Tergitol-15-S-9 inactivates SARS-CoV-2 and boosts immunoassay signals

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ABSTRACT
Inactivation of SARS-CoV-2 virus is necessary to mitigate risk but may interfere with diagnostic assay performance. We examined the effect of heat inactivation on a prototype SARS-CoV-2 antigen immunoassay run on the ARCHITECT automated analyzer. Recombinant full-length SARS-CoV-2 nucleocapsid protein and virus lysate detection was reduced by 66 and 31%, respectively. Several nonionic detergents were assessed as inactivationalternatives based on infectivity in cultured Vero CCL81 cells. Incubation of SARS-CoV-2 in 0.1% Tergitol 15-S-9 for 10 min significantly reduced infectivity and increased the immunoassay signal for cultured lysate and patient specimens. Tergitol 15-S-9 can inactivate SARS-CoV-2 while preserving epitopes on the nucleocapsid protein for enhanced detection by immunoassay antibodies.

METHOD SUMMARY
Treatment of SARS-CoV-2 virus lysate or patient specimens in viral transport medium with 0.1–0.5% Tergitol 15-S-9 for 10 min inactivates the virus, as measured by infectivity in cell culture, and does not interfere with detection of the SARS-CoV-2 nucleocapsid by a prototype nucleocapsid antigen immunoassay run on the automated ARCHITECT platform.

KEYWORDS:
immunoassay • SARS-CoV-2 • Tergitol 15-S-9 • viral inactivation

Handling of active cultures of highly infectious viruses, such as SARS, MERS and SARS-CoV-2, must be conducted in a biosafety level 3 (BSL-3) laboratory [1]. Inactivation of the virus in patient specimens can reduce infectivity and mitigate risk to lab personnel [2]. Previous studies of SARS reported effective inactivation with heat, UV irradiation and solvent/detergents such as Triton X-100, Tween 80 and sodium cholate [3]. Ideally, virus inactivation should occur in the transport media for biological specimens; however, recent studies of SARS-CoV-2 inactivation have reported that some methods and reagents may interfere with diagnostic assay performance [4]. Certain salts [5] and heat inactivation protocols [6] have been shown to reduce the accuracy of molecular assays, whereas heat inactivation of patient specimens was shown to reduce the amount of anti-SARS-CoV-2 IgG and IgM detected by fluorescence immunochromatography [7], but not by ELISA [8].

SARS-CoV-2 antigen tests are immunoassays useful for the diagnosis of COVID-19, with test results prompting further molecular testing, quarantine, contact tracing and treatment [9]. Several SARS-CoV-2 antigen assays are currently approved under emergency use authorization [10] to detect specific SARS-CoV-2 antigens in nasopharyngeal or nasal swab specimens. Few studies have examined whether viral inactivation methods affect SARS-CoV-2 antigen assay performance. Here, we evaluated the effect of SARS-CoV-2 inactivation on virus infectivity in cell culture and detection by a prototype SARS-CoV-2 antigen immunoassay run on the ARCHITECT automated analyzer (Abbott Laboratories, IL, USA).

Materials & Methods
Prototype SARS-CoV-2 antigen assay
A prototype sandwich immunoassay specific for the SARS-CoV/SARS-CoV-2 nucleocapsid antigen was developed on the Abbott ARCHITECT automated immunoassay analyzer. In this immunoassay, paramagnetic microparticles coated with a mouse monoclonal antibody serves as the solid phase reagent for capturing the viral nucleocapsid target. Some results obtained with Abbott's ARCHITECT prototype sandwich immunoassay involved the use of a monoclonal antibody against SARS-CoV provided by the National Institute of Infectious Diseases in Japan. The captured antigen is detected with a mouse monoclonal antibody conjugated with acridinium, which produces a chemiluminescent signal upon target binding, with signal quantified in relative light units (RLU).
Calibrator, stock virus lysate & patient specimens

