Testing for SARS-CoV2

0. **Viral Culture: Gold Standard**
   - Requires BSL-4 Lab
   - Long TAT
   - Not commercially feasible

1. **PCR: current Gold Standard; 4-6 hours to run test, batched samples of up to 92 per run.**
   a. In viral transport media/universal transport media, phosphate buffered saline
   b. Unclear infectious dose, semi-arbitrary cut off of 35ct value
      i. Lower the ct value = higher viral load
   Roche cobas
   Abbot M100
   BD Max

2. **Rapid PCR assays**
   a. Either single sample at a time or batched, 15-45 minutes per sample
   b. Must perform on DRY SWAB sample (else, dilutional effect reduces sensitivity)
   Abbot ID Now: 15 min
   Cepheid Xpert Xpress: 45 min

Limitations: all were NP swabs diluted in VCM
[https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7217789/pdf/main.pdf](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7217789/pdf/main.pdf)

**Table 2**

Positive and negative agreement of Abbott ID Now SARS-CoV-2 and Cepheid Xpert SARS-CoV-2 with Roche cobas SARS-CoV-2.

| Cobas C	ext{t} Category | ID Now (%, 95% CI) | Xpert (%, 95% CI) | Total |
|--------------------------|--------------------|-------------------|-------|
| Total Positive           | 65 (73.9, 63.2-82.3) | 87 (98.9, 92.9-99.9) | 88    |
| Low (> 30)               | 12 (34.3, 19.7-52.2) | 34+ (97.1, 83.4-99.8) | 35    |
| Medium (20-30)           | 38 (100, 88.6-100)  | 38 (100, 88.6-100)  | 38    |
| High (< 20)              | 15 (100, 74.7-100)  | 15 (100, 74.7-100)  | 15    |
| Negative                 | 25 (100, 83.4-100)  | 23 (92.0, 72.4-98.6) | 25    |

| Site          | Total Samples Tested | ACOV+ | INDCO+ | ACOV+/ INDCO+ | ACOV- | INDCO- | ACOV-/ INDCO- | Positivity | Positive Agreement | Negative Agreement | Performance Agreement (Kappa) |
|---------------|----------------------|-------|--------|---------------|-------|--------|---------------|------------|-------------------|----------------------|-------------------------|
| IMCC A        | 208                  | 13    | 13     | 1             | 161   | 22%    | 71.74 (55.32, 83.54) | 99.38 (95.09, 99.97) | .783 (.779, .788) |                       |                       |
| IMCC B        | 125                  | 39    | 17     | 0             | 69    | 44%    | 69.64 (55.74, 80.84) | 100.0 (93.43, 100.0) | .711 (.706, .717) |                       |                       |
| ED 1          | 105                  | 26    | 11     | 0             | 68    | 35%    | 70.27 (52.83, 83.56) | 100.0 (93.33, 100.0) | .751 (.744, .757) |                       |                       |
| ED 2          | 31                   | 12    | 3      | 0             | 16    | 50%    | 80.0 (51.37, 94.69)  | 100 (75.92, 100.0)   | .803 (.792, .814)  |                       |                       |
| ED 3          | 55                   | 29    | 3      | 1             | 22    | 60%    | 90.63 (73.83, 97.55) | 95.65 (76.03, 99.77) | .852 (.844, .861)  |                       |                       |
| Overall       | 524                  | 139   | 47     | 2             | 336   | 35%    | 74.73 (67.74, 80.67) | 99.41 (97.64, 99.89) |                       |                       |
Table 1. Positive percent agreement (PPA) of the Abbott ID Now and Diasorin Simplexa assays for the detection of SARS-CoV-2 was determined using a modified CDC assay as the reference standard

|                | Detected | Not Detected | PPA (95% CI) |
|----------------|----------|--------------|--------------|
| Abbott ID Now  | 90       | 6            | 94% (87-98%) |
| Diasorin Simplexa | 92   | 4            | 96% (90-99%) |
| Modified CDC assay | 96 | 0            | Not applicable |

