Philip M. Hemken, Eitan Israeli, Russell Taylor, Chris Jacobson, Maria Datwyler, Rene Geissler, Abbas Hadji, Nicolette Jeanblanc, Kinnari Pandya, Marilee Marcinkus, Ryan Piktel, M. Felicia Bogdan, Mary Rodgers, Mark Anderson, Robert Ziemann, Bryan C. Tieman, David Hawksworth, Jeffrey Moore, Kathy S. Otis, Christopher C. Marohnic, Josie Corby, Bailin Tu, Zhihong Lin, Alak Kar, James Hartnett, Carolyn Strobel, Svetoslava Gregory, Tracey Rae, A. Scott Muerhoff, Susan Brophy, John R. Hackett, Jr., David Daghfal, Matthew L. Faron, Amorina Cruz, Phaedre Mohr, Lori Sokoll and Gerard J. Davis*

Pre-analytical considerations in the development of a prototype SARS-CoV-2 antigen ARCHITECT automated immunoassay

https://doi.org/10.1515/cclm-2022-1292
Received December 20, 2022; accepted February 7, 2023;
published online February 20, 2023

Abstract

Objectives: To evaluate pre-analytical challenges related to high-volume central laboratory SARS-CoV-2 antigen testing with a prototype qualitative SARS-CoV-2 antigen immunoassay run on the automated Abbott ARCHITECT instrument.

Methods: Contrived positive and negative specimens and de-identified nasal and nasopharyngeal specimens in transport media were used to evaluate specimen and reagent on-board stability, assay analytical performance and interference, and clinical performance.

Results: TCID50/mL values were similar for specimens in various transport media. Inactivated positive clinical specimens and viral lysate (USA-WA1/2020) were positive on the prototype immunoassay. Within-laboratory imprecision was ≤0.10 SD (<1.00 S/C) with a ≤10% CV (≥1.00 S/C). Assay reagents were stable on board the instrument for 14 days. No high-dose hook effect was observed with a SARS-CoV-2 stock of Ct 13.0 (RLU>1.0×10^6). No interference was observed from mucin, whole blood, 12 drugs, and more than 20 cross-reactants. While specimen stability was limited at room temperature for specimens with or without viral inactivation, a single freeze/thaw cycle or long-term storage (>30 days) at −20 °C did not adversely impact specimen stability or assay performance. Specificity of the prototype SARS-CoV-2 antigen immunoassay was ≥98.5% and sensitivity was ≥89.5% across two ARCHITECT instruments. Assay sensitivity was inversely correlated with Ct and was similar to that reported for the Roche Elecsys® SARS-CoV-2 Ag immunoassay.

Conclusions: The prototype SARS-CoV-2 antigen ARCHITECT immunoassay is sensitive and specific for detection of SARS-CoV-2 in nasal and nasopharyngeal specimens. Endogenous proteases in mucus may degrade the target antigen, which limits specimen storage and transport times and complicates assay workflow.

Keywords: antigen; assay analytics; COVID-19; specimen pre-analytics.

Philip M. Hemken and Eitan Israeli contributed equally to this work.

Current affiliation: Eitan Israeli, The Barry Skolnick Biosafety Level 3 Unit, Faculty of Medicine, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.

***Corresponding author: Gerard J. Davis**, PhD, Assay Development, Abbott Laboratories, 100 Abbott Park Road, D09NC, AP20/3, Abbott Park, IL 60064, USA, Phone: 1 224 668 6562, E-mail: gerard.davis@abbott.com
Philip M. Hemken, Eitan Israeli, Robert Ziemann, Bryan C. Tieman, David Hawksworth, Jeffrey Moore, Kathy S. Otis, Christopher C. Marohnic, Josie Corby, Bailin Tu, Zhihong Lin, Alak Kar, James Hartnett, Carolyn Strobel, Svetoslava Gregory, Tracey Rae and A. Scott Muerhoff, Biologics Discovery, Abbott Laboratories, Abbott Park, IL, USA
Russell Taylor, Chris Jacobson, Maria Datwyler, Rene Geissler, Abbas Hadji, Nicolette Jeanblanc, Kinnari Pandya and Marilee Marcinkus, Assay Development, Abbott Laboratories, Abbott Park, IL, USA
Ryan Piktel and M. Felicia Bogdan, Process Design, Abbott Laboratories, Abbott Park, IL, USA
Mary Rodgers and Mark Anderson, ID Core Research, Abbott Laboratories, Abbott Park, IL, USA
Susan Brophy, John R. Hackett, Jr. and David Daghfal Abbott Diagnostics, Abbott Park, IL, USA
Matthew L. Faron and Amorina Cruz, Department of Pathology, The Medical College of Wisconsin, Milwaukee, WI, USA
Phaedre Mohr and Lori Sokoll, Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Introduction

