Application Note

Application of TAG-NGPM+™ in Rapid SARS-CoV-2 Antigen Tests

SUMMARY

In this application note, we demonstrated TAG-NGPM+ effectively inactivates viruses within 10 seconds of incubation with SARS-CoV-2.

We also tested TAG-NGPM+ as the sample reagent in three commercially available SARS-CoV-2 rapid antigen tests (lateral flow based) and demonstrated the following:

1) TAG-NGPM+ is compatible with the analyte detection mechanism of antigen test

2) TAG-NGPM+ does not interfere with test sensitivity in detecting positive controls

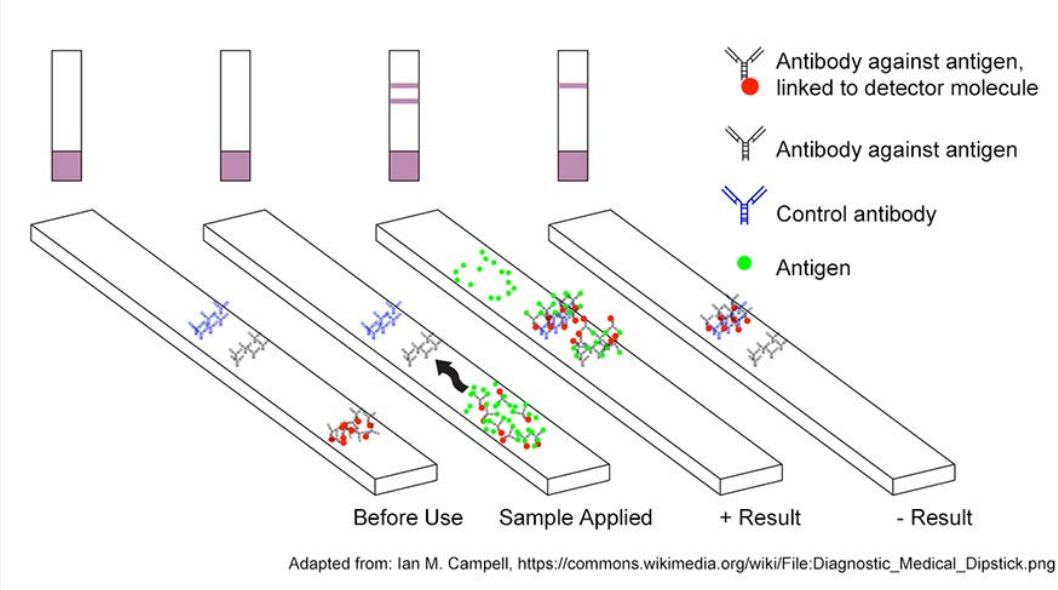
INTRODUCTION:

TAG-NGPM +™ is a molecular assay specimen collection and transport media with virus inactivation and biocide. It is designed to stabilize samples in ambient temperature and could serve as an excellent SARS-CoV-2 buffer reagent media for collection and transportation of samples to be used in PCR, RT-PCR, recombinase-based isothermal assays, and antigen tests.

The unique and proprietary formulation of TAG-NGPM+ allows dual compatibility with molecular and protein-based tests, and its effective virus inactivation function ensures safety of the patient and healthcare workers during sample collection, transport and testing.

Mechanism of Antigen Test: In brief, a sample is placed on a conjugation pad where the analyte (or antigen) of interest is bound by conjugated antibodies. The analyte-antibody mix subsequently migrates along a membrane by capillary flow across both 'test' and 'control' strips. These strips are coated with antibodies detecting the analyte of interest and a positive test is confirmed by the appearance of coloured control and test lines.

Test validity: Determined by the presence of a control line on the test.

