# Performance Characteristics of the Curative HT SARS-CoV-2 Test

Note: This assay has not been FDA approved or cleared but has been submitted to FDA for Emergency Use Authorization (EUA) and is currently under review.

- **Limit of Detection (LoD) - Analytical Sensitivity:**

**Table 1. LoD Data**

| CONCENTRATION | PASSING RATE (%) | |
|---|---|---|
| | Manual Extraction | KingFisher Extraction |
| 1,000 copies/mL | 6/6 (100%) | 6/6 (100%) |
| 500 copies/mL | 6/6 (100%) | 6/6 (100%) |
| 200 copies/mL | 6/6 (100%) | 6/6 (100%) |
| 100 copies/mL | 6/6 (100%) | 5/6 (83%) |
| 50 copies/mL | 5/6 (83%) | 1/6 (17%) |
| 20 copies/mL | 4/6 (67%) | 2/6 (33%) |

Based on the above results, the Curative HT SARS-CoV2 Test was determined to have an LoD of 200 copies/mL.

For the LoD Confirmation, 100% of samples processed with manual extraction passed, and 100% of samples processed with KingFisher extraction passed.

**Table 2. LoD Passing Rate**

| Concentration | Passing Rate (%) | |
|---|---|---|
| | Manual Extraction | KingFisher Extraction |
| 200 copies/mL | 20/20 (100%) | 20/20 (100%) |

- **Inclusivity:**

Based on a January 10, 2022, GSAID database analysis, 6,900,922 viral target genomes were in the GSAID database. Of these genomes 4,914 have 2 or more mismatch bases at any point within the sequences targeted by the Curative HT SARS-CoV-2 Test forward and reverse primers and probe. Treating these numbers as unlinked, randomly assorted, and assuming the 2 mismatches are adjacent and at either primer 3' end would be a worst-case scenario where

4,914/6,900,922 or 0.07% of SARS-CoV-2 genomes in the database are at risk of not being detected by the assay.

In addition, the ability of the Curative HT SARS-CoV-2 Test to detect the SARS-CoV-2 omicron variant, B.1.1.529, was evaluated by testing 20 omicron-positive patient samples and 20 negative simulated patient samples as shown in Table 3 below.

**Table 3. Inclusivity of the HT SARS-CoV-2 Test for the Detection of Omicron**

| | | Reference Standard (Sequenced and Contrived Samples) | |
|---|---|---|---|
| | | **Positive** | **Negative** |
| **The Curative HT SARS-CoV-2 Test** | **Positive** | 20 | 0 |
| | **Negative** | 0 | 20 |
| | **PPA** | 100.0% (95% C.I. 83.2%-100.0%)* | |
| | **NPA** | 100.0% (95% C.I 83.2%-100.0%)* | |

*Clopper-Pearson confidence intervals

Patient samples that tested positive for the omicron variant were identified through Curative's internal sequencing project which provides genomic surveillance and variant tracking for SARS-CoV-2 using de-identified residual positive patient samples. The data collected in this study indicates that the Curative HT SARS-CoV-2 Test detects the omicron variant with 100.0% PPA and 100.0% NPA.

- **Cross-reactivity (Analytical Specificity):**

To assess microbial interference, microorganisms were spiked into contrived SARS-CoV-2-positive samples with a concentration of SARS-CoV-2 that was 2x LoD. No interference was observed.

**Table 4. Microorganisms Tested for Cross-Reactivity and Microbial Interference**

| Microorganism | Test Concentration | Cross-Reactivity | Interference |
|---|---|---|---|
| Adenovirus 7A | $10^5$ TCID/mL | NONE | NONE |
| Human Metapneumovirus | $10^5$ TCID/mL | NONE | NONE |
| Parainfluenza virus 1 | $10^5$ TCID/mL | NONE | NONE |
| Parainfluenza virus 2 | $10^5$ TCID/mL | NONE | NONE |
| Parainfluenza virus 3 | $10^5$ TCID/mL | NONE | NONE |
| Parainfluenza virus 4 | $10^4$ TCID/mL | NONE | NONE |
| Influenza A | $10^5$ TCID/mL | NONE | NONE |
| Influenza B | $10^5$ TCID/mL | NONE | NONE |
| Enterovirus EV68 | $10^5$ TCID/mL | NONE | NONE |
| Respiratory Syncytial Virus | $10^5$ TCID/mL | NONE | NONE |
| Rhinovirus | $10^5$ TCID/mL | NONE | NONE |
| *Chlamydia pneumoniae* | $10^6$ CFU/mL | NONE | NONE |
| *Haemophilus influenzae* | $10^6$ CFU/mL | NONE | NONE |
| *Legionella pneumophila* | $10^6$ CFU/mL | NONE | NONE |
| *Mycobacterium tuberculosis* | $10^6$ CFU/mL | NONE | NONE |

