# Analytical Performance Report

# DIAQUICK COVID-19 Ag Cassette

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## 1. Analytical Sensitivity / Detection Limit

**Study objective:**
To evaluate the limit of detection of DIAQUICK COVID-19 Ag Cassette (Swab).

**Study design:**
Method:
The LOD for the DIAQUICK COVID-19 Ag Cassette (Swab) was established using limiting dilutions of a viral sample inactivated by heating at 65°C for 30 minutes, the material was supplied at a concentration of 4.6 x $10^5$ $TCID_{50}$/mL. In this study, designed to estimate the LOD of the assay when using a direct nasal swab, the starting material was spiked into a volume of pooled human nasal matrix obtained from healthy donors and confirmed negative for SARS-CoV-2. An initial range finding study was performed testing devices in triplicate using a 10-fold dilution series. At each dilution, 50 µL samples were added to swabs and then tested in the DIAQUICK COVID-19 Ag Cassette (Swab) using the procedure appropriate for patient nasal swab specimens. A concentration was chosen between the last dilution to give 9 positive results and the first to give 9 negative results. Using this concentration, the LOD was further refined with a 2-fold dilution series. At each dilution, then tested in 60 replicates tested in the same way using three lots of DIAQUICK COVID-19 Ag Cassette (Swab).

Materials:
- DIAQUICK COVID-19 Ag Cassette (Swab)
  Lot 1: 2006189; Lot 2: 2006190; Lot 3: 2006191
- SARS-CoV-2 cultured virus confirmed by PCR and has been inactivated by heating at 65°C for 30 minutes, the material was supplied at a concentration of 4.6 x $10^5$ $TCID_{50}$/mL.
- Pooled human nasal matrix obtained from healthy donors and confirmed negative for SARS-CoV-2.

Test procedure:
Perform the test according to the test procedure in the package insert.

Acceptance criteria:
Acceptable Limit of Detection level: Positive agreement ≥90%.

**Results:**
*Table 1.1: The result of 10-fold dilution series*

| Dilution series | Concentration | DIAQUICK COVID-19 Ag Cassette | | | NO. Positive/ Tested |
|---|---|---|---|---|---|
| | | Lot 1 | Lot 2 | Lot 3 | |
| Neat | 4.6 x $10^5$ $TCID_{50}$/mL | 3/3 | 3/3 | 3/3 | 9/9 |
| 1/10 | 4.6 x $10^4$ $TCID_{50}$/mL | 3/3 | 3/3 | 3/3 | 9/9 |
| 1/100 | 4.6 x $10^3$ $TCID_{50}$/mL | 3/3 | 3/3 | 3/3 | 9/9 |
| 1/1000 | 4.6 x $10^2$ $TCID_{50}$/mL | 3/3 | 3/3 | 3/3 | 9/9 |
| 1/10000 | 4.6 x 10 $TCID_{50}$/mL | 0/3 | 0/3 | 0/3 | 0/9 |

*Table 1.2: The results of 2-fold dilution series.*

| Dilution series | Concentration | DIAQUICK COVID-19 Ag Cassette | | | NO. Positive /Tested | Positive agreement |
|---|---|---|---|---|---|---|
| | | Lot 1 | Lot 2 | Lot 3 | | |
| Neat | 4.6 x $10^2$ $TCID_{50}$/mL | 20/20 | 20/20 | 20/20 | 60/60 | 100% |
| 1/2 | 2.3 x $10^2$ $TCID_{50}$/mL | 20/20 | 20/20 | 20/20 | 60/60 | 100% |
| 1/4 | 1.15 x $10^2$ $TCID_{50}$/mL | 20/20 | 20/20 | 20/20 | 60/60 | 100% |
| 1/8 | 5.75 x 10 $TCID_{50}$/mL | 6/20 | 3/20 | 5/20 | 14/60 | 23.3% |
| 1/16 | 2.88 x 10 $TCID_{50}$/mL | 0/20 | 0/20 | 0/20 | 0/60 | 0% |

**Conclusion:**
From the study above:
The Limit of Detection of the DIAQUICK COVID-19 Ag Cassette (Swab) is 1.15 x $10^2$ $TCID_{50}$/mL.