Table 2. Clinical performance comparison of three sample-to-answer EUA molecular assays for the detection of SARS-CoV-2 (n = 108).

| Molecular Assay | Reference Standard a | (± 95% CI) b | Kappa (κ)d | PPA | NPA |
|-----------------|----------------------|--------------|------------|-----|-----|
| Xpert Xpress    | Positive | 57 | 0 | 0.98 (1-0.95) | 98.3% (0.91-1) | 100% (0.93-1) |
|                 | Negative | 1 | 50 |  |  |  |
| ID NOW a       | Positive | 50 | 0 | 0.87 (0.96-0.78) | 87.7% (0.76-0.95) | 100% (0.93-1) |
|                 | Negative | 7 | 50 |  |  |  |
| ePlex           | Positive | 53 | 0 | 0.91 (0.99-0.83) | 91.4% (0.81-0.97) | 100% (0.93-1) |
|                 | Negative | 5 | 50 |  |  |  |

these were NP swabs in VCM.
3. Rapid Antigen Testing

https://www.fda.gov/media/137885/download

a. Quidel Sofia 2 test

Study 1: Due to the limited availability of direct swabs, the clinical performance of the SOFIA 2 SARS Antigen FIA was established with a study using one hundred forty-three (143) previously characterized frozen NP swabs originally collected in 3-mL viral transport media.

| SARS-CoV-2 Molecular | POS | NEG | Total | PPA | 95% CI |
|----------------------|-----|-----|-------|-----|--------|
| Sofia 2 SARS Antigen FIA Assay | POS | 47  | 0     | 47  | NPA    |
|                       | NEG | 12  | 84    | 96  | PPV    |
| Total                | 59  | 84  | 143   | NPV | 88%    |

Prevalence: 41%, 34%, 49%
% agreement: 92%
4. Antibody Testing

12 tests EUA approved at this time

Study 2:
A limited study using forty-eight (48) direct nasal swabs was performed. The samples were sequentially enrolled from four locations and tested fresh. The SOFIA 2 SARS Antigen FIA was compared to the Lyra SARS-CoV-2 Assay (EUA200016/A002), an extracted RT-PCR assay.

|                      | Lyra SARS-CoV-2 Assay EUA200016/A002 |                |                |
|----------------------|--------------------------------------|----------------|----------------|
|                      | POS                                  | NEG            | Total          |
| Sofia 2 SARS         | 4                                    | 0              | 4              |
| Antigen FIA Assay    |                                      |                |                |
|                      | 1                                    | 43             | 44             |
|                      | Total                                | 5              | 43             | 48             |
|                      | Sensitivity                          | 80.0%          | 37.6%          | 96.4%          |
|                      | Specificity                          | 100.0%         | 91.8%          | 100.0%         |
|                      | PPV                                  | 100.0%         | 51.0%          | 100.0%         |
|                      | NPV                                  | 97.7%          | 88.2%          | 99.6%          |
|                      | Prevalence                           | 10.4%          | 4.5%           | 22.2%          |
|                      | % agreement                          | 97.9%          |                |                |

Some qualitative, some quantitative, some IgG, some combo of IgG/M, some IgG, IgM, IgA

Timing is very important in regards to when antibodies may be present
- IgG testing may be best to consider at least 2 weeks or more after disease
- Unclear assay sensitivity in immunocompromised patients
- Unclear if temporary or sustained antibody presence

Unclear if these are neutralizing antibodies (e.g., protective antibodies)

https://www.fda.gov/media/137383/download (Abbott IgG quantitative)

- The SARS-CoV-2 IgG assay is intended for use as an aid in identifying individuals with 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 SARS-CoV-2 IgG assay should not be used to diagnose acute SARS-CoV-2 infection.
• IgG 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.

Still unknown:

1) Infectious Dose
2) Number of Asymptomatic Patients and their viral loads (?lower viral load if asymptomatic)
3) How long shedding of viable and non-viable virus present in nares, NP, sputum, etc. in different patients.