

Gyrolab® Assays

SARS-CoV-2 Total Antibody Assay

INTRODUCTION

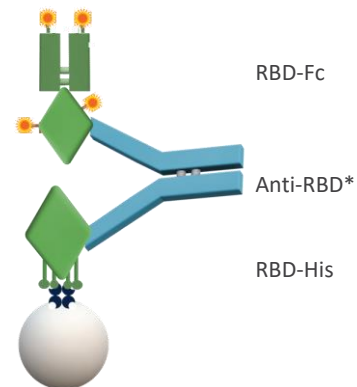
We have developed a three-step bridging Gyrolab assay with a broad assay range for qualitative detection of total antibodies generated against the spike protein (RBD) of SARS-CoV-2 in human serum samples. This assay has been developed for research use only and is not intended for diagnostic use.

Key benefits of the Gyrolab SARS-CoV-2 Total Antibody Assay are:

Low reagent consumption	
Capture	100 µg RBD-His => ~1 000 CDs (112 000 data points)
Detection	100 µg RBD-Fc => ~1 200 CDs (134 400 data points)
Low sample volume	4 µL diluted sample
Quick data analysis time	~1 h for 1 CD (112 data points)
Broad assay range	Double digit ng/mL to ~200 µg/mL levels of antibody can be measured

ASSAY DESIGN

The assay was set up as a three-step bridging assay with biotinylated SARS-CoV-2 (2019-nCoV) Spike RBD-His recombinant protein as the capture molecule and SARS-CoV-2 (2019-nCoV) Spike RBD-Fc recombinant protein labeled with Alexa Fluor® 647 as the detection molecule. Rabbit polyclonal anti-SARS-CoV-2 (2019-nCoV) Spike RBD antibody has been used as positive control.



* Total antibodies (IgG, IgA and IgM)

ASSAY CHARACTERISTICS

Assay range

Although the assay should be considered as qualitative, Gyrolab SARS-CoV-2 Total Antibody Assay demonstrates the ability to detect antibodies in a broad range of concentrations. Here, a negative control (prepared from a pre-pandemic human serum pool) and positive controls (prepared from a pre-pandemic human serum pool spiked with various amounts of rabbit polyclonal anti-spike RBD antibodies) were analyzed in duplicates achieving an assay range from approximately 40 to 200 000 ng/mL (Figure 1, Table 1).

Conc PC (µg/mL)	Average response (RU)	CV (%)
NC	0.038	50.0
0.040	0.121	5.0
0.200	0.425	17.5
1	1.69	N/A
4	9.30	9.6
10	24.7	9.1
40	97.9	4.7
100	179	1.3
200	265	0.5

Table 1 Average responses and CV obtained for a negative control (NC) and positive controls ranging from 40 ng/mL to 200 µg/mL (concentrations in neat serum) in Rexxip H containing 25% human serum. Rabbit polyclonal anti-SARS-CoV-2 (2019-nCoV) Spike RBD antibody was used as positive control

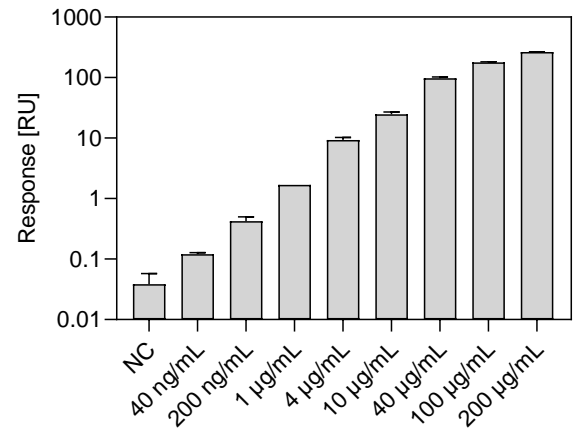


Figure 1 Average responses obtained for a negative control (NC) and positive controls ranging from 40 ng/mL to 200 µg/mL (concentrations in neat serum) in Rexxip H containing 25% human serum. Rabbit polyclonal anti-SARS-CoV-2 (2019-nCoV) Spike RBD antibody was used as positive control

Confirmed specificity of the assay

The specificity of the obtained responses in the positive controls (PCs) was confirmed in a competition assay where variable concentrations of unlabeled RBD protein were mixed with the sample. The response in the PC was effectively inhibited (Table 2).

Table 2 Inhibition of a positive control (PC, neat conc = 1 µg/mL) by addition of various amounts of unlabeled RBD protein to the sample

Sample	Conc RBD in sample (µg/mL)	% inhibition
PC	0.25	91
	2.5	98
	25	100

Screening of pre-pandemic human serum samples

Pre-pandemic human serum samples from 50 mixed gender individuals were analyzed. All individual samples showed responses in the same range as the negative controls (Figure 2).

