

CaptiSwab: Technical sheet describing development of a novel swab for DNA and RNA specimen collection

Background: The Captigen™ CaptiSwab is cross-platform precision microbial sampling device designed for optimized culture yield, RT-PCR yield, and next-generation sequencing (NGS). The Captiswab is intended to improve absorption and release of the specimen, while ensuring ease-of-use in a sterile field.

In this report, we describe how the Captiswab was:

- (1) Developed via **comparative materials testing (surface area adjusted) for absorption and extraction efficacy of planktonic bacteria**
- (2) Developed following **comparative materials testing (surface area adjusted) for collection, absorption and extraction efficacy of established biofilm**
- (3) Tested for absorption and extraction efficacy (surface area adjusted) in established biofilm **after 24 hour simulated transport specimen hold**
- (4) Assessed to see if a **nucleic acid buffer solution** was beneficial for yield

Thereafter, we:

- (5) Assessed the final device per the **CLSI M40-A2 standard for microbiological culture**.
- (6) Tested for its **ability to recover SARS-CoV-2 using RT-PCR and two RNA extraction methods**, in light of the SARS-CoV-2 pandemic.
- (7) Tested for its **ability to recover SARS-CoV-2 using RT-PCR and two RNA extraction methods** after a simulated transport (36 hour) dry holding period.

Technical specifications:

Color	White
Length	6" swab handle; 15 mm swab tip
Quantity	1
Handle material	Polypropylene
Tip shaft	Polypropylene
Tip material	Polyester proprietary knit
Sterilization	Gamma irradiated
Shelf life	6 months

(Experiment 1) Comparative testing of materials for absorption and extraction of planktonic Gram positive and Gram negative organisms on PCR

- **Aim:** Compare materials and weave patterns for optimal absorption and extraction performance.
- **Methods:** A panel of 15 candidate gauze and swab weave patterns were spiked with a standardized inoculum of Gram positive (*Staphylococcus aureus*), or Gram negative (*Escherichia coli*) planktonic organisms. Experimental conditions were repeated in triplicate.
- Following a holding period, each candidate sample was extracted and tested via PCR (Roche Lightcycler 480, Taqman 384 Protocol, UNG Program) alongside positive and negative controls. CP amplification thresholds were calculated.
- **Results:** Optimal candidates showing consistent yield results across Gram positive, Gram negative and fungal species were selected for further development. Detailed breakdown of this proprietary data is not shown.

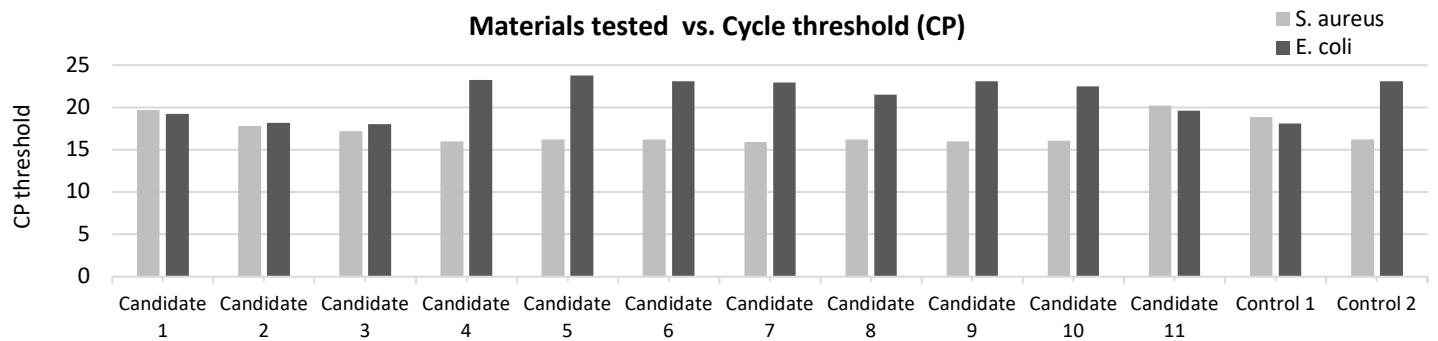


Figure 1. Data show comparison between candidate materials reaching cycle threshold when inoculated with either *S. aureus* or *E. coli*

#	Organism	Ct	Code
Candidate 1	<i>S. aureus</i>	19.68	3
Candidate 2	<i>S. aureus</i>	17.81	4
Candidate 3	<i>S. aureus</i>	17.17	6
Candidate 4	<i>S. aureus</i>	16.02	8
Candidate 5	<i>S. aureus</i>	16.22	9
Candidate 6	<i>S. aureus</i>	16.25	10
Candidate 7	<i>S. aureus</i>	15.93	11
Candidate 8	<i>S. aureus</i>	16.22	12
Candidate 9	<i>S. aureus</i>	15.98	13
Candidate 10	<i>S. aureus</i>	16.08	14
Candidate 11	<i>S. aureus</i>	20.21	15
Control 1	<i>S. aureus</i>	18.85	Neg
Control 2	<i>S. aureus</i>	16.18	Neg

#	Organism	Ct	Code
Candidate 1	<i>E. coli</i>	19.25	3
Candidate 2	<i>E. coli</i>	18.2	4
Candidate 3	<i>E. coli</i>	18.04	6
Candidate 4	<i>E. coli</i>	23.23	8
Candidate 5	<i>E. coli</i>	23.75	9
Candidate 6	<i>E. coli</i>	23.11	10
Candidate 7	<i>E. coli</i>	22.93	11
Candidate 8	<i>E. coli</i>	21.49	12
Candidate 9	<i>E. coli</i>	23.07	13
Candidate 10	<i>E. coli</i>	22.48	14
Candidate 11	<i>E. coli</i>	19.62	15
Control 1	<i>E. coli</i>	18.14	Neg
Control 2	<i>E. coli</i>	23.13	Neg

Tables 1a and 1b. Show minimum number of cycles (Ct) necessary to reach threshold for detection each material tested in *S.aureus* and *E.coli*, respectively

(Experiment 2) Comparative testing of materials for biofilm collection, absorption and extraction of Gram positive and Gram negative organisms on PCR

