SARS CoV-2 antibodies. Antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion. The assay does not detect levels of SARS-CoV-2-reactive antibodies below the test cutoff. Sensitivity of the New York SARS-CoV Microsphere Immunoassay for Antibody Detection for PCR-confirmed samples is 88% at 25 days post symptom onset but may be significantly lower for samples obtained earlier.

Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

False positive results for the New York SARS-CoV Microsphere Immunoassay for Antibody Detection may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

The New York SARS-CoV Microsphere Immunoassay for Antibody Detection is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The New York SARS CoV Microsphere Immunoassay (MIA) uses SARS-CoV-l antigen and relies upon the extensive antigenic similarity between SARS-CoV-l and SARS-CoV-2, such that sera from COVID-19 patients are highly cross reactive to SARS-CoV-1 antigens. The target antigen is full-length nucleocapsid (N) protein from SARS-CoV-1 that has 90% sequence homology with SARS-CoV-2 N protein. The recombinant N protein was produced by cloning the full-length sequence with a C-terminal hexahistidine tag into pET-28a(+) plasmid followed by expression in Rosetta™ BL21(DE3)pLysS E.coli cells and purification by metal chelation chromatography on a Ni-NTA column.

The test platform is the Luminex FlexMap dual laser cytometer, which allows analysis of suspension phase immunoassay performance on the surface of polystyrene or magnetic polystyrene microspheres that flow past lasers for fluorescence interrogation. In this assay, purified N antigen is covalently linked to the carboxy residue on the surface of a polystyrene microsphere via a carbodiimide linkage to form a peptide bond. The antigen-coupled beads are mixed with patient serum so that patient antibodies will bind to the antigen. Non-bound antibodies are washed away and then bound antibodies are reacted with a biotinylated secondary anti-human immunoglobulin reagent (reactive with human IgG, IgA, and IgM). After washing, beads are reacted with streptavidin labelled with Red Phycoerythrin (SA-RPE). After washing, beads are resuspended and surface bound antibody is detected with the Luminex analyzer, as the bead suspension passes in a flow cell past the lasers. The fluorescence measured is proportional to the amount of anti-SARS N protein antibodies in the patients' sera.

The assay was developed for use with serum specimens. Plasma specimens as well as samples containing interfering substances such as red cell debris or gross hemolysis samples are not suitable specimens for analysis.

COMPONENTS SPECIFIC TO THE TEST

Components specific to the New York SARS CoV Microsphere Immunoassay for Antibody Detection includes the full-length SARS-CoV N protein (aa 1 – 422) with a C-terminal hexahistidine tag (3.45mg/ml), coupled microspheres (Luminex 100 multi-analyte microspheres with carboxylated surface region), and positive and negative serum controls.

COMPONENTS REQUIRED BUT NOT SPECIFIC TO THE TEST

- Wash buffer (PBS, 0.05% Tween 20, pH 7.4), Sigma P-3563
- PBN (PBS, 1%BSA, 0.05% Azide, pH 7.4), Sigma P-3688 & Sigma S-8032
- Detecting Antibody Conjugate (Goat anti-human IgG/A/M antibody, biotin conjugate, affinity purified, 2mg), Noves by Life Technologies A18851
- SA-PE (Streptavidin, r-phycoerythrin (SAPE), 1mg/ml), Molecular Probes S866
- Calibration microspheres, Luminex F3D-CAL-K25
- Verification microspheres, Luminex F3D-PVER-K25
- Luminex sheath fluid, Luminex 40-5000
- MultiScreenHTS BV Filter Plate, 1.2 µm (Millipore #MSBVN1250)
- 12×75 mm disposable glass culture tubes
- 1 dram glass vial
- 3 dram glass vial
- Single and multi-channel pipettes: 5 µL-1000 µL
- Pipette tips: 5 µL-1000 µL
- Repeater pipette (optional)
- Reagent reservoirs
- Biosafety waste containers
- Absorbent bench pad

CONTROLS TO BE USED WITH THE NEW YORK SARS COV MICROSPHERE IMMUNOASSAY FOR ANTIBODY DETECTION

Positive and negative serum controls are included in each run. For the assay to be considered valid, the median fluorescence intensity (MFI) value of the positive control must be greater than the established cutoff value of the coupled bead lot, and the MFI value of the negative control must be lower than the cutoff value. Cutoff value for the bead lot used is recorded on the QC log and the assay worksheet. In case less than 50 events per bead region are obtained, the sample needs to be repeated to ensure accuracy.