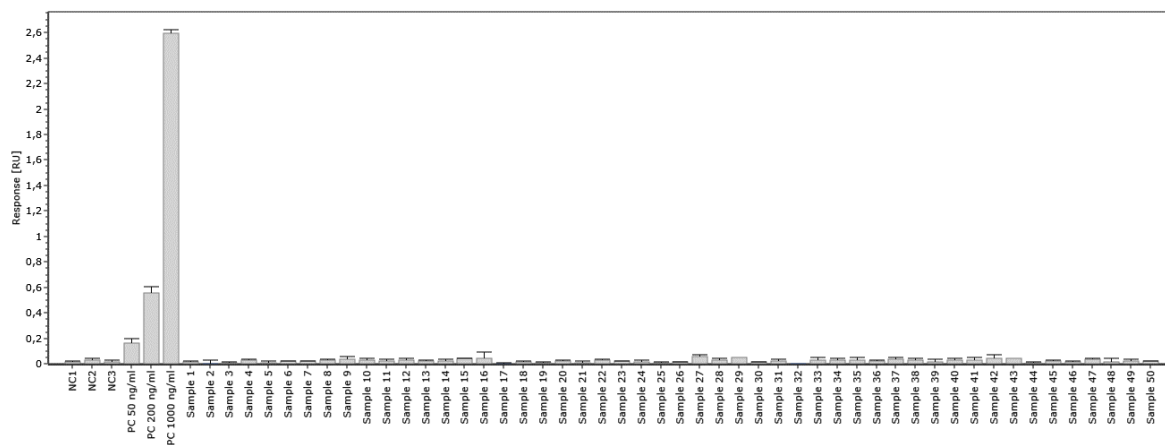


Figure 2 Measurements of human serum samples from 50 individuals (Sample 1-50), negative controls (NC1-NC3) and positive controls (PC) ranging from 50 ng/mL to 1000 ng/mL (concentrations in neat serum) in Rexxip H containing 25% human serum

MATERIALS AND METHODS

The assay was developed on a Gyrolab system using the Gyrolab Bioaffy 200 CD. The assay was set up using a three-step Gyrolab method with two wash solutions (200-3W-002-A) and a 5% PMT setting. The assay buffer was REXXIP H with 25% human serum. SARS-CoV-2 (2019-nCoV) Spike RBD-His Recombinant Protein from Sino Biological (40592-V08H) was biotinylated according to the Gyrolab biotinylation protocol (Gyrolab User Guide), with a 1:8 challenge ratio of biotin and used at a concentration of 70 nM, diluted with PBS-T containing 630 nM biotinylated BSA.

SARS-CoV-2 (2019-nCoV) Spike RBD-Fc Recombinant Protein from Sino Biological (40592-V02H), 100 µg reconstituted in 200 µL 0.1 M bicarbonate buffer, was labeled with one vial of Alexa Fluor® 647 according to the Gyrolab standard protocol (Gyrolab User Guide), and used at a concentration of 20 nM, diluted in REXXIP F. SARS-CoV-2 (2019-nCoV) Spike RBD rabbit polyclonal antibody from Sino Biological (40592-T62) diluted in REXXIP H with 25% human serum was used as positive control.

Summary

Capture	70 nM biotinylated SARS-CoV-2 (2019-nCoV) Spike RBD-His Recombinant Protein (Sino Biological, 40592-V08H) + 630 nM biotinylated BSA (Vector Laboratories, B-2007) in PBS-T
Detection	Alexa Fluor 647-labeled SARS-CoV-2 (2019-nCoV) Spike RBD-Fc Recombinant Protein (Sino Biological, 40592-V02H), 20 nM in REXXIP F
Positive control antibody	SARS-CoV-2 (2019-nCoV) Spike RBD rabbit polyclonal antibody (Sino Biological, 40592-T62) in REXXIP H with 25% human serum
CD-type	Bioaffy 200 CD
Method	200-3W-002-A
Wash buffer for needles	Wash buffer 1: PBS-T Wash buffer 2: Gyrolab Wash Buffer pH 11
PMT-setting	5%
Approximate range using particular PC	Approximately 10 ng/mL to 50 000 ng/mL (40 ng/mL to 200 000 ng/mL in neat human serum). The range can be modified, see Further development opportunities, below
Time to result	Approx. 1 hour
Sample volume	4 µL (sample diluted 1:4)
Throughput	56 samples in duplicate/CD

Further development opportunities

Data given in this document should only be considered as guidance. Further development of the assay could be performed. For example, different CD types (for example Gyrolab Bioaffy 1000 for a more sensitive assay) different dilutions of samples or different sample types might be evaluated.

Safety

When working with samples that might contain infectious virus it is imperative that appropriate safety measures are considered. Small amounts of aerosols are generated by the Gyrolab system during use. Follow the guidelines on handling such samples as recommended by your local health institution and perform a safety assessment prior to analyzing your samples on the Gyrolab system. For viral inactivation of samples, please refer to your institutional biosafety guidelines and viral deactivation procedures.

For additional support contact your local Field Application Support

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