- **Aim:** Assess biofilm collection ability, absorption activity, and extraction activity of shortlisted materials.
- **Methods:** Biofilms consisting of monocultures of either *S. aureus* (ATCC 25923) or *E. coli* (ATCC 11303) were grown on the surface of 12mm Polycarbonate transwell inserts. Once the biofilms were grown, each insert was removed from its original growth well and transferred to a new well containing a single gauze testing material (approximately 1cm²; each sample was previously weighed for subsequent normalization). Optimal candidate materials from Experiment 1 were selected for testing. Each biofilm was removed via debridement (vortex with uniform force applied). The samples were then transferred for testing of absorptive, biofilm removal and extraction efficiencies. All sample conditions were tested in triplicate.
- Each sample was tested per standardized protocols at CLIA-certified MicroGenDx Laboratories (Lubbock, TX). In brief, samples were mechanically lysed and extracted using the Qiagen TissueLyser in combination with the Roche High Pure PCR Template Preparation kit. Each sample was tested for 16S abundance to verify species, and to test for possible contaminants obtained during the procedure.
- **Results:** Samples were separated by species and material. Values are shown in terms of a normalized Ct value. Lower values are indicative of increased nucleic acid present at the time of extraction. Captiswab material was validated as efficient for biofilm removal and extraction. This efficiency extended across both *S. aureus* and *E. coli* samples.

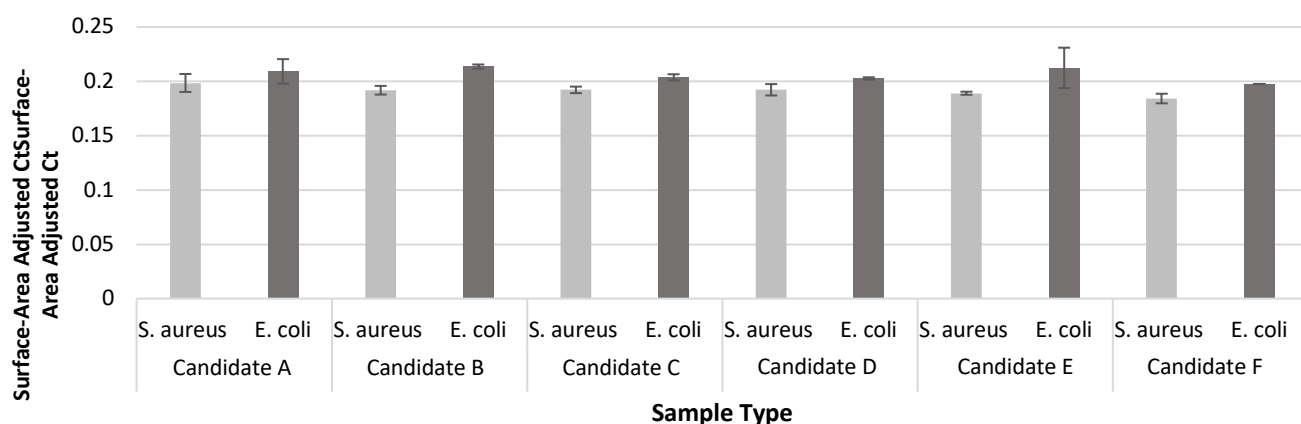


Figure 2. Graph represents comparative surface-area adjusted Ct values for materials between *S. aureus* and *E. coli*.

	Organism	Ct threshold	SA-Ct Value	SD
Candidate A	<i>S. aureus</i>	19.85	0.1985	0.008259
	<i>E. coli</i>	20.92	0.2092	0.011271
Candidate B	<i>S. aureus</i>	19.18	0.1918	0.003993
	<i>E. coli</i>	21.37	0.2137	0.001877
Candidate C	<i>S. aureus</i>	19.22	0.1922	0.003011
	<i>E. coli</i>	20.37	0.2037	0.002871
Candidate D	<i>S. aureus</i>	19.23	0.1923	0.005232
	<i>E. coli</i>	20.27	0.2027	0.001058
Candidate E	<i>S. aureus</i>	18.91	0.1891	0.001411
	<i>E. coli</i>	21.24	0.2124	0.018639
Candidate F	<i>S. aureus</i>	18.42	0.1842	0.004424
	<i>E. coli</i>	19.76	0.1976	0

Table 2. Data represents comparative surface-area adjusted Ct value of optimal candidates between *S. aureus* and *E. coli*.

<i>S. aureus</i>	Candidate A	Candidate B	Candidate C	Candidate D	Candidate E	Candidate F
Candidate A		0.435631	0.181808	0.233145	0.14054	0.171239
Candidate B	0.435631		0.941714	0.927701	0.470825	0.108477
Candidate C	0.181808	0.941714		0.975644	0.082565	0.164392
Candidate D	0.233145	0.927701	0.975644		0.36399	0.284143
Candidate E	0.14054	0.470825	0.082565	0.36399		0.255665
Candidate F	0.171239	0.108477	0.164392	0.284143	0.255665	

<i>E. coli</i>	Gauze 2	Gauze 5	Gauze 22	Gauze 23	Gauze 24	Gauze 25
Candidate A		0.612584	0.452512	0.457031	0.858944	0.216586
Candidate B	0.612584		0.060304	0.002109	0.912867	0.004519
Candidate C	0.452512	0.060304		0.68763	0.482488	0.067218
Candidate D	0.457031	0.002109	0.68763		0.453765	0.014052
Candidate E	0.858944	0.912867	0.482488	0.453765		0.303627
Candidate F	0.216586	0.004519	0.067218	0.014052	0.303627	

GRAM NEG. VS GRAM POS.	P-Value	P-Value (1-Tail)
Candidate A	0.37878	0.18939
Candidate B	0.009283	0.004642
Candidate C	0.006465	0.003232
Candidate D	0.052233	0.026116
Candidate E	0.146111	0.073055
Candidate F	0.034463	0.017231

Table 3a, 3b, 3c. P-value comparisons between sample types and Gram-Negative vs Gram-Positive bacteria.

