# Overview of FIND methodology for antibody evaluations

## Introduction:

SARS-CoV-2-specific antibodies are part of the immune response to infection, and may be detected during the early, late, convalescent, and post-recovery phases of disease. They are most useful for identifying recent or prior infection.

## Overview of the Studies:

### 1. Study Methodology:

#### Retrospective diagnostic evaluation

We conducted retrospective, multicentre diagnostic evaluation studies of COVID-19 serological assays using archived, frozen serum/plasma. The index tests included novel immunoassays (RDTs or manual ELISAs) that detect antibodies specific to recombinant SARS-CoV-2 proteins, including IgA, IgM, IgG or a combination thereof. For lateral flow assays, two independent operators at each site interpreted the test results, with discrepant results interpreted by a third operator. All operators were blind to the reference result. For ELISAs, samples were tested in duplicate where possible. We are presenting site-specific data and combined data across sites, where relevant.

#### Interference testing using specimens from the FIND biobank

Samples for the interference testing were selected from the FIND specimen bank that included plasma samples from febrile patients collected in Nothern Malawi from April 2017 to April 2018. The sample set included 10 plasma samples from patients with a confirmed Malaria RDT and PCR positive result, and 11 plasms samples that tested positive in dengue serology (IgG and/or IgM ELISA).

#### NIBSC reference panel testing

The COVID-19 convalescent plasma panel is composed of 6 members: 5 plasma samples from COVID-19 recovered patients (20/120, 20/122, 20/124, 20/126, 20/130) and a negative plasma pool from healthy donors collected before 2019 (20/128-negative). The panel is intended to be used for the development an  evaluation of serological assays for the detection of antibodies against SARS-CoV-2. The five samples from COVID-19 recovered patients are intended to be used as positive controls with varying antibody titres.

### 2. Analysis Methodology:

The estimates of sensitivity and specificity were calculated by fitting a generalized linear mixed-effect model accounting for the difference between sites and/or time-from-symptoms-onset groups. To fit the model we used the R function glmer with binomial family (logit model link function) including as random effects the site and the time- from-symptoms-onset. Two different models were used, based on two possible outcomes:

- estimates from data from all sites, for a specific time-from-symptoms-onset group: generalized linear mixed effect model with intercept as single fixed effect, with site as random effect;
- estimates from data for all sites, grouping all time-from-symptoms-onset groups: generalized linear mixed effect model with intercept as single fixed effect, with site as random effect and time from symptom onset group nested within the site.

For tests that measure multiple isotypes separately, sensitivity and specificity were calculated for each antibody isotype (e.g. IgM, IgG, IgA, and total Ig, as applicable) separately and were calculated in a combined manner, where a positive result for any isotype was interpreted as a positive test result and a negative result meant that a sample tested negative for all isotypes that could be detected by the assay. All tests were performed according to the manufacturer's instructions for use. An invalid index result (as defined according to the instructions for use) was repeated, pending availability of sufficient sample volume and test stock. Invalid index results which were not repeated, were not included in the analysis. Final analysis was calculated using valid Antibody results only.

## 3. Overview of Study Sites:
### a. Evaluations of Rapid Diagnostic Tests (RDTs)

**Bioaster Microbiology Technology Institute (Bioaster, France):** Samples for sensitivity estimates were collected from in-patients with a documented positive SARS-CoV-2 RT-PCR result. Samples were from adults only and collected between day 7 and day 70 post symptom onset. Samples for specificity estimates originated from patient samples from Hospices Civils of Lyon and Biomnis (Central lab, Lyon), collected between 2011 and December 2019.

**Boston Children's Hospital (BCH, USA):** Samples for sensitivity estimates were collected from in-patients and out-patients documented with a positive RT-PCR result, including a subset from health care workers, whose samples were tested at Boston Children's Hospital. Samples were from both children and adults. Samples for specificity estimates originated from two prior studies: one conducted in 2016-2018 focused on sepsis and the other which took place prior to 2019 focused on C. difficile.

**IS Global – Barcelona Institute for Global Health (ISG, Spain):** Samples for the sensitivity estimates were collected from individuals with a documented positive SARS-CoV-2 RT-PCR result, including from health care workers from the Hospital Clinic de Barcelona participating in a sero-conversion study and individuals who attended the Clinica of the Universidad de Navarra, Spain. Samples for the specificity estimates originated from healthy volunteers without respiratory symptoms, collected prior to December 2019.

