510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K231013

B Applicant

Zymo Research

C Proprietary and Established Names

DNA/RNA Shield SafeCollect Saliva Collection Kit

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QBD	Class II	21 CFR 866.2950 - Microbial Nucleic Acid Storage And Stabilization Device	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for the Zymo Research DNA/RNA Shield SafeCollect Saliva Collection kits for the collection, transport and storage of saliva specimens to the laboratory for downstream testing.

B Measurand:

Storage and stability of nucleic acids from SARS-CoV-2 in saliva

C Type of Test:

SARS-CoV-2 nucleic acid storage and stabilization device

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The DNA/RNA Shield SafeCollect Saliva Collection Kit is intended for the collection, inactivation, stabilization, and transportation, of unprocessed saliva specimens suspected of containing SARS-CoV-2. The DNA/RNA Shield SafeCollect Saliva Collection Kit is intended to transport and store saliva specimens at ambient temperature (20-25°C) from the collection site to the laboratory. Specimens collected and preserved in a DNA/RNA Shield SafeCollect Saliva Collection kit sample collection tube are suitable for use with legally marketed molecular diagnostic devices.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

None.

IV Device/System Characteristics:

A Device Description:

The DNA/RNA Shield SafeCollect Saliva Tube consists of a tube pre-filled with DNA/RNA Shield transport media. DNA/RNA Shield is a transport media that ensures stability of SARS-CoV-2 RNA during sample transport/storage at ambient temperatures and is intended to inactivate SARS-CoV-2, effectively lyses cells from collected saliva specimens.

The DNA/RNA Shield SafeCollect Saliva Tube contains a foil seal barrier that sequesters the DNA/RNA Shield transport media inside of the tube, until the cap, with a Safe Puncture tip is used to seal the DNA/RNA Shield SafeCollect Saliva tube. When the foil seal barrier is broken by the Safe Puncture Tip, the specimen is then allowed to mix with the DNA/RNA Shield transport media.

The DNA/RNA Shield SafeCollect Saliva Collection Kit consists of a DNA/RNA Shield SafeCollect Saliva Tube, a funnel designed for the collection of human saliva samples, and a cap with a Safe Puncture tip. Sample collection is conducted under the supervision of a healthcare provider. The user deposits their saliva into the collection tube with the aid of the attached funnel, the user removes the funnel and replaces it with the cap. Upon twisting and closing the Safe Puncture tip cap, the DNA/RNA Shield is released into the tube and mixes with the saliva.

B Principle of Operation:

The media components are intended to inactivate SARS CoV-2 capsids, disrupt/lyse lipid membranes, denature proteins, inactivate enzymes, and stabilize SARS CoV-2 RNA. The transport device is designed for storage of saliva for up to 21 days.

Do not mix with sodium hypochlorite. The media contains the following reagents:
- Inactivation buffer
- Salts
- pH buffer
- Water

V Substantial Equivalence Information:

A Predicate Device Name(s):
DNA/RNA Shield Collection Tube

B Predicate 510(k) Number(s):
K202641

C Comparison with Predicate(s):

Device & Predicate Device(s):	Device: K231013	Predicate: K202641
Device Trade Name	DNA/RNA Shield SafeCollect Saliva Collection Kit	DNA/RNA Shield Collection Tube
General Device Characteristic Similarities		
Intended Use/Indications For Use	The DNA/RNA Shield SafeCollect Saliva Collection Kit is intended for the collection, inactivation, stabilization, and transportation, of unprocessed saliva specimens suspected of containing SARS-CoV-2. The DNA/RNA Shield SafeCollect Saliva Collection Kit is intended to transport and store saliva specimens at ambient temperature (20-25°C) from the collection site to the laboratory. Specimens collected	The DNA/RNA Shield collection tube is intended for the stabilization and inactivation of upper and lower respiratory human specimens suspected of containing SARS-CoV-2. These devices can be used for collection transport and storage of specimens at ambient temperatures (20-25°C). Specimens collected and stored in a DNA/RNA Shield collection tube are suitable for use with legally marketed

	and preserved in a DNA/RNA Shield™ SafeCollect Saliva Collection kit sample collection tube are suitable for use with legally marketed molecular diagnostic devices.	molecular diagnostic devices.
Analyte	SARS-CoV-2 RNA	Same
Sample stability	20-25°C	Same
Collection media	DNA/RNA Shield media	Same
Special conditions for use	For prescription use only For *in-vitro* diagnostics use only	Same
General Device Characteristic Differences		
Sample collection	Saliva	Upper and lower respiratory specimens
RNA Stabilization at room temperature	Saliva: up to 21 days	Upper/lower respiratory tract samples: up to 28 days

VI Standards/Guidance Documents Referenced:

Special controls that are applicable to regulation 21 CFR 866.2950

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

   N/A

2. Linearity:

   N/A

3. Analytical Specificity/Interference:

   N/A

4. Assay Reportable Range:

N/A

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Shelf life
The shelf life for the DNA/RNA Shield SafeCollect Saliva Collection kit is six months after the date of manufacture, based on current real-time stability data. The real-time stability of the DNA/RNA Shield SafeCollect Saliva Collection kit with media was assessed using a total of three lots. Stability studies looked for bacterial and fungal growth in the media along with properties of the media, appearance, pH, voltage resistance and density.