Since the emergence of SARS-CoV-2 in 2019, several diagnostic assays have been developed to aid in surveillance and infection control, as well as patient screening and management. Early during the pandemic, molecular assays to detect SARS-CoV-2 viral nucleic acid became the backbone of the surveillance effort. These PCR-based assays were able to detect low levels of viral nucleic acid, even in asymptomatic individuals; however, molecular test positivity was not an accurate indicator of infectivity, and these assays were not as useful for guiding individual behaviors necessary for local infection control (e.g., isolation, mask wearing). SARS-CoV-2 antigen assays were developed to detect the viral nucleocapsid protein. When used often, these assays were able to detect early-stage infection during the window of greatest viral load and infectivity [1, 2] and thus better guided individual behaviors to mitigate transmission. Abbott Laboratories developed several SARS-CoV-2 molecular and antigen assays, including the rapid, point-of-care (POC) BinaxNOW™ COVID-19 – Antigen Self-Test Kit that is widely used today. To address the potential need for larger-scale central laboratory SARS-CoV-2 antigen testing, for example, for surveillance in schools and businesses, Abbott also developed an automated prototype SARS-CoV-2 antigen immunoassay run on the ARCHITECT *i*2000SR system. Similar assays were developed by Roche Diagnostics and Siemens Healthineers and received FDA emergency use authorization (EUA); however, turnaround times from specimen collection to test result limited their utility and POC SARS-CoV-2 antigen testing emerged as the preferred assay format for infection control.

Unlike POC testing, centralized high-throughput SARS-CoV-2 antigen testing would involve a more complex workflow, including transportation to the laboratory and ensuring specimen stability. Nasal and nasopharyngeal patient specimen collection and viral inactivation, specimen handling, and storage conditions could affect SARS-CoV-2 antigen stability and detection accuracy and limit the utility of large-scale, centralized antigen testing. Central lab testing would also have a longer turnaround time for reporting test results compared to POC tests. Here, we describe the development of the Abbott SARS-CoV-2 antigen immunoassay run on the automated high-throughput ARCHITECT *i*2000SR instrument, highlighting the pre-analytical challenges encountered during specimen collection through testing workflow that may affect assay performance and constrain the utility of this testing format for infection control. As these logistical and stability considerations are unique for centralized testing and ultimately prevented further development of the Abbott SARS-CoV-2 antigen immunoassay, this work and its findings can help inform the design and optimization of assays for use in future pandemics.

Materials and methods

Clinical specimens

Remnant nasal and nasopharyngeal specimens used in the stability study were originally collected from March 30, 2020 to April 4, 2020 in a separate IRB-approved study at the Medical College of Wisconsin (Milwaukee, WI, n=18). Remnant specimens from the Abbott Rapid Diagnostics Division (ARDx, Scarborough, ME; n=25) were also used in the stability study; these specimens were originally collected under separate IRB-approved protocols at the University of Rochester (collected September 20, 2020 – May 7, 2022) and Beaumont Health System (Royal Oak, MI) and University of Florida College of Medicine (Gainesville, FL; collected September 23, 2020 – May 7, 2022). All patient specimens were de-identified prior to transfer and were processed by Abbott Diagnostics (Abbott Park, IL) for testing. Negative specimens for the stability study were pooled from the Abbott In-House Collections.

A total of 300 negative and 150 positive de-identified nasopharyngeal specimens used in the clinical performance study were obtained from Cerba Xpert (Cerba Research, Ghent, Belgium); these specimens were collected in the late spring and summer of 2021 at French national screening sites during the Delta variant surge under a separate IRB-approved protocol. FLOQSwabs Flexible mini-tip (Copan Diagnostics, Murrieta, CA) were used to collect nasopharyngeal specimens. After specimen collection, the swabs were swirled five times in Universal Transport Medium (UTM; Copan Diagnostics) without viral inactivating agent and incubated for 1 min. The swab was then pressed against the side of the tube to recover liquid and discarded. Specimens were stored at 2–8, −20, or −80 °C and transported to the central laboratory. The study was performed in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki.