(Experiment 3) Comparative testing of materials for absorption and extraction of Gram positive and Gram negative organisms on PCR, following 24 hours of simulated transport specimen hold

- **Aim:** Assess absorption and extraction activity of shortlisted swab materials following 24-hour dry specimen hold.
- **Methods:** Monocultures consisting of either *S. aureus* (ATCC 25923) or *E. coli* (ATCC 11303) were grown in 12-well plates. After 24 hours each gauze sample was briefly submerged in culture. The samples were then transferred into tubes for 24 hours at 25°C to mimic the time of transport. Samples were then assayed to test of absorptive and nucleic acid extraction efficiencies. All sample conditions were tested in triplicate. Each sample was tested per standardized protocols at CLIA-certified MicroGenDx Laboratories (Lubbock, TX). Samples were mechanically lysed and extracted using the Qiagen TissueLyser in combination with the Roche High Pure PCR Template Preparation kit. Each candidate sample was extracted and tested via PCR (Roche Lightcycler 480)
- **Results:** Prior to testing, each sample was measured in order to normalize for sample surface area. Results compare each sample by test species, as well as after normalization. Lower Ct values correlate to higher amounts of bacteria present in the sample. Table 2 represents the p-value of each same tested against the negative control (Ct=30).

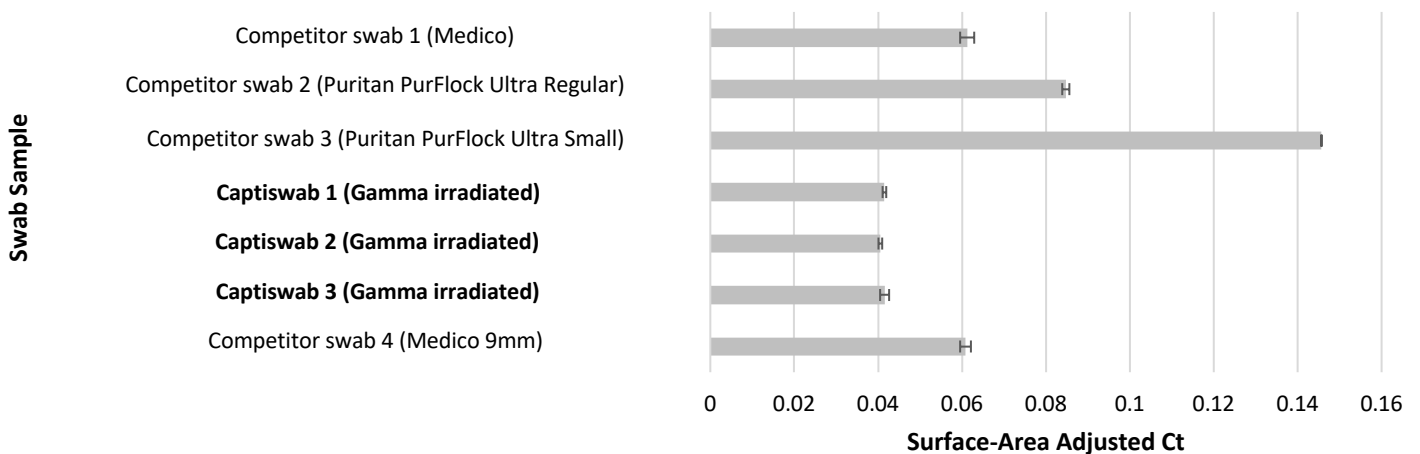


Figure 3. Data represents comparative surface-area adjusted Ct value between swab samples inoculated with *S. aureus* following 24-hour specimen hold.

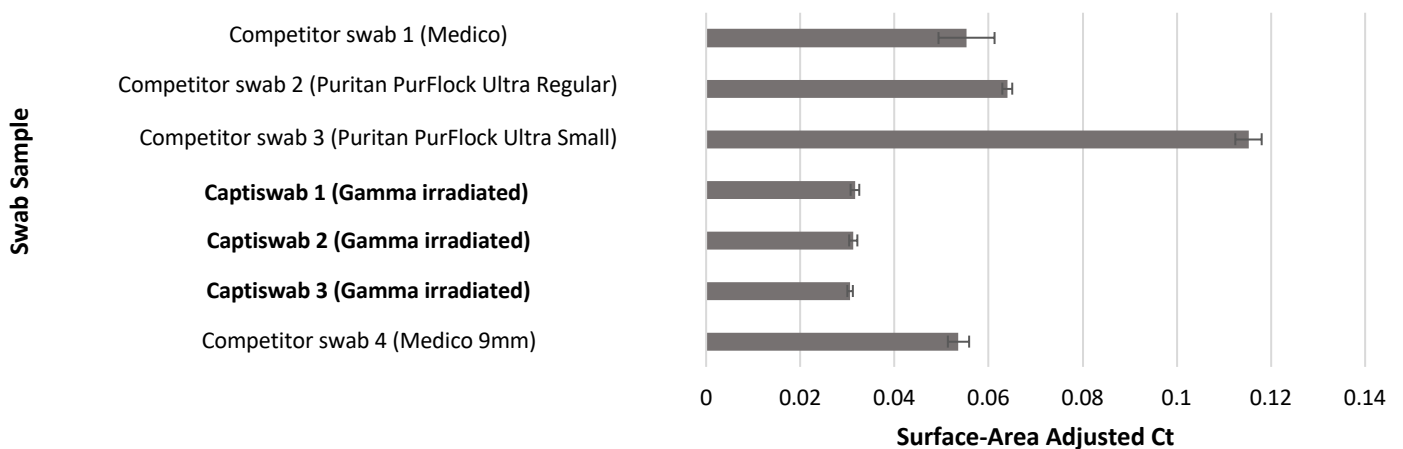


Figure 4. Data represents comparative surface-area adjusted Ct value between swab samples inoculated with *E. coli* following 24-hour specimen hold.

Species/Sample	Ct	Surface area Adjusted Ct	P-value
S. aureus – Competitor Swab 1 (Medico 9mm)	29.34333	0.060828	0.103822968
S. aureus – Competitor Swab 2 (Puritan PurFlock Ultra; Regular)	29.65667	0.084728	0.091771
S. aureus – Competitor Swab 3 (Puritan PurFlock Ultra; Small)	30	0.145666	N/A
S. aureus – Captiswab 1 (Gamma Irradiated)	29.24667	0.041544	0.113045
S. aureus – Captiswab 2 (Gamma Irradiated)	28.52667	0.040521	0.007317
S. aureus – Captiswab 3 (Gamma Irradiated)	29.21333	0.041496	0.023224
S. aureus – Competitor Swab 4 (Medico)	29.53333	0.061222	0.211325
E. coli – Competitor Swab 1 (Medico 9mm)	25.86333	0.053614	0.011195
E.coli – Competitor Swab 2 (Puritan PurFlock Ultra; Regular)	22.39333	0.063977	0.000392
E.coli – Competitor Swab 3 (Puritan PurFlock Ultra; Small)	23.73	0.115222	0.001404
E.coli – Captiswab 1 (Gamma Irradiated)	22.25667	0.031615	0.001159
E.coli – Captiswab 2 (Gamma Irradiated)	22.00333	0.031255	0.000979
E.coli – Captiswab 3 (Gamma Irradiated)	21.53333	0.030587	0.000391
E.coli – Competitor Swab 4 (Medico)	26.68667	0.055321	0.091871

Table 4. Data shows surface-area adjusted Ct values. P-value represents the statistical difference (T-test) between the Ct of the negative control (non-innoculated well) and each sample condition. Each sample condition was tested in triplicate.