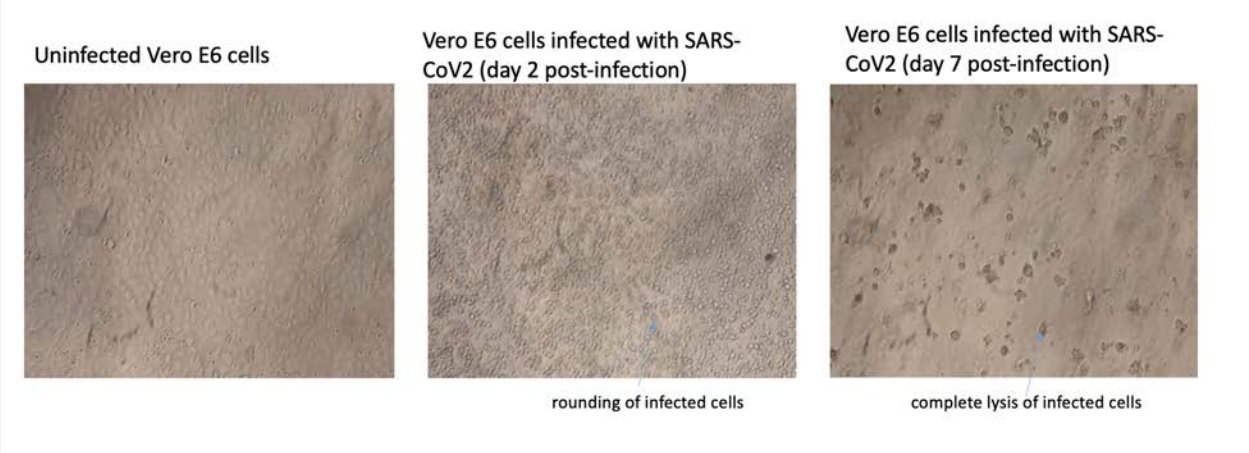


Adapted from: Ian M. Campell, https://commons.wikimedia.org/wiki/File:Diagnostic_Medical_Dipstick.png

Measuring viral titer using Cytopathic Effect

Cytopathic effect (CPE) refers to the morphologic changes in the host cells due to viral infection. CPE includes rounding of the infected cells, fusion with adjacent cells to form syncytia, and the appearance of nuclear and cytoplasmic inclusion bodies. It is widely used as a tool to visualize viral infection in host cells and evaluate the effectiveness of antiviral compounds.

CPE can be used as the measurement readout to quantify live viral titer in the Median Tissue Culture Infectious Dose (TCID50) assay, performed by adding serial dilution of virus-containing sample to cultured, adherent cells in plate wells.



MATERIALS AND METHODS

Virus Inactivation of SARS-CoV-2 in nasal matrix

High stock concentration of SARS-CoV-2 virus in nasal matrix was inoculated into TAG-NGPM+ medium and incubated at room temperature. PBS incubation for thirty (30) minutes will be used as a control. All conditions will be performed with five (5) replicates.

Due to the cytotoxicity of TAG-NGPM+, the reactions were subjected to column purification using Pierce Detergent Removal 0.5mL (Lot: WC320233 MFG: Thermo Scientific) purification column following the manufacturer's instructions. The viability of the virus was measured after each time point in the TAG-NGPM+ medium by TCID50 assay.

SARS-CoV-2 culture

SARS-CoV-2 (Isolate Japan/Ty-7-503/2021) was obtained from BEI resources. This virus aliquot was used to create SARS-CoV-2 stocks for the inactivation studies using TAG-NGPM+. All activities with live virus took place in a biosafety level 3 (BSL3) laboratory at MRIGlobal's Kansas City facility with adherence to established safety guidelines.

Rapid antigen test

LifeSign Status™ COVID-19 Flu A&B (LifeSign), Access Bio CareStart Rapid-Antigen Tests (Access Bio), and MP Bio Rapid SARS-CoV-2 Antigen Test Card Kit (MP Biomedicals) were purchased. Each antigen test was performed following the manufacturer's instructions and general laboratory practices and guidelines. In the case of positive control, control swabs containing inactivated and dried SARS-CoV-2 virus were used. TAG-NGPM+ was used in place of the sample buffer included in the kits in all TAG-NGPM+ experiments.

RESULTS

TAG-NGPM+ SARS-CoV-2 Inactivation

The objective of the inactivation study is to evaluate the effectiveness of TAG-NGPM+ to inactivate SARS-CoV-2 virus. High concentrations of SARS-CoV-2 virus (9X10^6 TCID50/ml) in negative upper respiratory sample matrices are inoculated into TAG-NGPM+ in a 1:3 ratio and incubated at room temperature for different time points that are 10s, 30s, 1min, 15min, and 30min. PBS incubation for 30 minutes is used as control. Due to the strong cytotoxicity of TAG-NGPM+ (> 3.0 log reduction), the incubated samples are subjected to immediate column purification for the removal of TAG-NGPM+ before inoculating onto Ver oE6 cells, incubating for four days and measuring the cytopathic effect (CPE).

The results for the inactivation study confirmed > 3.0 log reduction in viral titer after exposure time as short as 10 seconds in TAG-NGPM+ (Table 1) and demonstrated effective viral inactivation > 99.5%.