Sterilization
The DNA/RNA Shield SafeCollect Saliva Tube with media are not sold as sterile nor are they intended to be sterilized by the user. These vials are single use devices that do not require cleaning by the operator.

6. Performance Studies:

a) Detection Limit:
An analytical sensitivity study was conducted to determine the Limit of Detection (LoD) of SARS-CoV-2 when detected in saliva samples collected using the DNA/RNA Shield SafeCollect Saliva Collection kit in combination with the authorized Quick SARS-CoV-2 rRT-PCR Kit for SARS-CoV-2 detection. To determine a preliminary LoD, SARS-CoV-2 negative saliva was used as a clinical matrix collected in DNA/RNA Shield SafeCollect Saliva Collection Kit and spiked with inactivated SARS-CoV-2. The preliminary LoD was determined with the lowest concentration for which 5/5 independent replicates tested positive. Results for the preliminary LoD determination are in Table 1. below.

Table 1: Preliminary LoD

Concentrations Tested (GEC/mL)	Replicates	SARS-CoV-2 Targets	Interpretation	Call Rate
8.3×10^4 (5,000 GEC/rxn)	1	27.33	Positive	5/5
	2	28.03	Positive	
	3	27.73	Positive	
	4	27.60	Positive	
	5	27.73	Positive	
8.3×10^3 (500 GEC/rxn)	1	29.77	Positive	5/5
	2	28.83	Positive	
	3	29.33	Positive	
	4	29.64	Positive	
	5	29.97	Positive	
8.3×10^2 (50 GEC/rxn)	1	32.37	Positive	5/5
	2	32.95	Positive	
	3	32.31	Positive	
	4	32.88	Positive	
	5	32.49	Positive	
83	1	39.54	Positive	5/5

(5 GEC/rxn)	2	34.16	Positive	
	3	38.87	Positive	
	4	32.54	Positive	
	5	33.36	Positive	
8.3 (0.5 GEC/rxn)	1	N/A	Negative	1/5
	2	44.00	Inconclusive	
	3	37.89	Positive	
	4	40.94	Inconclusive	
	5	N/A	Negative	

To confirm the LoD, inactivated SARS-CoV-2 was spiked into negative saliva specimens and 20 replicates were independently processed. The lowest concentration at which all 5 replicates were positive in the preliminary LoD (i.e., 83 GEC/mL) was used as a starting point for the confirmatory LoD study. Therefore, concentrations above 83 GEC/mL (increasing by factor 2) were tested until ≥ 19/20 replicates tested positive. The final LoD for saliva was determined to be at the lowest concentration at which ≥ 19/20 replicates test positive. The final LoD was determined to be 250 GEC/ml (15 GEC/rxn). Results of the confirmatory LoD study for saliva specimens are in Table 2 below.

Table 2: Confirmatory LoD Determination

Concentrations Tested (GEC/mL)	Replicates	SARS-CoV-2 Targets	Interpretation	Call Rate
250 (15 GEC/rxn)	1	34.62	Positive	19/20
	2	34.83	Positive	
	3	33.99	Positive	
	4	N/A	Negative	
	5	35.84	Positive	
	6	33.94	Positive	
	7	35.25	Positive	
	8	35.01	Positive	
	9	33.99	Positive	
	10	33.69	Positive	
	11	34.01	Positive	
	12	34.03	Positive	
	13	35.32	Positive	
	14	34.46	Positive	
	15	35.84	Positive	
	16	35.56	Positive	
	17	33.76	Positive	
	18	35.33	Positive	
	19	34.00	Positive	
	20	33.56	Positive	
166 (10 GEC/rxn)	1	35.57	Positive	17/20
	2	35.35	Positive	
	3	35.78	Positive	
	4	N/A	Negative	
	5	33.80	Positive	

	6	34.42	Positive	
	7	35.19	Positive	
	8	35.01	Positive	
	9	34.87	Positive	
	10	36.17	Positive	
	11	N/A	Negative	
	12	34.25	Positive	
	13	37.80	Positive	
	14	N/A	Negative	
	15	34.64	Positive	
	16	35.44	Positive	
	17	35.51	Positive	
	18	35.46	Positive	
	19	36.05	Positive	
	20	34.66	Positive	
83 (5 GEC/rxn)	1	41.48	Inconclusive	13/20
	2	37.02	Positive	
	3	37.57	Positive	
	4	37.07	Positive	
	5	N/A	Negative	
	6	36.54	Positive	
	7	37.17	Positive	
	8	37.25	Positive	
	9	35.21	Positive	
	10	N/A	Negative	
	11	37.08	Positive	
	12	N/A	Negative	
	13	35.57	Positive	
	14	N/A	Negative	
	15	N/A	Negative	
	16	N/A	Negative	
	17	34.74	Positive	
	18	35.01	Positive	
	19	35.03	Positive	
	20	35.54	Positive	