Assay development

Twenty-nine IgG antibodies and nine VHHs (the antigen binding domain of a camelid antibody heavy chain, also referred to as nanobodies) produced in house and five external antibodies were screened for use in the prototype SARS-CoV-2 antigen ARCHITECT immunoassay, for a total of approximately 350 antibody pairs. Initial screening was performed with the external antibodies as internal antibodies were not yet available. The initial assay format was selected based on sensitivity, specificity, and preliminary testing of positive and negative patient swab specimens in viral transport medium (VTM, CDC, Atlanta, GA) with known Ct values from PCR-based testing with the Abbott SARS-CoV-2 RealTi*m*e assay run on the *m*2000 instrument (Abbott Laboratories, Abbott Park, IL). A one-step assay format was found to give the best sensitivity: the specimen, capture, and detection reagent are added together, briefly incubated, then washed prior to signal generation. One of the external antibodies in the initial format was not manufacturable, so an internal antibody was substituted as the capture antibody. The final format included dual capture using two internal antibodies (Ab1 and Ab2) and dual detection using one internal antibody (Ab3) and one external antibody (Ab4) from the National Institute for Infectious Diseases in Japan found to be robust to SARS-CoV-2 variants. Figure 1 illustrates the final prototype SARS-CoV-2 antigen ARCHITECT immunoassay format [3], with chemiluminescence signals quantified as relative light units (RLUs). Extensive epitope mapping of

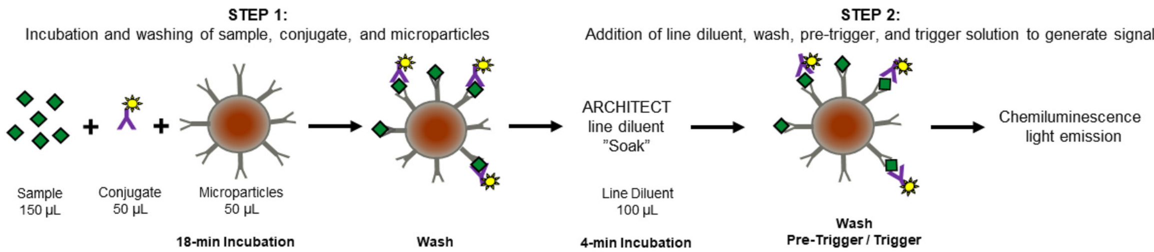

Figure 1: Prototype SARS-CoV-2 antigen ARCHITECT immunoassay. The assay is a dual antibody qualitative chemiluminescent microparticle immunoassay (CMIA). The assay uses a two-step reagent pipetting protocol in which the specimen, capture, and detection reagents are combined first and incubated for 18 min to allow antibody binding, followed by a 4 min soak/wash step. Two antibodies against the SARS-CoV-2 nucleocapsid protein are used for capture (Ab1 and Ab2) and detection (Ab3 and Ab4). The chemiluminescence signal is quantified as relative light units (RLUs). Nasal or nasopharyngeal swab specimens in universal transport medium (UTM) are inactivated with 0.2% Tergitol 15-S-9 based on a previous study [5]. Specimens are run at a final volume of 150 µL.

the four antibodies predicted robust detection of various circulating SARS-CoV-2 variants, including Omicron BA.1 and BA.2 and other variants of concern, when used in the dual capture/dual detection format. A recent study also demonstrated antibody performance in detecting SARS-CoV-2 variants across the Abbott Binax, PanBio, ARCHITECT, and immunoblot assays [4].

BIAcore binding kinetics

A BIAcore 4000 (GE Healthcare, Chicago, IL) was desorbed and sanitized per the manufacturer's protocols, and a Series S CM4 Sensor Chip (Cytiva, Marlborough, MA) was conditioned with short, duplicate injections of 100 mM HCl (Sigma, St. Louis, MO), 50 mM NaOH (Sigma), 0.1% SDS (Cytiva), and 150 mM H_3PO_4 (Fisher Scientific, Hampton, NH). BIAnormalizing solution was used for hydrodynamic addressing and normalization of the conditioned chip in 1x HBS-N prior to successfully passing the instrument's System Check with BIAtest solution (Cytiva). Mouse capture antibody reagent was immobilized onto the prepared CM4 chip using prepared Amine Coupling reagents per the manufacturer's protocols for a Mouse antibody capture kit (Cytiva).