Recombinant full-length nucleocapsid protein at calibrator levels was gravimetrically prepared in calibrator diluent (Cal Dil) consisting of HEPES 50 mM pH 7.2–7.4, NaCl 150 mM, 1% Tergitol 15-S-40 and protease-free 1% bovine serum albumin (BSA). Stock virus lysate (USA-WA1-2020 strain, GenBank accession MN985325.1) was obtained from BEI Resources (VA, USA; established by NIAID and managed by American Type Culture Collection to provide emerging infectious disease agents to the research community; NR-52281, lot# 70036318, TCID50 of $1.6 \times 10^6$ per ml) and diluted in viral transport medium (VTM; HBSS 1X, HI-FBS 2%, Amphotericin B 0.5 μg/ml and Gentamicin 100 μg/ml). For detergent inactivation experiments, BEI stock virus lysate was diluted in a base VTM diluent (10 mM phosphate-buffered saline, 2% BSA protease-free, 0.1% ProClin 300 and 10 ppm antifoam). Residual deidentified patient specimens in VTM were obtained from New York Biologics (NY, USA) and generation of Abbott high-titer calibrator stocks of SARS-CoV-2 was performed as described previously [BERG MG, ZHEN W, LUCIC D ET AL. DEVELOPMENT OF THE REALTIME SARS-COV-2 QUANTITATIVE LABORATORY DEVELOPED TEST AND CORRELATION WITH VIRAL CULTURE AS A MEASURE OF INFECTIVITY. J. CLIN. VIROL. (2021); SUBMITTED MANUSCRIPT].

Heat inactivation

Recombinant full-length SARS-CoV-2 nucleocapsid protein was diluted then heat inactivated at 65°C for 30 min. Stock virus lysate was inactivated at 60°C for 90 min in a BSL-3 lab before dilution in VTM, then heat inactivated again at 65°C for 30 min. Patient specimens in VTM had been previously heat inactivated at 65°C for 30 min for molecular testing on the Abbott m2000 platform.

Detergent inactivation

Tergitol 15-S-9, Tergitol 15-S-40, BrijL23, Tween-80, Tween-20, Surfynol 465, line diluent and blends of these nonionic detergents were combined with stock SARS-CoV-2 lysate (BEI NR-52281, lot# 70036318, TCID50 of $1.6 \times 10^6$ per ml). Virus lysate in VTM (∼10⁸ cp in 100 μl) was mixed 1:1 v/v with 100 μl VTM (no detergent control) or 100 μl of various detergents and blends to achieve a final detergent concentration of 0.1–0.5%. The mixtures were incubated at room temperature for 10, 20 or 30 min.

SARS-CoV-2 infection & in vitro cell culture

Vero CCL81 cells (3 × 10⁵ cells) were plated in a 96-well format the night before infection. Virus/detergent mixtures were applied to cultured Vero CCL81 cells for 2 h. In initial experiments, we found that above 0.0025%, some individual detergents or blends were toxic to Vero CCL81 cells after the 2-h incubation (data not shown). To reduce toxicity to cells, after the detergent inactivation period and immediately before infection, the virus/detergent mixtures were diluted in DMEM media lacking fetal bovine serum (FBS) (DMEM + penicillin/streptomycin [P/S]) to a final detergent concentration of ≤0.0025%. The diluted mixtures were applied to the cells and incubated for 2 h. Cells were then washed with PBS, placed in 100 μl complete media (DMEM + P/S + 10% FBS), and incubated for ≥96 h before assessment of infectivity by Viral Tox Glo assay.

