**Supplementary information**

**Analytical performance studies**

Precision was evaluated by following the CLSI EP05-A3 [1]. Within-run precision study was performed with 20 replicates of low (approximately 2.51 ng/L) and high (approximately 8.68 ng/L) mixed SARS-CoV-2 positive nasopharyngeal swab samples in 1 complete run. Between-day precision was assessed using the same mixed SARS-CoV-2 positive Nasopharyngeal swab samples daily over 20 days. The mean, standard deviation (SD), and coefficient of variation (CV, %), defined as (SD × 100)/mean, for each precision series were determined.

The limit of quantitation (LoQ) was determined as described by *the* Clinical and Laboratory Standards Institute (CLSI) EP17-A2 [2]. To establish the LoQ, five samples with concentrations of 0.318 ng/L, 0.637 ng/L, 0.955 ng/L, 1.274 ng/L and 1.592 ng/L were prepared. The study was performed on one MAGLUMI X8 analyzer using one calibration curve and one lot of reagent (lot No. 43322031001). Each sample was run in 3 days, with 5 replicates per run each day. A calibration curve was plotted to determine the LoQ, which was the concentration corresponding to the 20% coefficient of variation (CV).

Linearity was assessed by using a series of mixed 6 sample pools. As specified in the CLSI EP06 [3], a high-level of SARS-CoV-2 antigen concentration from one patient’s nasopharyngeal sample was serially diluted with a low nasopharyngeal sample. Diluted sample pools were measured in triplicate. Linear regression analysis was employed to assess the performance of recovery.

In order to assess the analytical specificity of the system, forty-eight commercialized microbial specimens were studied respectively. For detailed information of the 48 microbial specimens, please see Supplemental Table 2. Healthy human nasopharyngeal specimens (n=48) were measured (healthy controls) and then spiked with one of the virus to the target concentration, and tested with the MAGLUMI SARS-CoV-2 Ag assay (spiked-in samples). In addition, 10 non-COVID-19 respiratory specimens from clinical patients with upper respiratory virus infection were measured, 2 of whom were diagnosed with seasonal coronavirus infection, 2 were adenovirus, 2 influenza A, 2 influenza B and 2 were respiratory syncytial infection. None of these samples were SARS-CoV-2 positive (clinical samples). Specimens used in analytical validation were not used for subsequent studies.

To comprehensively evaluate the detection performance on different virus variants, 1 different variants of SARS-CoV-2 were added to a pool of nasopharyngeal samples obtained from healthy donors respectively. The variants were from ZeptoMetrix, LLC., with the initial concentration unit of TCID50/mL. Serial dilutions with 5 concentrations for each variants were prepared for SARS-CoV-2 Antigen testing. The mean of results obtained from triplicate tests were plotted. And a fitted linear regression model was plotted against the corresponding theoretical variant concentration.

**Analytical performance of MAGLUMI SARS-CoV-2 Ag (CLIA) tests**

The default unit for the analyte detected by the MAGLUMI SARS-CoV-2 antigen tests was manufacturer’s arbitrary unit (AU)/mL. To ensure our final results are shown in an SI derived unit, we carried out a unit conversion experiment prior to the studies. A linear relationship between AU/mL and ng/L was found (correlation coefficient factor R2=0.9976) in the range between 0.763 ng/L to 6.104 ng/L, and the conversion factor to obtain ng/L is 1.592 (i.e. 1 AU/mL = 1.592 ng/L).

At the concentration of 2.51ng/L and 8.68ng/L, the average percentage of coefficient of variation (CV%) for within-run and between-day were both shown to be less than 6%, (Supplemental Table 1).

Analytical validation established limit of quantitation (LoQ) at 0.399 ng/L and linear range from 0.40 to 3184.00 ng/L (Figure 1A,1B). A maximum of 20-fold dilution can be performed to achieve the clinical reportable range of 0.40 to 63680.00 ng/L.