Purified mouse antibody (Abbott Laboratories) was diluted into Running Buffer (1x HBS-EP+ [Cytiva], 0.1% BSA [Sigma], 0.1% Carboxymethyl-Dextran [Sigma], plus an additional 1 M NaCl [Sigma]) to 7.5 µg/mL and captured onto the immobilized mouse antibody capture surface for 7 min at a flow rate of 10 µL/min. After all antibodies were captured, the flow rate was changed to 30 µL/min and the biosensor was equilibrated for 2 min at an increased flow rate. High-performance injections of purified SARS-CoV-2 nucleocapsid diluted into Running Buffer at 0.457–3,000 nM (3-fold dilution series) occurred for 3 min immediately followed by 6 min of Running Buffer alone over capture (captured antibody) and reference (no antibody) surfaces. All concentrations were injected randomly, and each concentration was tested twice with multiple blank injections (0 nM). The biosensor was regenerated with one 3-min injection of the Mouse Antibody Regeneration Solution (Cytiva).

After removing all warm-up/conditioning cycles, the double, reference-subtracted sensorgrams were fit to a 1:1 binding model accounting for mass transfer using the BIAcore 4000 evaluation software.

Specimen stability

Stability of native nasal and nasopharyngeal specimens (Medical College of Wisconsin and ARDx), individual and pooled patient specimens, native positive spiked, viral lysate, and recombinant nucleocapsid protein (rAg) in UTM (Copan Diagnostics), calibrator diluent, and negative patient pooled human nasal wash was evaluated. rAg and high positive samples (pooled from 18 nasal and nasopharyngeal specimens with an average Ct value of 23.11) were spiked into a negative nasal and nasopharyngeal specimen matrix. The pooled high positive sample yielded RLUs of >3 million and was diluted in negative matrix to reach the appropriate RLU target concentrations as a function of the signal-to-cutoff ratio (S/C), based on the 6.5 RLU value for calibrator diluent. rAg spiked in negative patient specimen matrix was stored at room temperature (RT) 15–30 °C for 6 and 20 h. Negative and high positive nasal and nasopharyngeal specimens were stored at 2–8 °C for 24, 48, 72 h, and 7 days and at room temperature (15–30 °C) for 1, 3, 6, and 24 h. To inactivate virus, specimens were pretreated for 10 min with a 50X Tergitol 15-S-9 solution in UTM diluted to a final concentration of 0.2% Tergitol 15-S-9 and 0.0016% sodium azide [5]. Specimens were run on ARCHITECT *i*2000SR or Beckman Coulter Access 2 (Access SARS-CoV-2 Antigen Assay) instruments after storage under various conditions. Clinical specimens stored at −20 or −80 °C were also assessed for stability and the impact of freeze/thaw on assay performance on the ARCHITECT *i*2000SR instrument. Stability was reported as % difference in the S/C or a positive shift in the S/C ratio. An S/C% difference of <20% was designated as stable for samples with an S/C of >1.00. An S/C shift of <0.20 for samples with an S/C of <1.00 was designated as stable.

Assay analytical performance

FDA defines the limit of detection (LoD) as the lowest concentration of SARS-CoV-2 at which 19 of 20 replicates per concentration generate a reactive test result (≥1.0 cutoff index [COI]), expressed as the immunoassay tissue culture infective dose (TCID50)/mL. The LoD for specimens in UTM, VTM, and sterile saline (0.9%) was determined for the prototype SARS-CoV-2 antigen ARCHITECT immunoassay in limiting dilution studies (two-fold serial dilution series) using an inactivated viral lysate from BEI Resources (USA-WA1/2020, Cat. No. NR-52281). We evaluated the within-laboratory imprecision of the prototype SARS-CoV-2 antigen

ARCHITECT immunoassay using Clinical and Laboratory Standards Institute (CLSI, Malvern, PA) EP12-A2 as a guideline.

While the prototype SARS-CoV-2 antigen ARCHITECT immunoassay is a qualitative assay, we also assessed whether very high titer positive specimens have a high-dose hook (HDH) effect that could produce false negative results. An HDH effect occurs when a sample at an extremely high concentration falsely reads below the assay cutoff, while a more diluted sample shows higher results. We determined whether the prototype SARS-CoV-2 antigen ARCHITECT immunoassay exhibits and is critically susceptible to a HDH effect using CLSI EP34 as a guideline. HDH was evaluated with highest concentration of inactivated SARS-CoV-2 stock (Ct 13.0) available.

To determine the performance of the prototype SARS-CoV-2 antigen ARCHITECT immunoassay when a reagent kit remained on board the ARCHITECT *i*2000SR instrument for a minimum number of days, we conducted an in-use stability study using a classical, real-time testing protocol as defined by CLSI EP25-A. We also evaluated calibration curve stability.