INTERPRETATION OF RESULTS

Ninety samples collected from upstate New York and New York City blood donors prior to 2019 were used to set a low stringency (mean MFI plus 3 SD) and high stringency (mean MFI plus 6 SD) cutoff values.

Result interpretation

Final results are interpreted by the operator based on the MFI values reported by the Luminex instrument. **Table 1** summarizes the interpretation of results obtained with the New York SARS-CoV Microsphere Immunoassay for Antibody Detection.

Table 1. Interpretation of results.

New York SARS-CoV Microsphere Immunoassay Result	Test Result Interpretation
<3SD cutoff MFI	**Negative**: Antibodies to SARS-CoV-2 were not detected.
>3SD cutoff MFI and <6SD cutoff MFI	**Indeterminate**: Results are inconclusive for the presence of SARS-CoV-2 antibodies. No interpretation can be made.
>=6SD cutoff MFI	**Positive**: Antibodies to SARS-CoV-2 were detected.

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

There is no standard reference SARS-CoV-2 antigen material available; accordingly, absolute analytical sensitivity cannot be calculated.

2) Analytical Specificity

Reactivity/lnclusivity
Although mutations in the SARS-CoV-2 genome have been identified as the virus has spread, no serologically unique strains have been described relative to the originally isolated virus (this research is limited at present).

Cross reactivity
Cross-reactivity of the assay was evaluated using a set of 78 serum specimens with known antibodies to a diverse group of viral pathogens (Chikungunya, dengue, HCV, HIV, measles, mumps, rubella, VZV, West Nile virus, herpes simplex, Zika virus, enteroviruses, HBV, cytomegalovirus, Epstein Barr, Eastern Equine Encephalitis, and Yellow Fever viruses), as well as sera with Antinuclear antibodies and Rheumatoid Factor described below. One (1) specimen (from a subject who was positive for West Nile Virus antibodies) was positive, 2 specimens from subjects positive for chikungunta virus antibodies and 1 specimen from a subject who was positive to HIV antibodies were indeterminateby the New York SARS-CoV Microsphere Immunoassay (MIA) for Antibody Detection assay. The results are summarized in the **Table 2** below.

Table 2. Cross-Reactivity Results:

Antibody Positive Sera	Number of Samples Tested	New York SARS-CoV MIA Result		
		Positive	Indeterminate	Negative
Chikungunya virus	5	0	2	3
Dengue virus	5	0	0	5

HCV	5	0	0	5
HIV	5	0	1	4
Measles	5	0	0	5
Mumps	5	0	0	5
Rubella	5	0	0	5
Varicella Zoster virus	5	0	0	5
WNV	5	1	0	4
Herpes Simplex virus	5	0	0	5
ZIKV	2	0	0	2
Enterovirus	2	0	0	2
Rheumatoid Factor	5	0	0	5
HBV	5	0	0	5
CMV	5	0	0	5
EBV	2	0	0	2
WEEV/EEEV	1	0	0	1
Yellow Fever virus	1	0	0	1
Antinuclear antibodies	5	0	0	5
Total	78	1	3	74

In addition 286 presumed SARS-CoV-2 negative samples were evaluated for potential cross-reactivity. Out of 286, 256 specimens were collected in 2009 from blood donors, while 30 specimens were collected from patients with other respiratory infections. All 286 specimens were tested using the New York SARS-CoV Microsphere Immunoassay (MIA) for Antibody Detection assay. From the 256 samples collected prior to COVID-19, 255 out of 256 were negative with the New York SARS-CoV Microsphere Immunoassay (MIA) for Antibody Detection assay, resulting on a clinical specificity of 99.6%. From the 30 specimens with other respiratory illnesses, collected during COVID-19, 29 of them were negative with the New York SARS-CoV Microsphere Immunoassay (MIA) for Antibody Detection assay, resulting in 96.7% clinical specificity. A large proportion of this population is expected to have been exposed to other common respiratory infections.

3) Clinical Performance

Clinical Sensitivity

Six clinical studies were conducted, enrolling a total of 753 subjects at several US clinical collection sites. During these studies samples were collected from subjects confirmed PCR positive for SARS-CoV-2.