Analytical specificity was assessed with forty-eight commercially available virus forms in spiked-in human nasopharyngeal samples, respectively. Results showed negligible reaction to the SARS-CoV-2 antigen assay when compared to control specimens from healthy donors (Figure 1C). Similarly, compared with the control specimens, no significant change in relative light unit (RLU) was found in the tested respiratory samples of 10 SARS-CoV-2 negative respiratory specimens, including 2 seasonal coronavirus, 2 adenovirus, 2 influenza A, 2 influenza B and 2 respiratory syncytial virus positive specimens (Figure 1C). These results indicated a well-performed analytical specificity of the assay.

Five serial diluents of five SARS-CoV-2 variants of concerns (VOCs) were detected respectively with MAGLUMI SARS-CoV-2 Ag (CLIA) assay. The fitted linear regression model plotted against the corresponding theoretical variant concentrations showed a remarkable linear correlation between the antigen concentration versus the infectious dose for different VOCs (Figure 1D).

**Supplemental Table 1. Precision study on the MAGLUMI SARS-CoV-2 antigen assay.** The mean ± standard deviation (SD) was shown in the unit of ng/L.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample IDa** | **Mean**  **(n=80)b** | **Within-Run** | | **Between-Day** | |
| **SD** | **%CV** | **SD** | **%CV** |
| 1 | 2.51 | 0.089 | 3.53 | 0.151 | 5.99 |
| 2 | 8.68 | 0.335 | 3.85 | 0.441 | 5.08 |

**a.** Mixed SARS-CoV-2 positive nasopharyngeal swab samples with two different concentrations were prepared.

**b.** Within-run precision study was performed with 20 replicates of low (approximately 2.51 ng/L) and high (approximately 8.68 ng/L) mixed SARS-CoV-2 positive nasopharyngeal swab samples in 1 complete run. Between-day precision was assessed using the same mixed SARS-CoV-2 positive nasopharyngeal swab samples daily over 20 days.