Virus Tox Glo assay of infectivity

Cells were monitored by microscopy and cytopathic effects (CPE) were quantitatively measured using the Viral Tox Glo system (Promega, WI, USA), as described [BERG MG, ZHEN W, LUCIC D ET AL. DEVELOPMENT OF THE REALTIME SARS-COV-2 QUANTITATIVE LABORATORY DEVELOPED TEST AND CORRELATION WITH VIRAL CULTURE AS A MEASURE OF INFECTIVITY. J. CLIN. VIROL. (2021); SUBMITTED MANUSCRIPT]. The Viral Tox Glo assay measures cellular ATP as a surrogate of cell viability after viral infection. Luminescent signals measured in RLU indicate the level of ATP, which is inversely correlated with viral-infection-induced cytotoxicity. At day 4 after infection, 75 μl of medium was replaced

| Sample | RLU, n = 3, 2–8°C | RLU, n = 3, 65°C, 30 min | % shift from 2–8°C |
|--------|------------------|-------------------------|------------------|
| Recombinant nucleocapsid | | | |
| 0 pg/ml (Cal dil) | 108 | 81 | |
| 10 pg/ml | 1806 | 148 | -92 |
| 500 pg/ml | 75,958 | 35,430 | -53 |
| 1000 pg/ml | 148,587 | 68,663 | -54 |
| 5000 pg/ml | 684,426 | 232,031 | -66 |
| Virus lysate | | | |
| VTM | 141 | 122 | |
| 1:5000 (TCID₅₀/ml 320) | 1902 | 1500 | -21 |
| 1:100 (TCID₅₀/ml 16,000) | 108,003 | 65,834 | -39 |
| 1:50 (TCID₅₀/ml 32,000) | 204,525 | 137,982 | -33 |

N: Nucleocapsid protein; RLU: Relative light unit; VTM: Viral transport medium.
Results & discussion

Heat inactivation of virus diminishes anti-SARS-CoV-2 signal

Control (2–8 °C) and heated dilutions of nucleocapsid protein and virus lysate were run on the prototype ARCHITECT immunoassay. Heat inactivation reduced the assay signal an average of 66% for the recombinant nucleocapsid dilutions and 31% for the viral lysate dilutions (Table 1).

Tergitol 15-S-9 inactivates SARS-CoV-2

To avoid the significant loss of signal on the prototype ARCHITECT immunoassay following heat inactivation, we examined the use of detergent as an alternative approach to SARS-CoV-2 inactivation. Tergitol 15-S-9 alone and Tergitol 15-S-9 + Surlynol 465 at 0.1% concentration were able to inactivate the virus within 10 min; Tergitol + Tween-20 required 30 min (Figure 1). The other detergents...

Figure 1. Tergitol 15-S-9 alone and in blends inactivate SARS-CoV-2 virus after ≥10 min. SARS-CoV-2 virus lysate in VTM (10⁸ cp/100 μl) was mixed with 100 μl of various detergents and blends to a final concentration of 0.1% and incubated for the indicated times. The mixtures were then diluted to 0.00125% and applied to Vero CCL81 cells for 2 h. Four days after infection, Viral Tox Glo assay was used to detect ATP produced by remaining viable cells as a measure of infectivity. Comparison of “No detergent” negative controls (no virus) and “no detergent” positive controls (with virus) run in triplicate demonstrates an approximate 50% reduction in Viral Tox Glo assay signal due to cell death after 10 min. Tergitol 15-S-9 alone and with Surlynol inactivated virus after 10 min, as indicated by assay signals comparable to negative controls.

Figure 2. Tergitol 15-S-9 inactivates SARS-CoV-2 over a range of concentrations. Dose response of SARS-CoV-2 inactivation after incubation with decreasing concentrations of Tergitol 15-S-9 for 10 min. Infectivity was determined by the Viral Tox Glo assay. Blue indicates no virus controls, orange indicates virus-containing samples. No detergent (0.00%) negative controls (no virus) and positive controls (with virus) and each detergent condition were run in duplicate.
Table 2. Tergitol 15-S-9 Inactivation of SARS-CoV-2 Viral Lysate (BEI), RLU (n = 3).

