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Short communication

Assessment of an immunochromatographic kit for detection of severe acute respiratory syndrome coronavirus 2 and influenza viruses

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ABSTRACT

An immunochromatographic kit was developed to detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and influenza viruses (A and B) on two detection positions of a single strip. The sensitivity and specificity for SARS-CoV-2 were 97.4 % and 100 %, respectively, and those for influenza viruses were 100 %, respectively.

WHO (2022a) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) started in December 2019 and continues at the time of writing in 2021 (https://www.who.int/emergencies/diseases/novel-coronavirus-2019) with no predictable endpoint (Telenti et al., 2021). A worrisome problem is that an influenza epidemic or pandemic may occur during the COVID-19 pandemic because both viruses cause respiratory tract infections and are transmitted by respiratory droplets and/or aerosols (Telenti et al., 2021). We describe here production of an immunochromatographic assay (ICA) to detect both SARS-CoV-2 and influenza A and B (A/B) viruses.

Monoclonal antibodies were prepared by immunization of rats with recombinant SARS-CoV-2 nucleocapsid (N) protein (Oshiro et al., 2021) or N proteins of influenza A/B viruses (Flarebio Biotech, LLC, MD, USA and Sino Biological Inc, Beijing, China, respectively). The ICA was developed by coating nitrocellulose membranes with capture antibodies for SARS-CoV-2 (N1J7–1), and influenza A/B virus (K2A4 and K3B1, respectively) and control goat anti-rodent IgG antibody. Detection antibody-conjugated colloidal gold nanoparticles for SARS-CoV-2 (N3J3–1), and influenza A/B virus (K2A7 and K3B2, respectively) were immersed into glass fiber. The ICA kit, KMB LineCheck nCoV/Flu, was a single-strip, lateral-flow device comprising three detection positions on the strip, including a test position for SARS-CoV-2, a test position for influenza A/B viruses, and a control position (Fig. 1). To examine the stability of the ICA kit, the sensitivity and specificity of the kits were regularly determined using SARS-CoV-2 and influenza A/B virus antigens after stored at room temperature (5–30 °C). The sensitivity and specificity did not changed after 6 months storage. Further examination is now in progress. A nasopharyngeal swab sample (50 μL) obtained from a patient suspected COVID-19 or seasonal influenza was added in elution buffer (200 μL Tris-based buffer, pH 7.6). Three droplets of the solution were added into the sample well. To determine the detection limit of the ICA, culture supernatants of SARS-CoV-2 (2019-nCoV/JPN/TY/WK-521, 4.2 × 104 tissue culture infectious dose 50 (TCID50)/mL), influenza A virus [A/New Jersey/8/76(H1N1), 1.24 × 105 plaque forming unit (PFU)/mL] or influenza B virus (B/Taiwan/2/62, 1.72 × 104 PFU/mL) were diluted in PBS. An aliquot of diluted supernatant (10 μL) was further diluted in 240 μL of Tris-based buffer (pH 7.6), and 90 μL of the product was used to test the ICA. A total of 100 nasopharyngeal swab samples were collected from patients suspected of COVID-19 with symptoms such as fever, dry cough, fatigue, loss of taste/smell, nasal congestion, conjunctivitis, sore throat, headache, muscle/joint pain, skin rash, nausea/vomiting, diarrhea and/or chills/dizziness; during September 2020 to February 2021 at a university hospital in Tokyo. All samples were subjected to RT-PCR for the detection of SARS-CoV-2 according to protocol (Shirato et al., 2020). Of the 100 samples, 39 were RT-PCR-positive for SARS-CoV-2 (Table 1) among which 38 were ICA-positive for SARS-CoV-2. All 61 RT-PCR-negative samples were ICA-negative for SARS-CoV-2 (Table 1).
All 100 samples were ICA-negative for influenza (Table 1). The sensitivity and specificity of ICA for SARS-CoV-2 detection were 97.4 % and 100 %, respectively. The lower detection limit of ICA for SARS-CoV-2 was \(7.81 \times 10^{-2}\) TCID\(_{50}\)/mL. The ICA for influenza viruses was negative even at the highest concentration of \(2.5 \times 10^{3}\) TCID\(_{50}\)/mL of SARS-CoV-2 (data not shown). The samples in elution buffer containing SARS-CoV-2 at less than the lowest detection limit of ICA (4.88 \(\times 10^{-2}\) TCID\(_{50}\)/mL) were RT-PCR-positive for SARS-CoV-2.