Assay interference

Interference from endogenous substances was tested, including 0.5% bovine submaxillary gland mucin (Alfa Aesar, Ward Hill, MA; 5 mg/mL) and whole blood (4% v/v) negative for SARS-CoV-2 and SARS-CoV-2 antibodies (Boca Biolistics, Pompano Beach, FL). A swab containing 3 mL of a contrived positive specimen (+20% of cutoff by RLU) or 3 mL of a contrived negative specimen (−20% of cutoff by RLU) was inserted into 50 μL of each endogenous interferent solution, swirled, and incubated for 1 min. The swab was squeezed against the side of the tube and then discarded. 50X Tergitol pretreatment solution was added to each specimen at RT for a 15-min incubation prior to running on the prototype SARS-CoV-2 antigen ARCHITECT immunoassay [5].

To assess interference from drugs, a swab containing 3 mL of a contrived positive specimen (+20% cutoff by RLU) or 3 mL of a contrived negative specimen (−20% of cutoff by RLU) was inserted into 50 μL of each drug in solution, swirled, and incubated for 1 min. The swab was squeezed against the side of the tube and then discarded, and the specimen was run on the SARS-CoV-2 antigen ARCHITECT immunoassay. Swabs with 3 mL contrived positive specimen were mixed with 50 μL of each drug in solution and incubated for 1 min [6]. 50X Tergitol pretreatment solution was added to each specimen at RT for a 15-min incubation prior to running on the prototype SARS-CoV-2 antigen ARCHITECT immunoassay [5].

To test cross-reactivity, a swab was inserted into a tube containing 50 μL of each cross-reactant at its final concentration. The swab was then transferred into 3 mL of UTM, swirled five times, and incubated for 1 min at RT. Following the incubation, the swab was squeezed above the liquid line on the side of the tube and then discarded. The 50X Tergitol pretreatment solution was added to each specimen at RT for a 15 min incubation prior to running on the prototype SARS-CoV-2 antigen ARCHITECT immunoassay [5].

Clinical performance study

Specimens (Cerba Xpert) were tested in duplicate across two ARCHITECT *i*2000SR instruments using three calibrator lots. After arriving at the laboratory for testing, frozen specimens were thawed and all specimens were mixed by inversion 10 times. Three drops of 50X Tergitol pretreatment solution were added to 3 mL of each specimen in UTM [5], followed by gentle mixing by inversion 10 times. If present, visible precipitate was removed by centrifugation for 5 min. Specimens were incubated for 15 min at RT prior to running on the prototype SARS-CoV-2 antigen ARCHITECT immunoassay.

The sensitivity of the prototype assay run on the two ARCHITECT *i*2000SR instruments was determined using clinical specimens with known viral loads as reported by the vendor (Cerba Research; using the PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay run on the BioRad CFX384 instrument). Sensitivity data for the prototype assay was compared to that of the Elecsys® SARS-CoV-2 Ag immunoassay (Roche, Basel, Switzerland), as reported in the package insert [7].

Results

Assay analytical performance

BIAcore was used to verify the binding kinetics of the capture and detection antibodies used in the prototype SARS-CoV-2 antigen ARCHITECT immunoassay (Table 1). TCID50/mL values were similar for specimens in UTM (5), VTM (3.75), and calibrator diluent (3.75). All 20 positive specimens with viral inactivation were positive on the prototype immunoassay (≥1.0 COI), as was inactivated viral lysate (USA-WA1/2020). Furthermore, the TDIC50 for the prototype SARS-CoV-2 antigen ARCHITECT immunoassay in UTM, VTM, and sterile saline were approximately 4-fold lower than those reported for the EUA Elecsys SARS-CoV-2 Ag immunoassay (Table 2). Within-laboratory imprecision over the 5-day study was ≤0.10 SD (<1.00 S/C) with a ≤10% CV (≥1.00 S/C). Assay reagents were stable when stored on board the instrument for 14 days, with a less than −20% shift from baseline (≥1.00 S/C). No HDH effect was observed with the highest concentration of SARS-CoV-2 stock available (Ct 13.0, RLU>1.0×10^6). Endogenous interference with the assay is shown in Table 3; purified mucin (0.5%) and whole blood (4% v/v) did not interfere with assay performance (−0.06 and −0.01 S/CO, respectively). Drug interference and cross-reactivity results are shown in Supplementary Table 1 and Table 4, respectively. No interference was observed from any of the drugs evaluated, and no cross reactivity was observed.

Table 1: SPR analysis via BIAcore of the antibodies used in the prototype SARS-CoV-2 antigen ARCHITECT immunoassay.