| BEI virus dilutions | 0.1% (base) | 0.1% (CDC) | Tergitol 15-S-9 Concentration in base VTM | 0.1% | 0.2% | 0.3% | 0.4% | 0.5% |
|---------------------|-------------|------------|------------------------------------------|------|------|------|------|------|
| VTM only            | 51          | 47         | 45                                       | 46   | 44   | 43   | 46   |
| 1:800,000 (TCID50/mL 2) | 122         | 163        | 238                                      | 261  | 278  | 273  | 251  |
| 1:400,000 (TCID50/mL 4) | 172         | 306        | 470                                      | 484  | 519  | 557  | 474  |
| 1:100,000 (TCID50/mL 16) | 706         | 1100       | 1719                                     | 1853 | 1814 | 1824 | 1741 |
| 1:5000 (TCID50/mL 320) | 11916       | 26132      | 34985                                    | 35998| 37816| 36428| 35684|
| RLU difference from base diluent (%) |            |            |                                          |      |      |      |      |
| 1:800,000          | 34          | 95         | 114                                      | 128  | 124  | 106  |
| 1:400,000          | 77          | 173        | 181                                      | 201  | 212  | 175  |
| 1:100,000          | 56          | 144        | 163                                      | 157  | 158  | 147  |
| 1:5000             | 119         | 194        | 202                                      | 217  | 206  | 199  |
| Average RLU difference (%) | 72          | 151        | 165                                      | 176  | 175  | 157  |

RLU: Relative light unit; VTM: Viral transport medium.

Table 3. Tergitol 15-S-9 Inactivation of SARS-CoV-2 Patient Specimens in VTM, RLU (n = 1).

| Sample ID | PCR Ct | 0% (control) | Tergitol 15-S-9 Concentration in VTM | 0.10% | 0.20% | 0.30% | 0.40% |
|-----------|--------|--------------|--------------------------------------|-------|-------|-------|-------|
| 1         | 25.01  | 1521         | 5635                                 | 6567  | 10358 | 7985  |
| 2         | 25.14  | 1093         | 5500                                 | 7131  | 2500  | 12812 |
| 3         | 25.60  | 1014         | 6456                                 | 7871  | 6728  | 7292  |
| 4         | 26.69  | 225          | 508                                  | 493   | 446   | 679   |
| 5         | 27.05  | 619          | 1910                                 | 1633  | 1842  | 1740  |
| RLU difference from control (%) |        |              |                                      | 309   | 369   | 314   | 500   |
| Avg. RLU difference (%) |      |              |                                      |       |       |       |       |
| Negative specimens | ND    | 61           | 65                                   | 64    | 57    | 59    |
| 7         | ND     | 71           | 73                                   | 78    | 72    | 74    |
| 8         | ND     | 50           | 50                                   | 48    | 51    | 51    |
| 9         | ND     | 52           | 48                                   | 48    | 50    | 48    |
| 10        | ND     | 49           | 47                                   | 58    | 53    | 54    |

ND: Not detected; RLU: Relative light unit; VTM: Viral transport medium.

tested (Tergitol 15-S-40, Tween-20, Tween-80, Surlynol 465, and line diluent) displayed no effect on viral infectivity at 0.1% (Figure 1). Virus inactivation was repeated at a higher detergent concentration (0.5% final), which confirmed the results at 0.1% and additionally showed that the detergent Brij-L23 can inactivate SARS-CoV-2 when incubated for ≥20 min (data not shown).

Two different strains of SARS-CoV-2 were evaluated to confirm Tergitol 15-S-9 inactivation of the virus at 0.5% and 0.1%, but not at 0.01% (data not shown). To determine the optimal concentration of Tergitol 15-S-9 for inactivation, a dilution series was performed with concentrations ranging from 0.01%-0.5% with a 10 min incubation. We observed inactivation with Tergitol 15-S-9 at concentrations from 0.5% to 0.1% (Figure 2). At 0.05%, only one of the two replicates showed evidence of CPE, while at 0.01% there was no inactivation and both cell culture wells were heavily infected, similar to the no detergent controls (0.0%).