(Experiment 4) Comparative buffer testing

- **Aim:** To assess the buffer transport utility
- **Methods:** Biofilms consisting of monocultures of either *Staphylococcus aureus* (ATCC 25923) or *Escherichia coli* (ATCC 11303) were grown on the surface of 12mm Polycarbonate transwell inserts. Once the biofilms were grown, each insert was removed from its original growth well and transferred to a new well containing a single gauze testing material (approximately 1cm²; each sample was previously weighed for subsequent normalization). Each biofilm was removed via debridement (vortex with uniform force applied). The samples were then transferred into different buffers for testing of absorptive, biofilm removal and nucleic acid extraction efficiencies. All sample conditions were tested in triplicate.
- Each sample was tested per standardized protocols at CLIA-certified MicroGenDx Laboratories (Lubbock, TX). Samples were mechanically lysed and extracted using the Qiagen TissueLyser in combination with the Roche High Pure PCR Template Preparation kit. Each sample was tested for 16S abundance to verify species and test for possible contaminants obtained during the testing procedure.
- **Results:** Each buffer was compared to its corresponding “Dry” condition and gauze material as shown in the following graphs. Sample results are presented by organism and buffer condition. Values are shown in terms of a normalized (surface-area adjusted) Ct Value. Lower values are indicative of increased nucleic acid present at the time of extraction. There was minimal, if any, difference noted between the buffers tested vs. dry 36-hour hold conditions.

Graphs for Gram positive *S.aureus* testing (following surface-area adjustment): H₂O, Saline, Zymo, Amies buffers

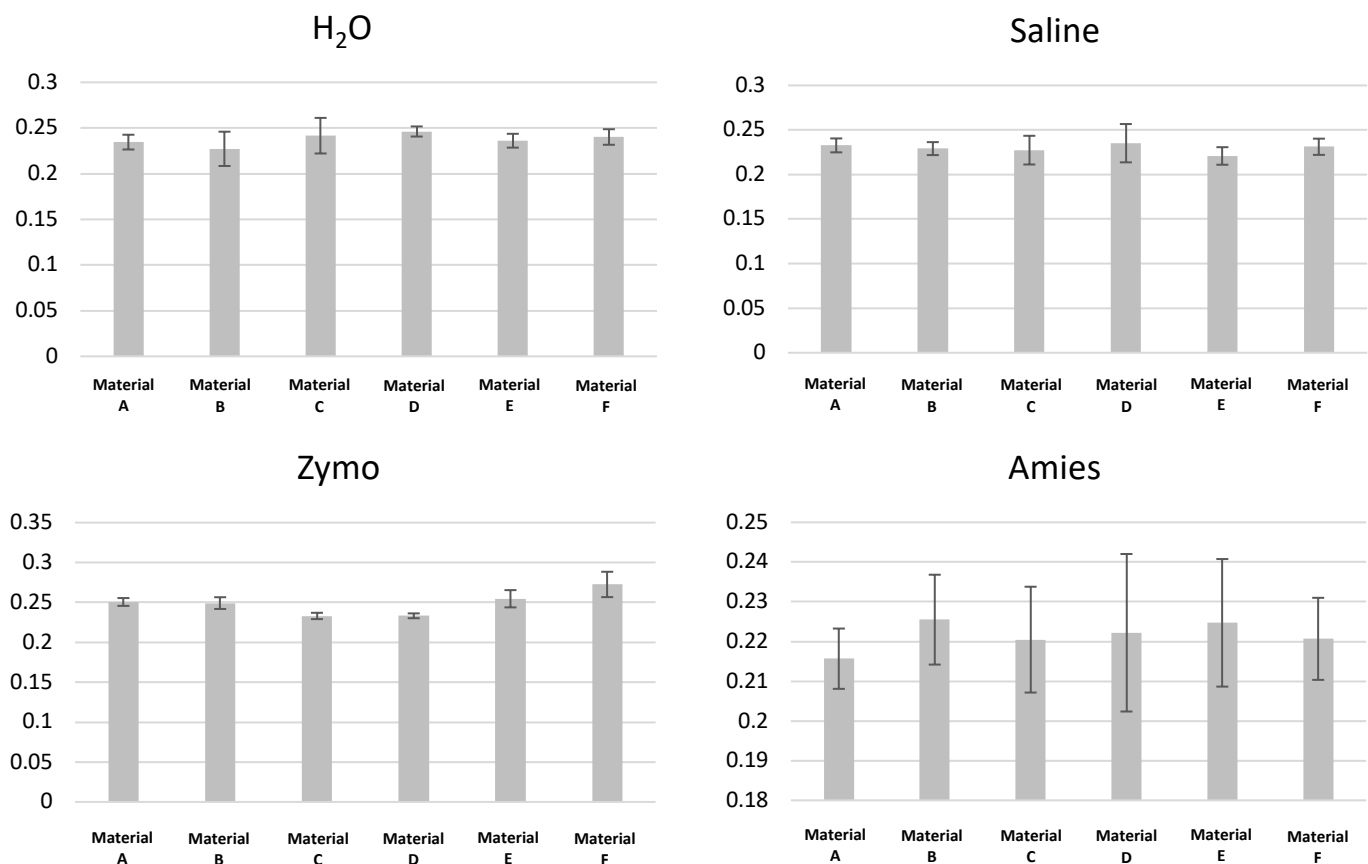


Figure 5. Graphs show comparisons between gauze type (n=3 replicates) and transport conditions (water, saline, Zymo DNA/RNA buffer, Amies buffer) when inoculated with *S. aureus*

Graphs for Gram negative *E.coli* testing (following surface-area adjustment): H₂O, Saline, Zymo, Amies transport