Table 1. Summary of SARS-CoV-2 inactivation in TAG-NGPM+

	TAG-NGPM+ 10s incubation	TAG-NGPM+ 30s incubation	TAG-NGPM+ 1min incubation	TAG-NGPM+ 15min incubation	TAG-NGPM+ 30min incubation	PBS 30min incubation
SARS-CoV-2 Variant Japan/Ty-7-503/2021 Average titer (TCID50/mL)	<3.16E+01	<3.16E+01	<3.16E+01	<3.16E+01	<3.16E+01	6.93E+04
Viral Titer log reduction (1 X 10x TCID50)	> 3	> 3	> 3	> 3	> 3	0

TAG-NGPM+ in Rapid Antigen testing

The validity of antigen lateral flow tests is determined by the presence of a control test line at the end of the incubation period. To evaluate whether TAG-NGPM+ interferes with the test validity and detection, TAG-NGPM+ was used as the sample extraction buffer at a volume appropriate for three commercially available SARS-CoV-2 antigen kits and replacing the kit sample extraction buffers, which are saline-based and does not effectively inactivate the SARS-CoV-2 virus. We were able to purchase LifeSign Status™ COVID-19 Flu A&B (LifeSign), Access Bio CareStart Rapid-Antigen Tests (Access Bio), and MP Bio Rapid SARS-CoV-2 Antigen Test Card Kit (MP Biomedicals). LifeSign Status™ COVID-19 Flu A&B and Access Bio CareStart Rapid-Antigen Tests have obtained EUA from FDA for use of in vitro diagnostics (IVDs) for the detection and/or diagnosis of the virus that causes COVID-19, whereas MP Bio Rapid SARS-CoV-2 Antigen Test Card Kit is for research use only.

Negative control swabs that did not come in contact with SARS-CoV-2 samples were used, and positive control swabs containing inactivated and dried SARS-CoV-2 viruses were used for positive control tests.

After appropriate incubation time for each antigen test, distinct control test lines appeared on all test cassettes, indicating TAG-NGPM+ does not denature proteins and does not interfere with antigen-antibody interaction. The test lines are present in the positive controls of all three antigen tests, with varied test sensitivities indicated by the boldness/faintness of the T line in comparison to the C line.

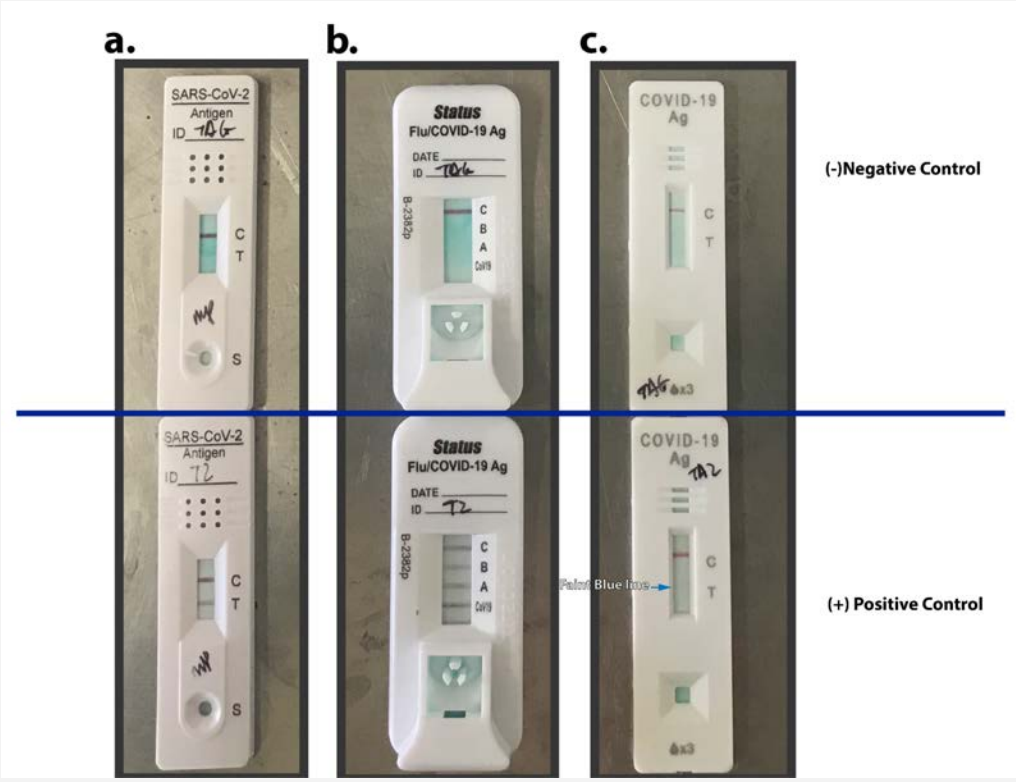


Figure 1: Antigen tests with TAG-NGPM+. a. MP Bio Rapid SARS-CoV-2 Antigen Test Card Kit (MP Biomedicals), b.LifeSign Status™ COVID-19 Flu A&B (LifeSign), c. Access Bio CareStart Rapid-Antigen Tests (Access Bio).