Antibodies	k_a, $\times 10^5$ L/Ms	k_d, $\times 10^{-4}$ L/s	K_D, nM
Ab1 capture	1.5	2.1	1.4
Ab2 capture	1.1	2.7	2.5
Ab3 detection	4.9	34	6.9
Ab4 detection	0.86	2.4	2.8

Table 2: TCID50 for the prototype SARS-CoV-2 antigen ARCHITECT immunoassay and competitor assay (Elecsys SARS-CoV-2 Ag immunoassay).

	ARCHITECT[a]	Elecsys[b]
UTM	5.0	22.5
VTM	3.9	22.5
Sterile saline (0.9% NaCl)	3.8	37.5

UTM, universal transport medium (Copan Diagnostics); VTM, viral transport medium (CDC). [a]All 20 specimens were positive (≥1.0 COI). [b]TCID50 for the Elecsys SARS-CoV-2 Ag immunoassay as reported in the package insert [7].

Table 3: Endogenous interference for the prototype SARS-CoV-2 antigen ARCHITECT immunoassay.

Specimen	S/C	Shift from un-spiked S/C
Positive >1.0 S/C un-spiked	1.17	N/A
Positive >1.0 S/C spiked with 5 mg/mL mucin	1.10	−0.06
Positive >1.0 S/C spiked with 4% v/v whole blood	1.16	−0.01
Negative >1.0 S/C un-spiked	0.87	N/A
Negative <1.0 S/C spiked with 5 mg/mL mucin	0.82	−0.05
Negative <1.0 S/C spiked with 4% v/v whole blood	0.86	−0.01

S/C, signal-to-cutoff ratio.

Specimen stability

Loss of sample stability over time under various storage conditions was comparable for samples run on the prototype SARS-CoV-2 antigen ARCHITECT immunoassay or the Access SARS-CoV-2 Antigen Assay on the Beckman Coulter Access 2 instrument (Supplementary Figure 1). Positive patient specimens run under multiple conditions to simulate different sample handling and collection processes showed limited stability at RT with and without viral inactivation (Supplementary Figure 2A). Pre-inactivated specimens had a stability of <6 h at RT, and post-inactivation stability was <1 h. A single freeze/thaw cycle did not adversely impact specimen stability or antigen detection by the prototype assay, with all but one specimen remaining positive (S/C≥1.00) at RT (Supplementary Figure 2B). While purified mucin did not interfere with assay performance, the presence of mucus in positive clinical specimens was found to be a major driver of specimen instability, likely due to the activity of mucal proteases (Figure 2). Long-term storage

Table 4: Cross-reactivity of the prototype SARS-CoV-2 antigen ARCHITECT immunoassay.

Description	Specimen	n	S/C	Shift from UTM S/C	Final concentration
Calibrator	Index cal 6.5 pg/mL (cutoff)	3	1.00	0.93	N/A
Controls	UTM	3	0.07	0.00	N/A
	Viral lysate 2.8 TCID50/mL	3	1.13	1.06	N/A
Viral	Influenza A	3	0.09	0.01	1×10^5
	Influenza B	3	0.07	0.00	1×10^5
	Adenovirus	3	0.08	0.00	1×10^5
	Rhinovirus	3	0.11	0.03	1×10^5
	Enterovirus	3	0.07	0.00	1×10^5
	Human para i nfluenza 1	3	0.09	0.02	1×10^5
	Human para influenza 2	3	0.07	0.00	1×10^5
	Human para influenza 3	3	0.07	0.00	1×10^5
	Human para influenza 4B	3	0.07	−0.01	1×10^5
	Human metapneumovirus (hMPV)	3	0.09	0.02	1×10^5
	Human coronavirus 229E	3	0.07	0.00	1×10^5
	Human coronavirus NL63	3	0.21	0.14	1×10^4[a]
	Betacoronavirus 1 (OC43)	3	0.10	0.03	1×10^4[a]
	Respiratory syncytial virus A	3	0.13	0.06	1×10^5
Bacterial	*Staphylococcus epidermis*	3	0.07	0.00	1×10^6
	Streptococcus pneumoniae	3	0.06	−0.01	1×10^6
	Bordetella pertussis	3	0.07	0.00	1×10^6
	Haemophilus influenzae	3	0.06	−0.01	1×10^6
	Chlamydia pneumoniae	3	0.09	0.01	1×10^6
	Legionella pneumophila	3	0.03	−0.04	1×10^5[a]
	Mycoplasma pneumoniae	3	0.22	0.15	1×10^6
	Staphylococcus aureus	3	0.07	0.00	1×10^6
	Streptococcus pyogenes	3	0.07	0.00	1×10^6
Yeast	*Candida albicans*	3	0.07	0.00	1×10^6
Human nasal matrix	Pooled human nasal wash	3	0.20	0.12	N/A

S/C, signal-to-cutoff ratio; UTM, universal transport medium (Copan Diagnostics). [a]Concentration lowered by 1 log due to stock titer limitations.