**Supplemental Table 2. Overview of virus forms examined in the cross-reaction study.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Virus type** | **Manufacturer** | **Catalogue number** | **Final concentration for the assay** |
| HKU1 | Invent Diagnostica GmbH | DSPA 4.1.9.14.1 | 1.5×105 TCID50/mL |
| OC43 | ZeptoMetrix, LLC | 0810024CF | 1.5×105 TCID50/mL |
| NL63 | ZeptoMetrix, LLC | 0810228CF | 1.5×105 TCID50/mL |
| 229E | ZeptoMetrix, LLC | 0810229CF | 1.5×105 TCID50/mL |
| H1N1 (seasonal) | ZeptoMetrix, LLC | 0810244CF | 1.5×105 TCID50/mL |
| H3N2 | ZeptoMetrix, LLC | 0810238CF | 1.5×105 TCID50/mL |
| H5N1 | BEI Resources | NR-31131 | 1.5×105 TCID50/mL |
| H7N9 | BEI Resources | NR-44078 | 1.5×105 TCID50/mL |
| H1N1 (2009) | American Type Culture Collection | VR-1894™ | 5.2×105 TCID50/mL |
| Influenza B (Yamagata) | ZeptoMetrix, LLC | 0810518CF | 3.8×105 TCID50/mL |
| Influenza B (Victoria) | ZeptoMetrix, LLC | 0810571CF | 1.5×105 TCID50/mL |
| HPIV I | ZeptoMetrix, LLC | 0810014CF | 1.26×105 TCID50/mL |
| HPIV II | ZeptoMetrix, LLC | 0810015CF | 1.5×105 TCID50/mL |
| HPIV III | ZeptoMetrix, LLC | 0810016CF | 8.51×105 TCID50/mL |
| RSV type A | ZeptoMetrix, LLC | 0810040ACF | 1.5×105 TCID50/mL |
| RSV type B | ZeptoMetrix, LLC | 0810040CF | 1.5×105 TCID50/mL |
| Rhinovirus A | ZeptoMetrix, LLC | 0810012CFN | 1.5×105 TCID50/mL |
| Rhinovirus B | ZeptoMetrix, LLC | 0810038CF | 1.5×105 TCID50/mL |
| Rhinovirus C | ZeptoMetrix, LLC | 0810053CF | 1.9×105 TCID50/mL |
| Adenovirus 1 | ZeptoMetrix, LLC | 0810050CF | 3.09×105 TCID50/mL |
| Adenovirus 2 | ZeptoMetrix, LLC | 0810110CF | 1.5×105 TCID50/mL |
| Adenovirus 3 | ZeptoMetrix, LLC | 0810062CF | 1.5×105 TCID50/mL |
| Adenovirus 4 | ZeptoMetrix, LLC | 0810070CF | 1.5×105 TCID50/mL |
| Adenovirus 5 | ZeptoMetrix, LLC | 0810020CF | 2.7×105 TCID50/mL |
| Adenovirus 7 | ZeptoMetrix, LLC | 0810021CF | 1.5×105 TCID50/mL |
| Adenovirus 55 | ZeptoMetrix, LLC | 0810022CF | 3.6×105 TCID50/mL |
| Enterovirus A | ZeptoMetrix, LLC | 0810236CF | 1.5×105 TCID50/mL |
| Enterovirus B | ZeptoMetrix, LLC | 0810543CF | 1.5×105 TCID50/mL |
| Enterovirus C | ZeptoMetrix, LLC | 0810343CF | 5.5×105 TCID50/mL |
| Enterovirus D | ZeptoMetrix, LLC | 0810300CF | 2.2×105 TCID50/mL |
| EBV | ZeptoMetrix, LLC | 0810008CF | 1.5×105 TCID50/mL |
| Measles virus | ZeptoMetrix, LLC | 0810025CF | 1.5×105 TCID50/mL |
| HCMV | ZeptoMetrix, LLC | 0810003CF | 1.5×105 TCID50/mL |
| Rotavirus | ZeptoMetrix, LLC | 0810041CF | 2.4×105 TCID50/mL |
| Norwalk virus | BIOIVT & ELEVATING SCIENCE | HMN48675 | 1.5×105 TCID50/mL |
| Mumps virus | ZeptoMetrix, LLC | 0810079CF | 1.5×105 TCID50/mL |
| VZV | BIOIVT & ELEVATING SCIENCE | HMN48824 | 3.2×105 TCID50/mL |
| hMPV | ZeptoMetrix, LLC | 0810159CF | 1.5×105 TCID50/mL |
| **M. pneumonia** | ZeptoMetrix, LLC | 0801579 | 2.70×106 CCU/mL |
| C. **pneumoniae** | ZeptoMetrix, LLC | 0804392 | 1.70×106 IFU/mL |
| Haemophilus influenzae | ZeptoMetrix, LLC | 0801679 | 6.97×106 CFU/mL |
| S. **aureus** | ZeptoMetrix, LLC | 0804125 | 2.51×106 CFU/mL |
| S. pneumoniae | ZeptoMetrix, LLC | 0801439 | 1.34×106 CFU/mL |
| K. pneumoniae | ZeptoMetrix, LLC | 0801506 | 1.14×106 CFU/mL |
| M. tuberculosis | ZeptoMetrix, LLC | 0801660 | 1.49×106 CFU/mL |
| Canidia Albicans | ZeptoMetrix, LLC | 0801504 | 4.76×106 CFU/mL |
| MERS-CoV | ZeptoMetrix, LLC | 0810575UV | 1.5×105 TCID50/mL |
| SARS-CoV | BEI Resources | NR-9323 | 1.5×105 TCID50/mL |

**References**

1. McEnroe RJ, Durham AP, Goldford MD, Kondratovich MV, Lababidi S, Magari R, et al. Evaluation of Precision of Quantitative Measurement Procedure; Approved Guideline. Clinical Laboratory Standards Institute 2014.
2. Pierson-Perry JF, Vaks JE, Vore TEK, Durham AP, Fischer C, Gutenbrunner C, et al. Evaluation of detection capability for clinical laboratory measurement procedures; approved guideline. Clinical Laboratory Standards Institute 2012.
3. McEnroe RJ, Durham AP, Kondratovich MV, Johansen JV, Meyers PG, Souers RJ, Vaks JE. Evaluation of linearity of quantitative measurement procedures. EP06. Clinical and Laboratory Standards Institute 2020.