| Microorganism | Test Concentration | Cross-Reactivity | Interference |
|---|---|---|---|
| *Streptococcus pneumoniae* | $10^6$ CFU/mL | NONE | NONE |
| *Streptococcus pyogenes* | $10^6$ CFU/mL | NONE | NONE |
| *Bordetella pertussis* | $10^6$ CFU/mL | NONE | NONE |
| *Mycoplasma pneumoniae* | $10^6$ CFU/mL | NONE | NONE |
| *Pneumocystis jirovecii* | $10^6$ CFU/mL | NONE | NONE |
| *Candida albicans* | $10^6$ CFU/mL | NONE | NONE |
| *Pseudomonas aeruginosa* | $10^6$ CFU/mL | NONE | NONE |
| *Staphylococcus epidermis* | $10^6$ CFU/mL | NONE | NONE |
| *Streptococcus salivarius* | $10^6$ CFU/mL | NONE | NONE |
| Human coronavirus 229E | $10^4$ TCID/mL | NONE | NONE |
| Human coronavirus OC43 | $10^5$ TCID/mL | NONE | NONE |
| Human coronavirus HKU1 | $10^5$ copies/mL | NONE | NONE |
| Human coronavirus NL63 | $10^5$ TCID/mL | NONE | NONE |
| MERS-coronavirus | $10^5$ TCID/mL | NONE | NONE |
| Pooled human nasal wash | N/A | NONE | NONE |

- **Interfering Substances:**

**Table 5. Potentially Interfering Endogenous and Exogenous Substances**

| Substance | Active Ingredient | Test Concentration | Interference |
|---|---|---|---|
| Human DNA | Human DNA | 1,666.67 ng/L | NONE |
| Afrin® Original nasal spray | Oxymetazoline hydrochloride (0.05%) | 10% v/v | NONE |
| | | 15% v/v | YES |
| Mucin | Purified mucin protein | 2.5 mg/mL | NONE |
| Cepacol® lozenges | Benzocaine, menthol | 3 mg/mL | NONE |
| Chloraseptic® sore throat spray | Phenol, glycerin | 5% v/v | NONE |
| Robitussin® cough syrup | Dextromethorphan, guaifenisin | 5% v/v | NONE |
| Nicotine | Nicotine | 0.03 mg/mL | NONE |
| Human whole blood | Blood | 1% v/v | NONE |
| Inhaled bronchodilators | Albuterol sulphate | 0.83 mg/mL | NONE |
| Antibiotic nasal ointment | Mupirocin | 10 mg/mL | NONE |
| Antibiotic ointment | Tobramycin | 4 µg/mL | NONE |
| Saline nasal spray | Sodium chloride (0.65%) | 15% v/v | NONE |
| Neosynephrine® Original nasal drops | Phenylephrine hydrochloride (0.5%) | 15% v/v | NONE |
| Zycam® nasal gel | Oxymetazoline hydrochloride | 15% v/v | NONE |
| Nasal corticosteroid | Fluticasone propionate | 5 µg/mL | NONE |

- **Sample Stability:**

Patient samples may be stored in Specimen Transport Buffer for 144 hours (six days) at ambient temperature prior to being tested with the Curative HT SARS-CoV-2 Test.

- **Clinical Evaluation:**

When comparing symptomatic participants' results on the Curative HT SARS-CoV-2 Test and on a highly sensitive FDA-authorized (EUA) molecular comparator test, the Positive Percent Agreement (PPA) was 95.0% (95% confidence interval [95% CI]: 83.1% to 99.4%) and the Negative Percent Agreement (NPA) was 98.2% (95% CI: 93.5% to 99.8%). In total, 27.5% (11/40) of the positive samples had a low viral load as defined by FDA and as measured by the comparator test.

**Table 6. Performance of the Curative HT SARS-CoV-2 Test in Symptomatic Individuals**

| | **Comparator SARS-CoV-2** | | |
|---|---|---|---|
| **Curative HT SARS-CoV-2** | **Positive** | **Negative** | **Total** |
| **Positive** | 38 | 2 | 40 |
| **Negative** | 2 | 107 | 109 |
| **Total** | 40 | 110 | 149 |

Positive Percent Agreement (PPA): 95.0% (95% CI: 83.1%, 99.4%)
Negative Percent Agreement (NPA): 98.2% (95% CI: 93.5%, 99.8%)

When comparing the Curative HT SARS-CoV-2 Test to the comparator test among asymptomatic participants, the PPA was 95.0% (95% confidence interval [95% CI]: 75.1% to 99.9%) and the NPA was 99.1% (95% CI: 95.0% to 100.0%). In total 30% (6/20) of the positive samples had a low viral load as defined by FDA and as measured by the comparator test.

**Table 7. Performance of the Curative HT SARS-CoV-2 Test in Asymptomatic Individuals**

| | **Comparator SARS-CoV-2** | | |
|---|---|---|---|
| **Curative HT SARS-CoV-2** | **Positive** | **Negative** | **Total** |
| **Positive** | 19 | 1 | 20 |
| **Negative** | 1 | 109 | 110 |
| **Total** | 20 | 110 | 130 |

Positive Percent Agreement (PPA): 95.0% (95% CI: 75.1%, 99.9%)
Negative Percent Agreement (NPA): 99.1% (95% CI: 95.0%, 100.0%)