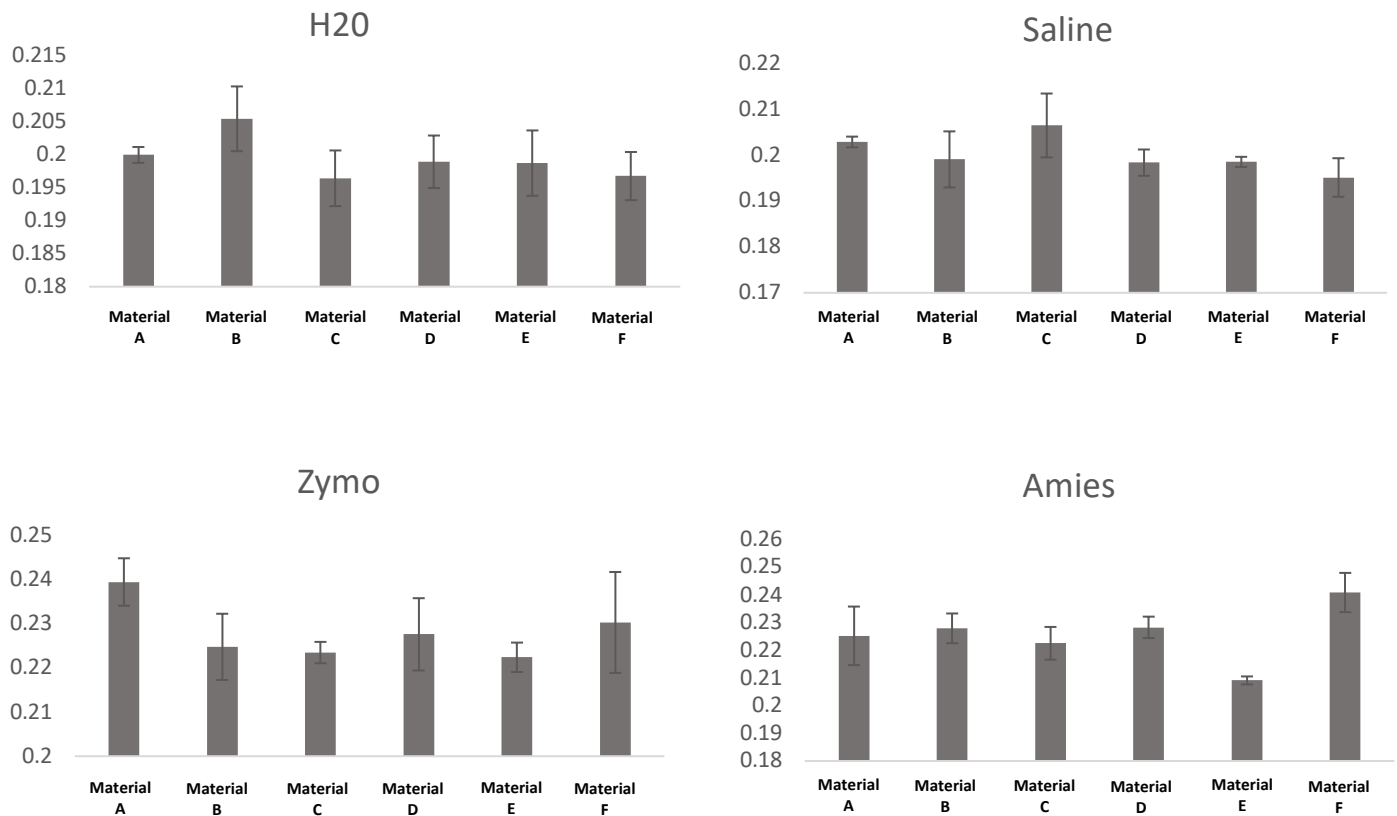


Figure 6: Data show comparisons between gauze type and transport conditions when inoculated with *E. coli*.

	H ₂ O	Saline	Zymo	Amies
S. aureus - Material A	0.018695	0.016944	0.017024	0.01252
S. aureus - Material B	0.005807	0.000235	0.003289	0.048156
S. aureus - Material C	0.068998	0.077802	0.054216	0.058013
S. aureus - Material D	0.100179	0.098315	0.096303	0.087852
S. aureus - Material E	0.00372	0.003425	0.004476	0.005001
S. aureus - Material F	0.002084	0.001292	0.011662	0.004821
<i>E. coli</i> - Material A	0.007405	0.007778	0.008317	0.007348
<i>E. coli</i> - Material B	0.042719	0.021587	0.09483	0.119329
<i>E. coli</i> - Material C	0.000453	0.000408	0.000508	0.000444
<i>E. coli</i> - Material D	0.063576	0.055302	0.097423	0.083757
<i>E. coli</i> - Material E	0.095764	0.12461	0.390899	0.208241
<i>E. coli</i> - Material F	0.001916	0.002017	0.00365	0.003423

Table 5. P-values comparing transport conditions to a “Dry” control.

Summary Graph Showing Buffer Performance (following surface area adjustment)