## 2. Analytical Specificity

### 2.1. Interfering Substances

**Study objective:**
To evaluate if the substances found in respiratory specimen interfere with the DIAQUICK COVID-19 Ag Cassette (Swab).

**Study design:**
Method:
Test simulated specimens using the DIAQUICK COVID-19 Ag Cassette (Swab) to evaluate if there are any substance interferences. These potential interfering substances were spiked separately into negative pooled human nasal matrix and SARS-CoV-2 cultured virus as the simulated specimens. 50 µL simulated specimens were added to swabs and then tested in the DIAQUICK COVID-19 Ag Cassette (Swab) using the procedure appropriate for patient nasal swab specimens.

Materials:
- DIAQUICK COVID-19 Ag Cassette (Swab)
  Lot 1: 2006189; Lot 2: 2006190; Lot 3: 2006191
- Limit of detection reference: SARS-CoV-2 cultured virus with concentration of $1.15 \times 10^2$ $TCID_{50}$/mL.
- Pooled human nasal matrix obtained from healthy donors and confirmed negative for SARS-CoV-2.
- Potential interfering substances: Human blood (EDTA anticoagulated), Mucin, Antiviral drugs (Oseltamivir phosphate, Ribavirin), Antibiotics/antibacterial drugs (Levofloxacin, Azithromycin, Meropenem, Tobramycin), Nasal sprays or nose drops (Phenylephrine, Oxymetazoline, 0.9% sodium chloride, A natural soothing ALKALOL), Nasal corticosteroids (Beclomethasone, Hexadecadrol, Flunisolide, Triamcinolone, Budesonide, Mometasone, Fluticasone, Fluticasone propionate).

Test Procedure:
Perform the test according to the test procedure in the package insert.

**Results:**
*Table 2.1.1. Test results of negative pooled human nasal matrix simulated specimens.*

| Potential interfering substances were spiked into Negative pooled human nasal matrix | | Concentration | DIAQUICK COVID-19 Ag Cassette | | |
|---|---|---|---|---|---|
| | | | Lot 1 | Lot 2 | Lot 3 |
| | Human blood (EDTA anticoagulated) | 20% (v/v) | - | - | - |
| | Mucin | 5mg/ml | - | - | - |
| Antiviral drugs | Oseltamivir phosphate | 5mg/ml | - | - | - |
| | Ribavirin | 5mg/ml | - | - | - |
| Antibiotics /antibacterial drugs | Levofloxacin | 5mg/ml | - | - | - |
| | Azithromycin | 5mg/ml | - | - | - |
| | Meropenem | 5mg/ml | - | - | - |
| | Tobramycin | 2mg/ml | - | - | - |
| Nasal sprays or nose drops | Phenylephrine | 20%(v/v) | - | - | - |
| | oxymetazoline | 20%(v/v) | - | - | - |
| | 0.9% sodium chloride | 20%(v/v) | - | - | - |
| | A natural soothing ALKALOL | 20%(v/v) | - | - | - |

| | | | | | |
|---|---|---|---|---|---|
| Nasal corticosteroids | Beclomethasone | 20%(v/v) | - | - | - |
| | Hexadecadrol | 20%(v/v) | - | - | - |
| | Flunisolide | 20%(v/v) | - | - | - |
| | Triamcinolone | 20%(v/v) | - | - | - |
| | Budesonide | 20%(v/v) | - | - | - |
| | Mometasone | 20%(v/v) | - | - | - |
| | Fluticasone | 20%(v/v) | - | - | - |
| | Fluticasone propionate | 20%(v/v) | - | - | - |