**Liverpool School of Tropical Medicine (LSTM, England):** Samples for the sensitivity estimates were collected from individuals enrolled in the FASTER trial, with a documented positive SARS-CoV-2 RT-PCR result (in-patients only). Samples for the specificity estimates originated from individuals diagnosed with other respiratory diseases prior to November 2019, including Influenza A, common circulating conronaviruses (229E, OC43) and tuberculosis. The sample set also included samples from individuals with non-respiratory diseases prior to November 2019, including dengue virus, HIV and parasitic infections caused by *Plasmodium falciparum*, *Schistosoma mansoni*, *S. heamotobium*, *Strongyloides stercoralis* and *Entomoeba histolytica.* 20 patient samples from April 2020 with respiratory symptoms but with a confirmed negative SARS-CoV-2 qPCR and SARS-CoV-2 IgG ELISA were also used.
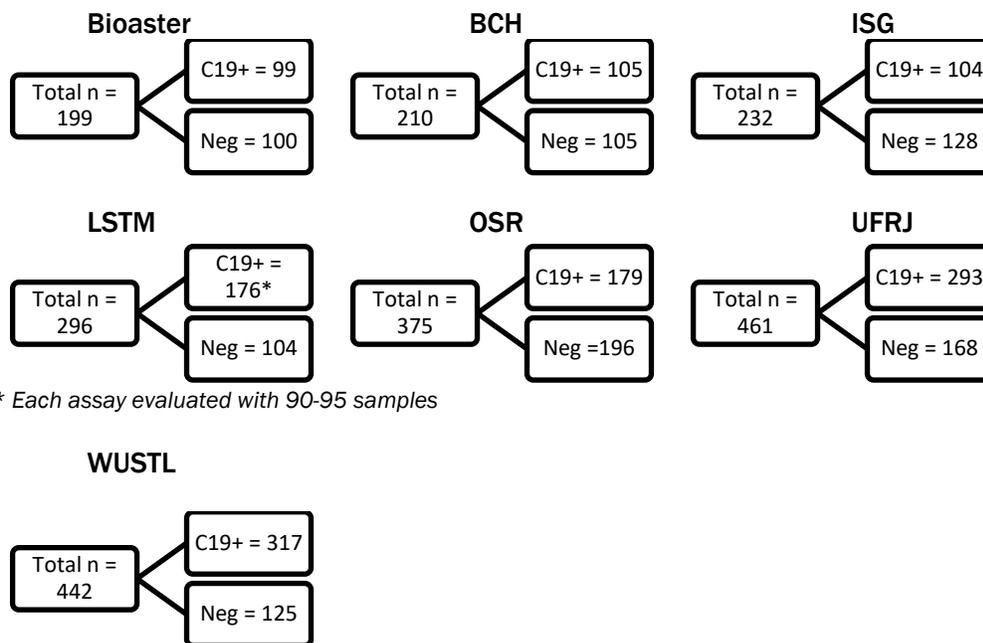
**Ospedale San Raffaele (OSR, Italy):** Samples for the sensitivity estimates were collected from individuals with a documented positive SARS-CoV-2 RT-PCR result, including both in-patients and out-patients. Samples for the specificity estimates originated from several sources: samples from individuals diagnosed with other respiratory diseases prior to November 2019 (preK) and healthy volunteers (preH).

**Universidade Federal do Rio de Janeiro (UFRJ, Brazil):** Samples for sensitivity estimates were collected

through an ongoing study "*Characterization of Risk Factors and Development of New Serological Tests for SARS-CoV-2 Infection*" in which symptomatic health care professional are enrolled and are followed periodically. All samples included were included from individuals with a documented positive SARS-CoV-2 RT-PCR result. Samples for the specificity estimates originated from healthy volunteers without respiratory symptoms, collected prior to November 2019.
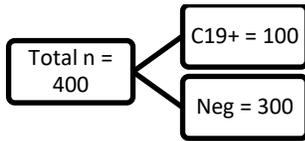
**Washington University, St. Louis (WUSTL, USA):** Samples for the sensitivity estimates were collected from individuals with a documented positive SARS-CoV-2 RT-PCR result including individuals with acute COVID-19 symptoms who were admitted to the hospital for care and provided blood samples as well as subjects who were COVID-19 symptom free for at least 14 days and then presented to donate convalescent plasma. Samples for the specificity estimates originated from several sources: samples collected from healthy adults (prior to December 2019) and sick individuals presenting at the hospital (March – May 2019). As well, archived samples from Uganda and Cote d'Ivoire collected for prior research purposes were included.

### Bioaster

Total n = 199 → C19+ = 99 / Neg = 100

### BCH

Total n = 210 → C19+ = 105 / Neg = 105

### ISG

Total n = 232 → C19+ = 104 / Neg = 128

### LSTM

Total n = 296 → C19+ = 176* / Neg = 104

### OSR

Total n = 375 → C19+ = 179 / Neg = 196

### UFRJ

Total n = 461 → C19+ = 293 / Neg = 168

*\* Each assay evaluated with 90-95 samples*

### WUSTL

Total n = 442 → C19+ = 317 / Neg = 125

## b. Evaluations of ELISAs

**Centre Hospitalier Universitaire Vaudois (CHUV, Switzerland):** Samples for sensitivity estimates were collected from patients documented with a positive SARS-CoV-2 RT-PCR result within the first 2 months post disease. Samples for specificity estimates were collected prior to November 2019 and included a subset with known reactivity to other human coronaviruses and other viruses, from patients with Lupus, and from individuals with undefined illness. Tests were evaluated in two stages. First, 180 samples were assessed (preliminary panel) and products that met ≥ 90% sensitivity and ≥ 97% were then tested with 217 additional negative samples and 3 positive samples (full panel, N = 400).

CHUV

```
┌──────────┐      ┌──────────────┐
│ Total n = │─────│  C19+ = 100  │
│   400    │      └──────────────┘
└──────────┘      ┌──────────────┐
                  │  Neg = 300   │
                  └──────────────┘
```
*Prelim: 97 Pos + 83 Neg*

## 4. Limitations:

Samples were purposefully (not randomly) selected for the antibody evaluations. Sample panel composition was different across sites, though inclusion/exclusion criteria were the same, introducing variability. Storage conditions and transit times for test kits may have been prolonged which could impact test integrity and performance.