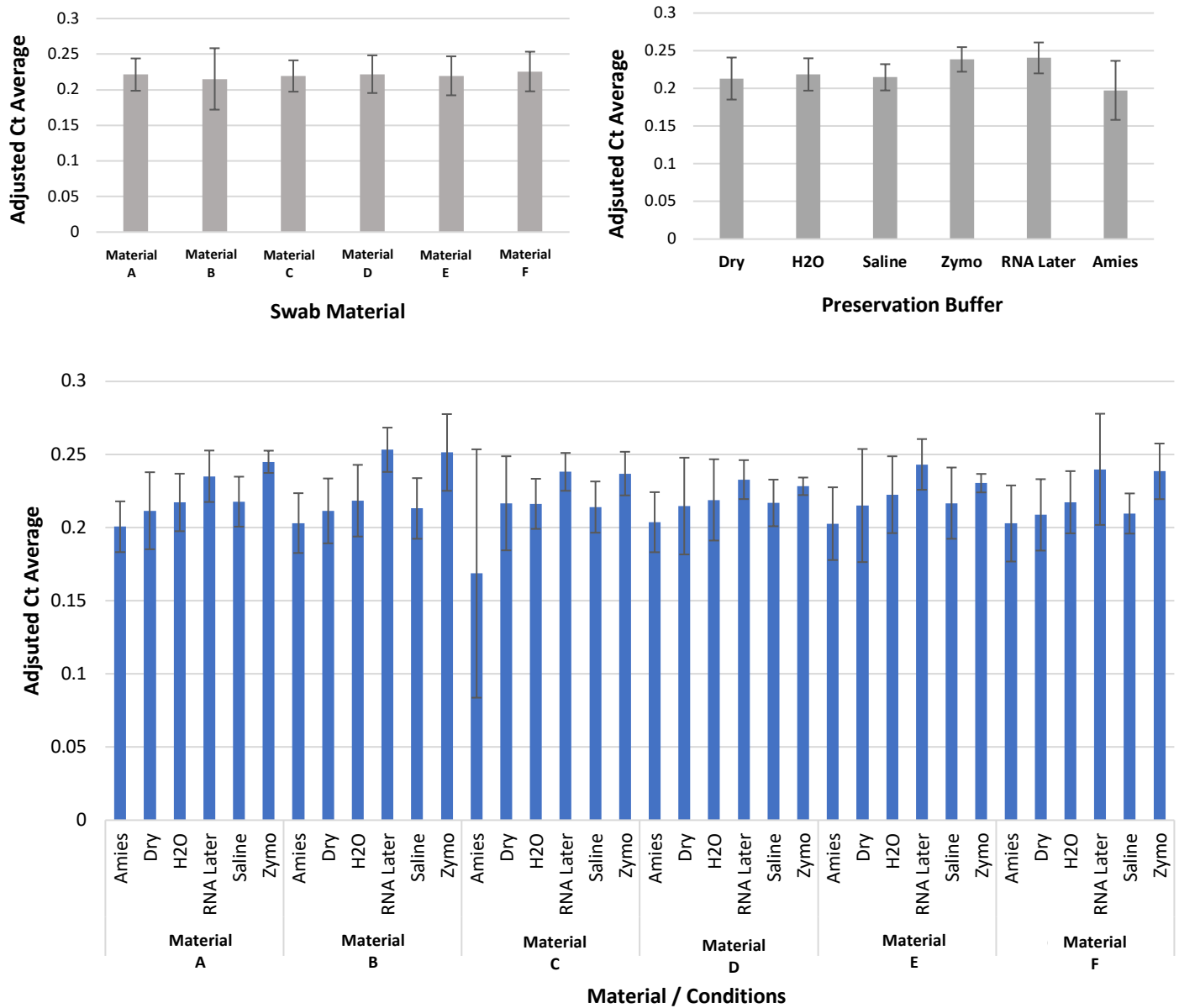


Figure 7(a), (b) and (c). Data show summary comparison between buffer performance for each material tested.

(Experiment 5) Assessment of Captiswab to the CLSI M40-A2 standard for microbiological culture.

- **Aim:** Captiswab validation per the CLSI M40-A2 standard for culture.
- **Methods:** The test procedures employed for determining bacterial viability performance were based upon the quality control methods described in Clinical Laboratory Standards Institute (CLSI) M40-A2. Test organisms utilized in this study were those specifically listed in M40-A2 for establishing performance claims and quality control of swab transport systems. These include a representative panel of aerobes, anaerobes and fastidious bacteria. Bacterial viability studies were performed on the Captiswab, as well as a widely utilized competitor BD swab, at room temperature. Swabs accompanying each transport system were inoculated in triplicate with 100µl of specific concentrations of organism suspension. Swabs were then placed in their respective transport medium tubes and were held for 0 hrs, 24 hrs or 48 hrs. At the appropriate time intervals, each swab was processed according to the Roll-Plate or Swab Elution Method.
- **Results of CLSI M40-A2 testing:**

	Swab	Temperature	Quantitative (Swab Elution) CFU/ml			Qualitative (Roll Plate) CFU			
			0hr	48hr	M40-A2 Compliance?	0hr	24hr	48hr	M40-A2 Compliance?
Propionibacterium acnes ATCC® 6919	Captiswab 1	Room Temp	4.80E+06	1.49E+07	✓	312		35	✓
	Captiswab 2		5.30E+06	1.61E+07	✓	174		14	✓
	BD swab		6.37E+06	1.52E+07	✓	242		59	✓
Neisseria gonorrhoeae ATCC® 43069	Captiswab 1	Room Temp	5.60E+06	1.62E+07	✓	149	76		✓
	Captiswab 2		6.18E+06	1.58E+07	✓	248	97		✓
	BD swab		6.38E+06	1.48E+07	✓	197	96		✓
Fusobacterium nucleatum ATCC® 25586	Captiswab 1	Room Temp	2.20E+07	1.95E+07	✓	142		47	✓
	Captiswab 2		2.18E+07	1.99E+07	✓	241		21	✓
	BD swab		2.20E+07	2.03E+07	✓	230		96	✓
Haemophilus Influenzae ATCC® 10211	Captiswab 1	Room Temp	1.49E+07	1.60E+07	✓	269		70	✓
	Captiswab 2		7.21E+05	3.00E+07	✓	320		82	✓
	BD swab		7.68E+06	2.41E+07	✓	264		75	✓
Streptococcus pneumoniae ATCC® 6305	Captiswab 1	Room Temp	1.76E+07	2.85E+07	✓	170		13	✓
	Captiswab 2		1.37E+07	1.90E+07	✓	182		28	✓
	BD swab		3.75E+06	5.44E+06	✓	134		11	✓
Streptococcus pyogenes ATCC® 19615	Captiswab 1	Room Temp	1.71E+07	1.42E+07	✓	284		76	✓
	Captiswab 2		6.35E+06	1.83E+07	✓	178		44	✓
	BD swab		1.88E+07	1.09E+07	✓	282		92	✓
Prevotella melaninogenica ATCC® 25845	Captiswab 1	Room Temp	1.58E+06	2.31E+07	✓	264		64	✓
	Captiswab 2		1.85E+07	8.50E+06	✓	134		61	✓
	BD swab		1.56E+07	1.35E+07	✓	178		31	✓
Bacteroides fragilis ATCC® 25285	Captiswab 1	Room Temp	8.95E+06	2.02E+07	✓	210		38	✓
	Captiswab 2		9.72E+06	4.80E+06	✓	196		97	✓
	BD swab		2.46E+06	1.81E+07	✓	160		83	✓
Peptostreptococcus anaerobius ATCC® 27337	Captiswab 1	Room Temp	2.08E+07	1.51E+07	✓	226		44	✓
	Captiswab 2		5.15E+06	9.14E+06	✓	140		37	✓
	BD swab		9.46E+06	1.75E+07	✓	280		63	✓

N.B. Pseudomonas aeruginosa ATCC® BAA-427 results are pending due to back order of this organism from ATCC with no availability anticipated until July 2020.