*Note: "-" = negative result*

*Table 2.1.2. Test Results of SARS-CoV-2 cultured virus simulated specimens*

| **Potential interfering substances were spiked into Limit of detection references（SARS-CoV-2 cultured virus with concentration of 1.15 x $10^2$ $TCID_{50}$/mL）** | | **Concentration** | **DIAQUICK COVID-19 Ag Cassette** | | |
|---|---|---|---|---|---|
| | | | **Lot 1** | **Lot 2** | **Lot 3** |
| | Human blood (EDTA anticoagulated) | 20% (v/v) | + | + | + |
| | Mucin | 5mg/ml | + | + | + |
| Antiviral drugs | Oseltamivir phosphate | 5mg/ml | + | + | + |
| | Ribavirin | 5mg/ml | + | + | + |
| Antibiotics /antibacterial drugs | Levofloxacin | 5mg/ml | + | + | + |
| | Azithromycin | 5mg/ml | + | + | + |
| | Meropenem | 5mg/ml | + | + | + |
| | Tobramycin | 2mg/ml | + | + | + |
| Nasal sprays or nose drops | Phenylephrine | 20%(v/v) | + | + | + |
| | oxymetazoline | 20%(v/v) | + | + | + |
| | 0.9% sodium chloride | 20%(v/v) | + | + | + |
| | A natural soothing ALKALOL | 20%(v/v) | + | + | + |
| Nasal corticosteroids | Beclomethasone | 20%(v/v) | + | + | + |
| | Hexadecadrol | 20%(v/v) | + | + | + |
| | Flunisolide | 20%(v/v) | + | + | + |
| | Triamcinolone | 20%(v/v) | + | + | + |
| | Budesonide | 20%(v/v) | + | + | + |
| | Mometasone | 20%(v/v) | + | + | + |
| | Fluticasone | 20%(v/v) | + | + | + |
| | Fluticasone propionate | 20%(v/v) | + | + | + |

*Note: "+"= positive results*

**Conclusion:**
According to the results, we can conclude that all the mentioned substances did not interfere with the DIAQUICK COVID-19 Ag Cassette (Swab).

## 2.2. Cross Reactivity

**Study objective:**
To evaluate the cross reactivity of DIAQUICK COVID-19 Ag Cassette (Swab) with potential cross-reactivity pathogens.

**Study design:**
Materials:

- DIAQUICK COVID-19 Ag Cassette (Swab)
  Lot 1: 2006189; Lot 2: 2006190; Lot 3: 2006191

Method:
To find if there is cross reaction with possible pathogens in the clinical specimens, use the DIAQUICK COVID-19 Ag Cassette (Swab) to test these specimens.

Specimen Preparation:
*Table 2.2.1. Specimen Information*

| Pathogens | |
|---|---|
| Respiratory syncytial virus Type A | Human coronavirus NL63 |
| Respiratory syncytial virus Type B | Human coronavirus HKU1 |
| Novel influenza A H1N1 virus (2019) | Parainfluenza virus 1 |
| Seasonal influenza A H1N1 virus | Parainfluenza virus 2 |
| Influenza A H3N2 virus | Parainfluenza virus 3 |
| Influenza A H5N1 virus | Parainfluenza virus 4 |
| Influenza B Yamagata | Haemophilus influenzae |
| Influenza B Victoria | Streptococcus pyogenes |
| Rhinovirus | Streptococcus pneumoniae |
| Adenovirus 3 | Candida albicans |
| Adenovirus 7 | Bordetella pertussis |
| EV-A71 | Mycoplasma pneumoniae |
| Mycobacterium tuberculosis | Chlamydia pneumoniae |
| Mumps virus | Legionella pneumophila |
| Human coronavirus 229E | Pooled human nasal wash |
| Human coronavirus OC43 | |

Procedure:
Test above specimen with DIAQUICK COVID-19 Ag Cassette (Swab). Add 50 µL specimen to the swab and perform the test according to the test procedure in the package insert. Three lots of the tests will be used per specimen, each specimen will be tested once per lot. Totally three operators, one operator perform one lot.

*Note:* If the test result is valid, test the specimen again. People who prepare the specimen do not participate in the specimen testing. Operators (A, B, C) do not prepare the specimen.