A total of 152 nasopharyngeal swab samples were collected from patients suspected of seasonal influenza with symptoms such as fever, cough, headache, muscle and joint pain, severe malaise, sore throat and a runny nose (WHO (2022b); during January to April 2016 in seven clinics in Fukuoka, Japan. All samples were subjected to RT-PCR for the detection of influenza A/B viruses. Of them, 42 were RT-PCR-positive for influenza A and 50 were positive for influenza B (Table 2) of which all were ICA-positive for influenza viruses. All RT-PCR-negative samples were ICA-negative for influenza viruses (Table 2). All the 152 samples were ICA-negative for SARS-CoV-2 (Table 2). The sensitivity and specificity of KBM LineCheck nCoV/Flu for influenza viruses were 100 %, respectively. The lower detection limits of ICA for influenza A/B viruses were \(1.21 \times 10^{-1}\) PFU/mL and \(1.68 \times 10^{-1}\) PFU/mL, respectively. The ICA for SARS-CoV-2 was negative even at the highest concentrations of \(1.55 \times 10^{-3}\) PFU for influenza A and \(2.15 \times 10^{-3}\) PFU for influenza B viruses, respectively (data not shown). The samples in elution buffer containing influenza viruses at less than the lowest detection limit of ICA (6.05 PFU/mL and 8.4 PFU/mL, respectively) were RT-PCR-positive for influenza viruses.

As shown in Table S1, recombinant N proteins of SARS-CoV-1 and SARS-CoV-2 were ICA-positive for SARS-CoV-2 and ICA-negative for influenza virus. Influenza A virus (H1N1) (A/Virginia/ATCC1/2009, A/Swine/1976/31, A/Swine/Iowa/15/30), influenza A virus (H3N2) A/HongKong/8/68(TC-adapted) and influenza B virus, B/Lee/20 were ICA-positive for influenza virus and ICA-negative for SARS-CoV-2 (Table S1). Three recombinant N proteins of MERS-coronavirus, human coronavirus OC43 and human coronavirus 299E, and 59 pathogens causing respiratory infections were ICA-negative for both SARS-CoV-2 and influenza viruses (Table S1).

KMB LineCheck nCoV/Flu is a useful kit to test for COVID-19 and influenza simultaneously, especially during an influenza season amid the COVID-19 pandemic.

**Authors’ contributions**

MA, JS and SO developed the kit. JS and YT acquired clinical samples. SO, NM and KS assessed the kit and analyzed the data. KF, MA and JS conducted RT-PCR. YT, TM, TT and TK supervised the study. All authors approved this final version manuscript.

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**Table 1**

Detection of SARS-CoV-2 by KBM LineCheck nCoV/Flu in 100 nasopharyngeal swab samples from COVID-19 suspected patients.

|                  | Positive | Negative | Total |
|------------------|----------|----------|-------|
| SARS-CoV-2 (influenza virus) | \(38 (0)\) | \(0 (0)\) | \(38 (0)\) |
| ICA for SARS-CoV-2 (influenza virus) | \(62 (100)\) | \(0 (100)\) | \(62 (100)\) |
| Total            | \(39 (0)\) | \(61 (100)\) | \(100 (100)\) |


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*a* Numbers of RT-PCR samples for SARS-CoV-2 (samples of RT-PCR-positive or negative for influenza virus).

*b* Numbers of samples ICA-positive or negative for SARS-CoV-2 (samples ICA-positive or negative for influenza virus).

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**Fig. 1.** Details of the immunochromatographic assay kit (KBM LineCheck nCoV/Flu). Samples showing a single line at the control position were negative for SARS-CoV-2 or influenza A and B viruses (a), whereas samples showing two lines, one each at the control and test position for SARS-CoV-2 or influenza A and B viruses, were positive for SARS-CoV-2 (b) or influenza A and B viruses (c).
Table 2
Detection of influenza A and B viruses by KBM LineCheck nCoV/Flu in 153 nasopharyngeal swab samples from influenza suspected patients.

|                     | RT-PCR |                       |
|---------------------|--------|-----------------------|
|                     | Influenza viruses (SARS-CoV-2) |          |
|                     | Positive | Negative | Total    |
| ICA                 |          |          |          |
| Influenza viruses (SARS-CoV-2) | 92 (0) | 0 (0) | 92 (0) |
| Negative            | 0 (0)    | 61 (153) | 61 (153) |
| Total               | 92 (0)   | 61 (153) | 153 (153) |

*Numbers of samples RT-PCR-positive or negative for influenza viruses (those of samples RT-PCR-positive or negative forSARS-CoV-2).

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Declaration of Competing Interest

MA and JS are employees of Kohjin Bio Co., Ltd.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jviromet.2022.114477.

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WHO. Coronavirus disease (COVID-19) pandemic. Coronavirus disease (COVID-19) (who.int).

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