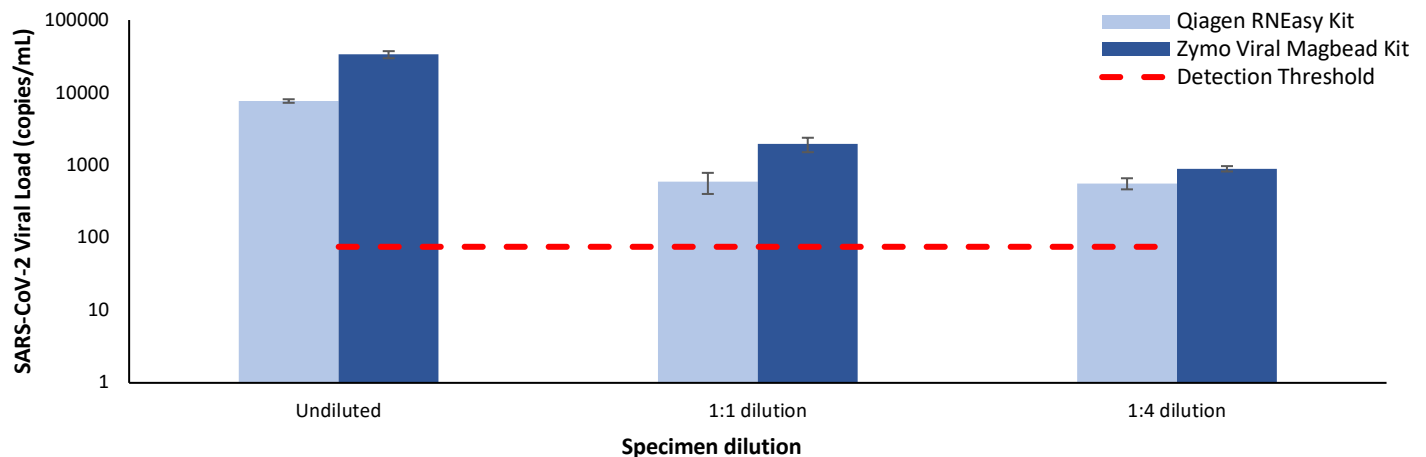
Experiment: Recovery of SARS-CoV-2 using RT-PCR and two RNA extraction methods, in light of the SARS-CoV-2 pandemic

- **Aim:** To assess the ability of the CaptiSwab to absorb and extract SARS-CoV-2 using the Qiagen RNeasy and Zymo Viral Magbead kits via RT-PCR (Roche).
- **Methods:** CaptiSwabs were inoculated with a positive patient specimen (>10,000 viral copies/ml) and then chemically extracted using the Qiagen RNeasy (200 µl) and Zymo Viral Magbead kit (400 µl) kits. Each specimen was run at the original positive concentration, at a 1:1 dilution, and at a 1:4 dilution in order to test spanning range of the assay and compare the kits at various positive levels. The SARS-CoV-2 Molecular Diagnostic Assay utilized is a modification of the Centers for Disease Control (CDC) EUA assay conducted at MicroGenDx Laboratories (Lubbock, TX). The modifications have been shown not to impact the performance of the assay. Testing meets or exceeds the requirements of the FDA for submission of EUA request; further documentation can be found at the FDA website [<https://www.fda.gov/media/137370/download>].

Name	Description	Oligonucleotide Sequence (5'>3')	Label1	Working Conc.
2019-nCoV_N1-F	2019-nCoV_N1 Forward Primer	5'-GAC CCC AAA ATC AGC GAA AT-3'	None	20 µM
2019-nCoV_N1-R	2019-nCoV_N1 Reverse Primer	5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'	None	20 µM
2019-nCoV_N1-P	2019-nCoV_N1 Probe	5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3'	FAM, BHQ-1	5 µM
2019-nCoV_N2-F	2019-nCoV_N2 Forward Primer	5'-TTA CAA ACA TTG GCC GCA AA-3'	None	20 µM
2019-nCoV_N2-R	2019-nCoV_N2 Reverse Primer	5'-GCG CGA CAT TCC GAA GAA-3'	None	20 µM
2019-nCoV_N2-P	2019-nCoV_N2 Probe	5'-FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1-3'	FAM, BHQ-1	5 µM
2019-nCoV_N3-F	2019-nCoV_N3 Forward Primer	5'-GGG AGC CTT GAA TAC ACC AAA A-3'	None	20 µM
2019-nCoV_N3-R	2019-nCoV_N3 Reverse Primer	5'-TGT AGC ACG ATT GCA GCA TTG-3'	None	20 µM
2019-nCoV_N3-P	2019-nCoV_N3 Probe	5'-FAM-AYC ACA TTG GCA CCC GCA ATC CTG-BHQ1-3'	FAM, BHQ-1	5 µM
RP-F	RNAse P Forward Primer	5'-AGA TTT GGA CCT GCG AGC G-3'	None	20 µM
RP-R	RNAse P Reverse Primer	5'-GAG CGG CTG TCT CCA CAA GT-3'	None	20 µM
RP-P	RNAse P Probe	5'-FAM – TTC TGA CCT GAA GGC TCT GCG CG – BHQ-1-3'	FAM, BHQ-1	5 µM

Table I: CDC designed probes for the detection of SARS-CoV-2. ¹TaqMan® probes labeled at the 5'-end with the reporter molecule 6-carboxyfluorescein (FAM) and with the quencher, Black Hole Quencher 1 (BHQ-1) (Biosearch Technologies, Inc., Novato, CA) at the 3'-end. 2019-nCoV Markers (N1 and N2), RNase P (Extraction Control), universal detection of SARS-like coronaviruses (N3 assay)

Results: Both RNA extraction kits were both effective at absorbing and extracting the SARS-CoV-2 target from Captiswab