**Results:**

*Table 2.2.2. Test Results*

| Pathogens | Concentration | DIAQUICK COVID-19 Ag Cassette | | |
|---|---|---|---|---|
| | | Lot1 | Lot2 | Lot3 |
| Respiratory syncytial virus Type A | $5.5x10^7$ PFU/mL | - | - | - |
| Respiratory syncytial virus Type B | $2.8x10^5$ TCID50/mL | - | - | - |
| Novel influenza A H1N1 virus (2019) | $1x10^6$ PFU/mL | - | - | - |
| Seasonal influenza A H1N1 virus | $1x10^5$ PFU/mL | - | - | - |
| Influenza A H3N2 virus | $1x10^6$ PFU/mL | - | - | - |
| Influenza A H5N1 virus | $1x10^6$ PFU/mL | - | - | - |
| Influenza B Yamagata | $1x10^5$ PFU/mL | - | - | - |
| Influenza B Victoria | $1x10^6$ PFU/mL | - | - | - |
| Rhinovirus | $1x10^6$ PFU/mL | - | - | - |
| Adenovirus 3 | $5x10^{7.5}$ $TCID_{50}$/mL | - | - | - |
| Adenovirus 7 | $2.8x10^6$ $TCID_{50}$/mL | - | - | - |
| EV-A71 | $1x10^5$ PFU/mL | - | - | - |
| Mycobacterium tuberculosis | $1x10^3$ bacteria/mL | - | - | - |
| Mumps virus | $1x10^5$ PFU/mL | - | - | - |
| Human coronavirus 229E | $1x10^5$ PFU/mL | - | - | - |
| Human coronavirus OC43 | $1x10^5$ PFU/mL | - | - | - |
| Human coronavirus NL63 | $1x10^6$ PFU/mL | - | - | - |
| Human coronavirus HKU1 | $1x10^6$ PFU/mL | - | - | - |
| Parainfluenza virus 1 | $7.3x10^6$ PFU/mL | - | - | - |
| Parainfluenza virus 2 | $1x10^6$ PFU/mL | - | - | - |
| Parainfluenza virus 3 | $5.8x10^6$ PFU/mL | - | - | - |
| Parainfluenza virus 4 | $2.6x10^6$ PFU/mL | - | - | - |
| Haemophilus influenzae | $5.2x10^6$ CFU/mL | - | - | - |
| Streptococcus pyogenes | $3.6x10^6$ CFU/mL | - | - | - |
| Streptococcus pneumoniae | $4.2x10^6$ CFU/mL | - | - | - |
| Candida albicans | $1x10^7$ CFU/mL | - | - | - |
| Bordetella pertussis | $1x10^4$ bacteria/mL | - | - | - |
| Mycoplasma pneumoniae | $1.2x10^6$ CFU/mL | - | - | - |
| Chlamydia pneumoniae | $2.3x10^6$ IFU/mL | - | - | - |
| Legionella pneumophila | $1x10^4$ bacteria/mL | - | - | - |
| Pooled human nasal wash | 100% | - | - | - |

*Note: "-"= Negative Results*

**Conclusion:**

The tested pathogens above showed no cross-reaction with the DIAQUICK COVID-19 Ag Cassette (Swab).

## 3. Precision

### 3.1. Intra-Assay

**Study objective:**
To evaluate the precision of DIAQUICK COVID-19 Ag Cassette (Swab) in lot.

**Study design:**
Method:
Use the same batch of tests the positive and negative specimen and observe the precision in lot. 50 µL specimens were added to swabs and then tested in the DIAQUICK COVID-19 Ag Cassette (Swab) using the procedure appropriate for patient nasal swab specimens. Three lots were assayed by three operators respectively. Testing at each lot consisted of 10 replicates for each specimen.

Materials:
- DIAQUICK COVID-19 Ag Cassette (Swab)
  Lot 1: 2006189; Lot 2: 2006190; Lot 3: 2006191
- Positive Specimen: SARS-CoV-2 cultured virus: Concentration of 1.15 x $10^2$ $TCID_{50}$/mL (LoD) and 4.6 x $10^2$ $TCID_{50}$/mL (4 x LoD)
- Negative Specimen: Pooled human nasal matrix obtained from healthy donors and confirmed negative for SARS-CoV-2.

Test Procedure:
Perform the test according to the test procedure in the package insert.

Acceptance Criteria:
Negative specimens get negative result and positive specimens get positive result.