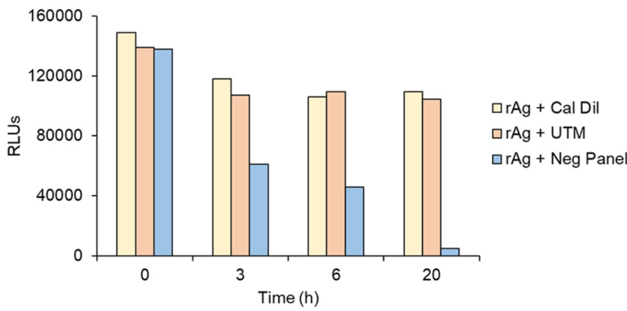

Figure 2: SARS-CoV-2 nucleocapsid instability. Recombinant nucleocapsid protein (rAg) was spiked into calibrator diluent, UTM alone, or a negative patient specimen panel in UTM and incubated at room temperature for the indicated times prior to running on the SARS-CoV-2 antigen ARCHITECT immunoassay. The presence of nasal mucus in the negative patient specimen panel significantly reduced nucleocapsid stability leading to a weaker assay signal (relative light units, RLUs) compared to rAg in diluent or UTM.

(>30 days) at −20 °C did not lead to a significant loss of detected antigen (Supplementary Table 2).

Assay clinical performance

Testing the positive and negative patient nasopharyngeal specimens in UTM, the prototype SARS-CoV-2 antigen immunoassay had a specificity of ≥98.5% and sensitivity of ≥89.5% across the two ARCHITECT instruments (Supplementary Figure 3). The assay cutoff signal ranged from ~4,000 to 5,500 RLUs and was set to the mean calibrator response of ~5,250 RLUs. All additional 150 negative specimens gave a response of <2,000 RLUs. The assay sensitivity was inversely correlated with Ct and was similar across the two instruments as well as with the Roche Elecsys immunoassay, based on values reported in the package insert [7] (Supplementary Table 3 and Figure 3).

Discussion

These studies indicated that the prototype automated SARS-CoV-2 antigen ARCHITECT immunoassay is capable of sensitive and specific detection of SARS-CoV-2 antigen in patient nasal and nasopharyngeal swab specimens in UTM. While the prototype ARCHITECT immunoassay format and analytical and clinical performance were similar to those of EUA SARS-CoV-2 antigen immunoassays, key pre-analytical challenges related to specimen stability are likely to limit the utility of central lab SARS-CoV-2 antigen testing. We observed relative instability of the target antigen in both nasal and nasopharyngeal swab specimens. The presence of endogenous proteases in mucus may degrade the target antigen, which would limit clinical specimen storage and transport times. While freezing specimens mitigated antigen instability, this requirement would add logistical complexity to specimen storage and transportation workflows. Current CDC guidance on specimen handling suggests respiratory specimens can be stored at 2–8 °C for up to 72 h [8]; it is possible that this guidance may not apply to specimen handling for SARS-CoV-2 antigen testing, as the RNA target for molecular testing may be more stable. Addition of protease inhibitors or other stabilizing reagents to the transport medium may be necessary to stabilize the antigen target during specimen transport to the central lab. Further studies are needed to determine the impact of alterations in transport medium or addition of additives with a pretreatment solution on specimen stability and assay performance, though these steps would add complexity to the workflow.

Thus, while our study demonstrated that the analytical and clinical performance of the prototype ARCHITECT SARS-CoV-2 antigen immunoassay is similar to the EUA immunoassay from Roche, specimen instability at RT, with or without viral inactivation, is an important pre-analytic factor that must be addressed at the time of specimen