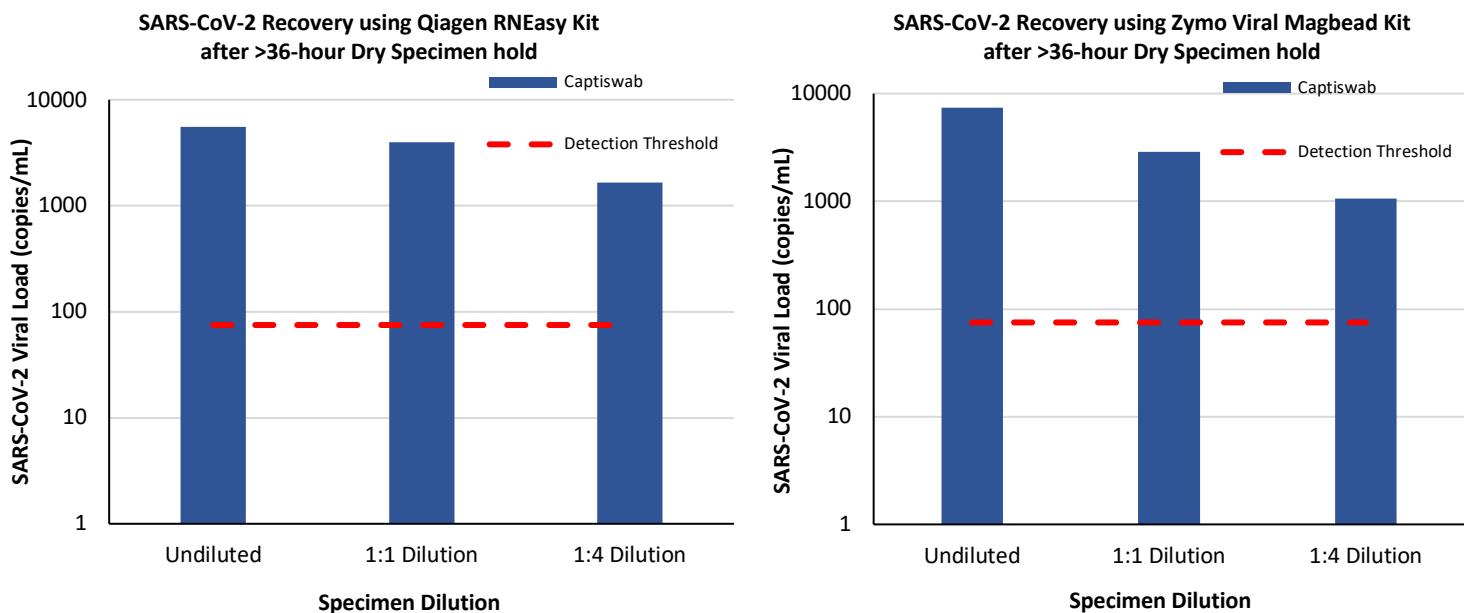
Graph I: Shows SARS-CoV-2 RT-PCR Recovery from Captiswab Specimens at Room Temperature, using the Qigen RNeasy kit and Zymo Viral Magbead kit. Detection threshold for this modification of the CDC EUA assay was 75 viral copies/ml.

Specimen dilution	RNA extraction kit	Mean Viral load (copies/ml)	SD	SE	Detection Threshold (copies/ml)
Undiluted	Qiagen RNeasy kit	7664.13	746.82	431.18	75
1:1 dilution	Qiagen RNeasy kit	592.13	331.09	191.15	75
1:4 dilution	Qiagen RNeasy kit	561.91	169.46	97.84	75
Undiluted	Zymo Viral Magbead kit	33517.73	6476.37	3739.13	75
1:1 dilution	Zymo Viral Magbead kit	1947.63	759.82	438.68	75
1:4 dilution	Zymo Viral Magbead kit	894.11	131.68	76.02	75

Table II: Shows SARS-CoV-2 recovery from Captiswab at undiluted, 1:1 dilution and 1:4 dilution inoculations using 2 extraction methods

Experiment: Recovery of SARS-CoV-2 using RT-PCR and two RNA extraction methods after 36-hour sample hold

- **Aim:** To determine the ability of the CaptiSwab to absorb, transport and extract SARS-CoV-2 using the Qiagen RNeasy and Zymo Viral Magbead kits, after 36 hour dry specimen hold at 25°C.
- **Methods:** CaptiSwabs were inoculated in standardized fashion with a positive patient specimen and then chemically extracted using the Qiagen RNeasy and Zymo Viral Magbead kits. Each specimen was run at the original positive concentration, at a 1:1 dilution, and at a 1:4 dilution in order to test spanning range of the assay and compare the kits at various positive levels. Samples were then maintained at 25°C prior to extraction and testing. The SARS-CoV-2 Molecular Diagnostic Assay utilized is a modification of the Centers for Disease Control (CDC) EUA assay conducted at MicroGenDx Laboratories (Lubbock, TX). The modifications have been shown not to impact the performance of the assay. Testing meets or exceeds the requirements of the FDA for submission of EUA request. Further documentation regarding this protocol can be found at the FDA website [<https://www.fda.gov/media/137370/download>].
- **Results:** The two extraction kits were effective at absorbing and extracting the viral COVID target from Captiswabs. The threshold for SARS-CoV-2 detection was 75 copies/ml. Means and SE of triplicate repeat experiments are shown.



Graph IIa and IIb: Show SARS-CoV-2 RT-PCR Recovery from Captiswab Specimens following >36-hour dry hold at Room Temperature, using the Qiagen RNeasy kit and Zymo Viral Magbead kits. Detection threshold for this modification of the CDC EUA assay was 75 viral copies/ml.

Specimen Dilution	RNA Extraction Kit	Swab tested	Mean viral load (copies/ml)
Undiluted	Qiagen RNeasy kit	Captiswab	5546.64182
1:1 Dilution	Qiagen RNeasy kit	Captiswab	3991.468
1:4 Dilution	Qiagen RNeasy kit	Captiswab	1665.909
Undiluted	Zymo Viral Magbead kit	Captiswab	7404.88
1:1 Dilution	Zymo Viral Magbead kit	Captiswab	2893.459
1:4 Dilution	Zymo Viral Magbead kit	Captiswab	1056.913

Table III: SARS-CoV-2 RT-PCR Recovery from Captiswab Specimens following >36-hour dry hold at Room Temperature, using the Qiagen RNeasy kit and Zymo Viral Magbead kits.