**Results:**
**Lot 1: 2006189**

| | | Specimens | | |
|---|---|---|---|---|
| | | Negative Specimen | 1.15 x $10^2$ $TCID_{50}$/mL | 4.6 x $10^2$ $TCID_{50}$/mL |
| Test | 1 | - | + | + |
| | 2 | - | + | + |
| | 3 | - | + | + |
| | 4 | - | + | + |
| | 5 | - | + | + |
| | 6 | - | + | + |
| | 7 | - | + | + |
| | 8 | - | + | + |
| | 9 | - | + | + |
| | 10 | - | + | + |

**Lot 2: 2006190**

| | | Specimens | | |
|---|---|---|---|---|
| | | Negative Specimen | $1.15 \times 10^2$ $TCID_{50}$/mL | $4.6 \times 10^2$ $TCID_{50}$/mL |
| Test | 1 | - | + | + |
| | 2 | - | + | + |
| | 3 | - | + | + |
| | 4 | - | + | + |
| | 5 | - | + | + |
| | 6 | - | + | + |
| | 7 | - | + | + |
| | 8 | - | + | + |
| | 9 | - | + | + |
| | 10 | - | + | + |

**Lot 3: 2006191**

| | | Specimens | | |
|---|---|---|---|---|
| | | Negative Specimen | $1.15 \times 10^2$ $TCID_{50}$/mL | $4.6 \times 10^2$ $TCID_{50}$/mL |
| Test | 1 | - | + | + |
| | 2 | - | + | + |
| | 3 | - | + | + |
| | 4 | - | + | + |
| | 5 | - | + | + |
| | 6 | - | + | + |
| | 7 | - | + | + |
| | 8 | - | + | + |
| | 9 | - | + | + |
| | 10 | - | + | + |

**Conclusion:**
The precision is fine and acceptable of DIAQUICK COVID-19 Ag Cassette (Swab).

### 3.2. Inter-Assay

**Study objective:**
To evaluate the precision of DIAQUICK COVID-19 Ag Cassette (Swab) between lot.

**Study design:**
Method:
Use different batches of test to test the same negative and positive specimen and observe the precision between lots. 50 µL specimens were added to swabs and then tested in the DIAQUICK COVID-19 Ag Cassette (Swab) using the procedure appropriate for patient nasal swab specimens.

Materials:
- DIAQUICK COVID-19 Ag Cassette (Swab)
  Lot 1: 2006189; Lot 2: 2006190; Lot 3: 2006191
- Positive Specimen: SARS-CoV-2 cultured virus: Concentration of $1.15 \times 10^2$ $TCID_{50}$/mL. (LoD) and $4.6 \times 10^2$ $TCID_{50}$/mL (4 x LoD).
- Negative Specimen: Pooled human nasal matrix obtained from healthy donors and confirmed negative for SARS-CoV-2.

Test Procedure:
Perform the test according to the test procedure in the package insert.

Acceptance Criteria:
Negative specimens get negative results and positive specimens get positive results.

**Result:**

| | | Negative Specimen | | |
|---|---|---|---|---|
| | | Lot 1 | Lot 2 | Lot 3 |
| Test | 1 | - | - | - |
| | 2 | - | - | - |
| | 3 | - | - | - |
| | 4 | - | - | - |
| | 5 | - | - | - |
| | 6 | - | - | - |
| | 7 | - | - | - |
| | 8 | - | - | - |
| | 9 | - | - | - |
| | 10 | - | - | - |

| | | $1.15 \times 10^2$ $TCID_{50}$/mL | | |
|---|---|---|---|---|
| | | Lot 1 | Lot 2 | Lot 3 |
| Test | 1 | + | + | + |
| | 2 | + | + | + |
| | 3 | + | + | + |
| | 4 | + | + | + |
| | 5 | + | + | + |
| | 6 | + | + | + |
| | 7 | + | + | + |
| | 8 | + | + | + |
| | 9 | + | + | + |
| | 10 | + | + | + |

| | | $4.6 \times 10^2$ $TCID_{50}$/mL | | |
|---|---|---|---|---|
| | | Lot 1 | Lot 2 | Lot 3 |
| Test | 1 | + | + | + |
| | 2 | + | + | + |
| | 3 | + | + | + |
| | 4 | + | + | + |
| | 5 | + | + | + |
| | 6 | + | + | + |
| | 7 | + | + | + |
| | 8 | + | + | + |
| | 9 | + | + | + |
| | 10 | + | + | + |

**Conclusion:**
The precision is fine and acceptable of DIAQUICK COVID-19 Ag Cassette (Swab).