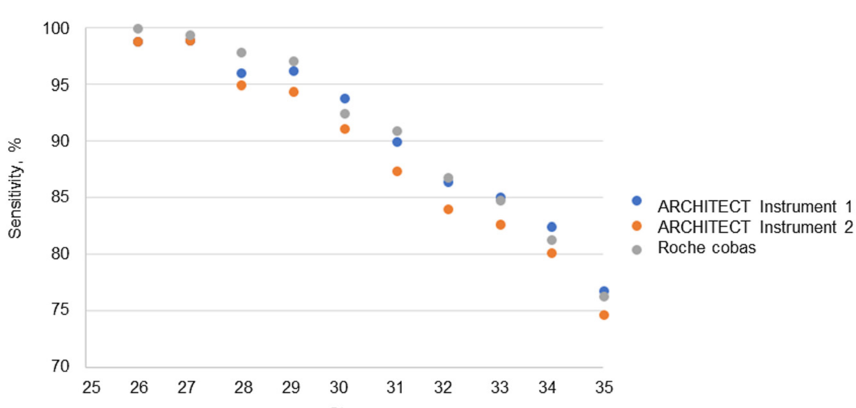

Figure 3: Clinical performance of the prototype SARS-CoV-2 antigen ARCHITECT immunoassay on nasopharyngeal specimens. Sensitivity across a range of Ct values was similar for the ARCHITECT immunoassay and the Roche Elecsys SARS-CoV-2 Ag immunoassay, based on the values provided in the package insert [7] (see also Supplementary Table 3).

collection, and currently limits the utility of centralized, high-throughput automated testing of SARS-CoV-2 antigen for infection control. Antigen assay formats other than rapid POC tests need to be monitored and characterized to maintain the validity of clinical specimens and accuracy of assay results. Understanding and controlling the causes of specimen instability will help further optimize current assays and direct future research to mitigating these areas of risk.

Acknowledgments: The authors would like to thank Sandhya Parameswaran for thoughtful support and guidance related to clinical studies. The authors would like to thank Brian Renley for statistical analyses of clinical data. Stacey Tobin, PhD, provided editorial assistance in the preparation of this manuscript. Portions of this work were previously presented at AACC in July 2022 in Chicago, IL.
Research funding: This study was funded by Abbott Laboratories. Writing assistance was provided by Stacey Tobin, PhD, funded by Abbott Laboratories.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Competing interests: PMH, RT, CJ, MD, RG, AH, NJ, KP, MM, RP, FB, MR, MA, RZ, BCT, DH, JM, KSO, CCM, JC, BT, ZL, AK, JH, CS, SG, TR, ASM, SB, JRH, DD, and GJD are employees of Abbott Laboratories and owners of Abbott stock. PM and LS receive research funding from Abbott Laboratories to Johns Hopkins University. EI, MF, and AC have no relevant conflicts of interest to report.
Informed consent: Not applicable.
Ethical approval: Local Institutional Review Boards provided approval for initial collection of patient specimens with informed consent in separate studies; de-identified remnant specimens were used in this study.

References

1. Killingley B, Mann AJ, Kalinova M, Boyers A, Goonawardane N, Zhou J, et al. Safety, tolerability and viral kinetics during SARS-CoV-2 human challenge in young adults. Nat Med 2022;28:1031–41.
2. Correa IA, Faffe DS, Galliez RM, Goncalves CCA, Maia RA, da Silva GP, et al. A SARS-CoV-2 negative antigen rapid diagnostic in RT-qPCR positive samples correlates with a low likelihood of infectious viruses in the nasopharynx. Front Microbiol 2022;13:912138.
3. Quinn FA, Scopp R, Lach A, Drake C, Mo M, Albright J, et al. A comparison of different sample matrices for evaluating functional sensitivity, imprecision and dilution linearity of the Abbott ARCHITECT i2000 TSH assay. Clin Chem Lab Med 2002;40:709–12.
4. Tieman BC, Kovacs S, Rodgers MA, Mohaimani A, Gregory S, Christensen D, et al. Methods to evaluate the impact of SARS-CoV-2 nucleocapsid mutations on antigen detection by rapid diagnostic tests. Biotechniques 2022;73:136–41.
5. Berg MG, Israeli E, Quaco E, Cloherty GA, Hemken PM. Tergitol-15-S-9 inactivates SARS-CoV-2 and boosts immunoassay signals. Biotechniques 2021;71:370–5.
6. Bose ME, McCaul KC, Mei H, Sasman A, He J, Kramp W, et al. Simulated respiratory secretion for use in the development of influenza diagnostic assays. PLoS One 2016;11:e0166800.
7. Elecsys® SARS-CoV-2 antigen immunoassay [instructions for use]. Basel, Switzerland: Roche Diagnostics; 2021.
8. Centers for Disease Control and Prevention. Interim guidelines for collecting and handling of clinical specimens for COVID-19 testing. Available from: https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html [Accessed 17 Jun 2022].

Supplementary Material: This article contains supplementary material (https://doi.org/10.1515/cclm-2022-1292).