Table 3 reports the results obtained after testing the 753 specimens with the New York SARS-CoV Microsphere Immunoassay for Antibody Detection at the NYS Wadsworth Center organized by days from onset of symptoms. The sensitivity was calculated for the early samples (<7 days after onset of symptoms), the samples between 7 and 10 days, between 11 and 15 days, 16 and 20 days and the later samples (≥ 20 days after onset of symptoms).

These data confirm that assay sensitivity increases relative to time from disease onset showing that the sensitivity of the New York SARS-CoV Microsphere Immunoassay for Antibody Detection for PCR-confirmed samples is 79.3% at 20 or more days from symptom onset.

Table 2. Sensitivity of the NY SARS-CoV MIA by days from symptom onset.

Days from onset	Samples tested (N)	RT-PCR result	NY SARS-CoV MIA Result as Compared to PCR			Sensitivity (95%CI)
			Positive	Indeter.	Negative	
<7	179	Pos	32	30	117	32/179 **17.9%** (12.96% - 24.15%)
7 – 10	67	Pos	21	19	27	21/67 **31.3%** (21.50% - 43.20%)
11 – 15	47	Pos	23	4	20	23/47 **48.9%** (35.28% - 62.76%)
16 – 20	126	Pos	62	19	45	62/126 **49.2%** (40.63% - 57.83%)
>20	334	Pos	265	28	41	265/334 **79.3%** (74.68% - 83.34%)
Total	753					

In addition, two clinical studies were conducted. A total of 108 samples were collected at Westchester County from subjects confirmed PCR positive for SARS-CoV-2 and evaluated with the New York SARS-CoV Microsphere Immunoassay for Antibody Detection test. Samples were obtained at least 25 days from the onset of symptoms and shown in the table below (**Table 4**).

This additional time point, of 25 days from onset of symptoms, shows that the sensitivity of the New York SARS-CoV Microsphere Immunoassay for Antibody Detection increases with respect to the 20 days from onset of symptoms showed in Table 3 to 88% at 25 days post symptom onset.

Table 3. Sensitivity of the NY SARS-CoV MIA for samples collected at least 25 days after the symptom onset.

Clinical Study	Number of Samples Tested	Results with NY SARS-CoV MIA		
		Positive (> 6SD)	Indeterminate (>3 SD, <6SD)	Negative (<3 SD)
Study 1	44	39	2	3
Study 2	64	56	4	4
Total Samples	108	95	6	7
Sensitivity	**88.0%** (95/108); 95%CI: 80.49% to 92.83%			

Clinical Specificity

Four different sera collections were used to evaluate cross-reactivity of the assay.

A first set of samples consisted of 256 serum samples collected in 2009 from blood donors (American Red Cross in Syracuse, NY, and New York Blood Center in New York City, NY).

A second set of samples consisted of 78 sera with known antibody reactivity to a diverse group of viral pathogens (Chikungunya, dengue, HCV, HIV, measles, mumps, rubella, VZV, West Nile virus, herpes simplex, Zika virus, enteroviruses, HBV, cytomegalovirus, Epstein Barr, Eastern Equine Encephalitis, and Yellow Fever viruses), as well as sera with Antinuclear antibodies and Rheumatoid Factor.

A third set of samples contained 30 specimens from patients with other respiratory infections collected in March of 2020, at Columbia University Medical Center (CUMC).

A fourth set of samples contained 69 specimens collected from patients with signs and symptoms consistent with other respiratory infections were tested with the New York SARS-CoV Microsphere Immunoassay for Antibody Detection.

Specificity of the assay is provided in **Table 5.**

Table 5. Assay specificity with different sets of sera**.**

	Sample set	**Specificity (95%CI)**
1	Blood donors	255/256 99.6% (97.8% - 99.93%)
2	Diverse group of viral pathogens	77/78 98.7% (93.1% - 99.8%)
3	Respiratory infections	29/30 96.7% (83.3% - 99.4%)
4	Other study with Respiratory infections	67/69 97.1% (90.0% - 99.2%)

LIMITATION

This test uses biotin-streptavidin technology, and it is possible that biotin will interfere with this assay, however biotin interference has not been evaluated for this assay. The assay might be susceptible to false results in patients taking biotin supplements.