Tergitol increases anti-SARS-CoV-2 signal
To assess the effects of increasing amounts of Tergitol 15-S-9 on the SARS-CoV-2 antigen signal in the prototype ARCHITECT immunoasay, virus lysate was inactivated with increasing concentrations of Tergitol 15-S-9 (0.1 – 0.5%) diluted in base VTM diluent. Each condition
was run in triplicate. Inactivation of the virus in VTM with 0.1%-0.5% Tergitol 15-S-9 significantly increased the immunoassay signal an average of 151% to 176%, with the highest RLUs seen with 0.3%-0.4% Tergitol 15-S-9 (Table 2).

We then tested the effect of inactivating virus in patient specimens with 0.1%-0.4% Tergitol 15-S-9 in VTM on detection in the prototype ARCHITECT immunoassay. As with the stock virus lysate, positive patient specimens inactivated in 0.1%-0.4% Tergitol 15-S-9 had significantly higher immunoassay signal compared to VTM control (Table 3). Negative patient specimens remained unchanged. Thus, treatment of stock SARS-CoV-2 lysate and patient specimens with 0.1% Tergitol 15-D-9 for 10 min not only inactivated the virus, but also increased the signal in the ARCHITECT prototype immunoassay.

**Conclusion**

SARS-CoV-2 heat inactivation requires 30 min and reduces immunoassay sensitivity. Tergitol 15-S-9 is a simple additive to VTM that can inactivate virus in 10 min while allowing improved detection by the ARCHITECT prototype sandwich immunoassay. A mid-range concentration of 0.2% Tergitol 15-S-9 for 10 min was selected for subsequent assay optimization. Tergitol 15-S-9 may preserve secondary/tertiary protein structures that heat inactivation destroys. We also found that Tergitol 15-S-9 increased the RLU signal in the ARCHITECT prototype sandwich immunoassay for SARS-CoV/SARS-CoV-2, suggesting that it may expose epitopes for enhanced detection by the immunoassay antibodies.

**Future perspective**

Future studies will need to examine whether Tergitol 15-S-9 affects other SARS-CoV-2 diagnostic assays, including molecular assays and rapid antigen tests.

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**Executive summary**

**Background**

- We determined the effect of different methods of SARS-CoV-2 inactivation on 1) virus infectivity in cell culture and 2) detection of the nucleocapsid antigen by a prototype SARS-CoV-2 immunoassay run on the ARCHITECT automated analyzer.

**Methods**

- Recombinant SARS-CoV-2 nucleocapsid antigen, stock virus lysate, and infected patient specimens were inactivated with heat and/or various types and combinations of detergents.
- The effect of inactivation was determined on infectivity of the virus in cultured Vero CCL81 cells measured by Viral Tox Glo assay and on detection of the nucleocapsid antigen by the prototype immunoassay.

**Results & discussion**

- Heat inactivation reduced the immunoassay signal an average of 66% for the recombinant nucleocapsid and 31% for viral lysate.
- Tergitol 15-S-9 was able to inactivate the virus within 10 min.
- Tergitol 15-S-9 increased the immunoassay signal an average of 151% to 176% in viral lysate and 309% to 500% in patient specimens.

**Conclusion**

- Tergitol 15-S-9 is a simple additive to viral transport media that can inactivate SARS-CoV-2 while preserving epitopes on the nucleocapsid protein for enhanced detection by immunoassay antibodies.

**Author contributions**

MG Berg planned and executed experiments and wrote the manuscript; E Israeli planned and executed experiments and wrote the manuscript; E Quaco executed experiments; GA Cloherty reviewed the manuscript; PM Hemken planned and executed experiments and wrote the manuscript. All authors reviewed and approved the manuscript for submission.

**Financial & competing interests disclosure**

This study was funded by Abbott Laboratories. All authors are employees of Abbott Laboratories. A patent application has been filed for the described method. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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