Table 2. Summary of Antigen tests results with TAG-NGPM+

Test Name		Antigen positive/negative control test with TAG-NGPM+	
	Sample+reagent vol needed per test (µl)	TAG-NGPM+ positive controls (Valid test Y/N, +/- test)	TAG-NGPM+ negative controls (Valid test Y/N, +/- test)
LifeSign Status™ COVID-19 Flu A&B (Box of 25)	250	Y, +	Y, -
AccessBio CareStart Rapid-Antigen Tests 20 Tests Per Kit	75	Y, +	Y, -
MP Bio Rapid SARS-CoV-2 Antigen Test Card Kit (RUO)	75	Y, +	Y, -

Next, we evaluated whether TAG-NGPM+ interfere with detection sensitivity in antigen testing in comparison with the sample extraction buffer that came with the kit, which was likely optimized for detection.

MP bio antigen test kit is used in this experiment due to its high sensitivity in detecting diluted antigen controls. A high concentration antigen stock was used to prepare 1: 2 dilutions either in MP bio sample reagent buffer or TAG-NGPM+, followed by serial dilutions with a dilution factor of 2 (1X, 2X, 4X, 8X, 16X dilution factor). The sample dilutions were used as input in MP Bio antigen tests. Both the TAG-NGPM+ test and MP bio sample reagent test were able to show a positive band up to a dilution factor of 8X. The result suggests that TAG-NGPM+ is able to maintain the test detection sensitivity without interference when used directly in place of the sample extraction buffer, ensuring test performance and preventing potential exposure to live virus by inactivating SARS-CoV-2 in samples.

	Kit buffer (Valid test Y/N, +/- test)	TAG-NGPM+ (Valid test Y/N, +/- test)
1X	Y, +	Y, +
2X	Y, +	Y, +
4X	Y, +	Y, +
8X	Y, +	Y, +
16X	Y, -	Y, -

Figure 2: MP Bio antigen tests with TAG-NGPM+ (bottom row) and MP Bio sample reagent (top row). Antigen controls were serial diluted, with 1 indicating highest antigen concentration, and 16 indicating lowest antigen concentration.

CONCLUSION

It's imperative to diagnose COVID-19 as quickly and reliably as possible in order to prevent the spread of this disease. The current rapid antigen tests can provide results in minutes instead of days from PCR testing, however, the testing uses an open-faced cassette and does not effectively inactivate SARS-CoV-2 virus, putting the operator at risk for exposure to live viruses.

Using a viral transport media with virus inactivating function can offer better protection against viral exposure, however, guanidine-containing virus-killing ITMs have harsh chemistry and can denature proteins and therefore not compatible with rapid antigen testing mechanisms.

TAG-NGPM + is a molecular assay specimen collection and transport media with virus inactivation and biocide. The reagent was designed to stabilize samples at ambient temperature so they can be safely transported for testing, potentially making it the perfect sample buàer reagent for SARS-CoV-2 specimens that are being submitted to downstream tests.

TAG-NGPM+ is a comprehensive, well-rounded formula that combines the best of PCR and antigen testing. This powerful dual compatibility means it can be used with both tests to get an accurate diagnosis for any patient in need.

TAG-NGPM+ is available now on Thomas Scientific with a range of packaging options suitable for ready-to-use sample collection, kitting, and test-developing needs.

APPENDIX: TAG PRODUCT LIST

Part No	Part Description
NGPM+-B-A	TAG-NGPM+™ (RUO) , 1 Liter, Bulk
NGPM+-T-A	TAG-NGPM+™ (RUO) , 10ml tube w/ 2ml fill
NGPM+-TS-A	TAG-NGPM+™ (RUO) 10ml tube w/ 2ml fill w/ swab
NGPM+-C-S	TAG-NGPM+™ (RUO) , 30ml in 40ml cup, Sample