Conclusion:
The DNA/RNA Shield SafeCollect medium used to collect saliva, and the Authorized Quick SARS-CoV-2 2rRT-PCR Kit reached a SARS-CoV-2 LoD of 250 GEC/mL (15 GEC/reaction) for saliva specimens, which is equivalent to the established LoD of the authorized reference assay.

b) Stability of SARS-CoV-2 in saliva specimens:
The room temperature (20-25 ºC) stability of SARS-CoV-2 in DNA/RNA Shield SafeCollect Saliva kit was established by spiking 3X LoD of SARS-CoV-2 (750 GEC/ml) (see LoD section VII.A.6.a above) into negative saliva specimens collected using the DNA/RNA Shield SafeCollect Saliva Collection Kit and stored at room temperature in a time course

study of 21 days. The room temperature stability of SARS-CoV-2 was measured using the Quick SARS-CoV-2 rRT-PCR Kit at day 0, 1, 2, 3, 4, 5, 6, 7, 14, and 21. Results are summarized in Table 3.

Table 3. SARS-CoV-2 Stability

Concentration Tested	Days at Room Temperature	Replicates	Average Ct (Standard Deviation)	Call Rate
Low Positive 3x LoD (750 GEC/mL)	Day 0	3	33.75 (0.21)	3/3
	Day 1	3	33.37 (0.22)	3/3
	Day 2	3	34.10 (0.35)	3/3
	Day 3	3	33.93 (0.32)	3/3
	Day 4	3	34.24 (0.97)	3/3
	Day 5	3	34.34 (0.24)	3/3
	Day 6	3	34.75 (0.87)	3/3
	Day 7	3	34.44 (0.41)	3/3
	Day 14	3	33.98 (0.36)	3/3
	Day 21	3	34.05 (0.48)	3/3

Conclusion:
There is no significant change in stability over time and the data are within the acceptance criteria (≤+/-10% deviation from day 0). Stability is determined by acceptable data that supports the stability claim and does not exceed day 0 by a 3-log range. Therefore, SARS-CoV-2 is stable in saliva specimens collected using the DNA/RNA Shield SafeCollect Saliva Collection kit for up to 21 days when stored at room temperature (20-25 ºC).

c) Inactivation:
An inactivation study was conducted to test DNA/RNA Shield SafeCollect Saliva Collection kit media ability to inactivate SARS-CoV-2. The inactivation study used a stock titer of $9x10^5$ PFU/mL for SARS-CoV-2. The study was performed in a Biosafety Level 3 (BSL-3) facility.

Stock SARS-CoV-2 was spiked into DNA/RNA Shield media (at a ratio of 1:3 using 100 µL of viral stock was mixed with 300 µL of DNA/RNA Shield SafeCollect medium). The virus and media mixture were incubated for 30 mins at room temperature. The mixture was then serial diluted in cell culture medium and added to confluent monolayers of VeroE6 cells. Cells and media were incubated at 37°C in 5% CO_2 for one hour with gentle rocking every 15 minutes. Media was aspired and 1.0 mL of pre-warmed overlay media was added. Cells were incubated for two days, fixed in 10% formaldehyde, and stained with 0.5% crystal violet to enable plaque visualization and enumeration. A positive control (untreated viral stock) and negative control (media only) were included in each run. A no-virus media control (DNA/RNA Shield media only) was also included to assess whether the media was cytotoxic. The study was replicated three independent times.

Inactivation rate:
The DNA/RNA Shield media showed no cytotoxicity on veroE6 cells when the media was diluted 1:1,000 with cell culture media. The 1:1,000 dilution factor is needed to avoid

cytotoxic effects the DNA/RNA Shield media has on the cell monolayer. The mixture of SARS-CoV-2 and DNA/RNA Shield media combined for 30 minutes demonstrated at least a 2-log reduction in SARS-CoV-2. Greater than a 2-log reduction of SARS-CoV-2 could not be quantified based on the starting concentration of the virus compounded by the need to dilute the DNA/RNA Shield media 1:1000. CPE could not be observed at < 3.0 logs due to the cytotoxic effects of the DNA/RNA Shield media.

Conclusion:
Zymo DNA/RNA shield inactivates SARS-CoV2 when incubated for at least 30 minutes at room temperature.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   N/A

2. Matrix Comparison:

   N/A

C Clinical Studies:

1. Clinical Sensitivity:

   N/A

2. Clinical Specificity:

   N/A

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   N/A

D Clinical Cut-Off:

N/A

E Expected Values/Reference Range:

N/A

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.