



**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY**

I Background Information:

A 510(k) Number

K240797

B Applicant

Permantis Public Health (PPH)

C Proprietary and Established Names

PPH 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 PPH Saliva Collection Kit for collection, transport, and storage of SARS-CoV-2 in saliva specimens to the laboratory for downstream testing.

B Measurand:

Storage and stabilization of nucleic acids from SARS-CoV-2.

C Type of Test:

Microbial 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 PPH Saliva Collection Kit is designed for use in the non-invasive collection, inactivation, and stabilization of viral nucleic acids for in vitro diagnostic testing of saliva samples. The device is intended to inactivate and stabilize human clinical saliva samples from the collection site to the laboratory at room temperature. The device is intended to be used by a health care provider for the collection of saliva samples suspected of containing SARS-CoV-2. The saliva sample is stabilized and 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 PPH Saliva Collection Device contains a plastic bulb pipette, paper cup, plastic tube with PPH Saliva collection buffer (0.3 ml). It is designed for use in the non-invasive collection, inactivation, and stabilization of viral nucleic acids for in vitro diagnostic testing of saliva samples.

Saliva is collected into the provided paper cup and then transferred into the tube containing PPH Saliva collection buffer with the bulb pipet. After transfer of the saliva sample, the tube should be approximately half full. The tube with sample is then capped and inverted 5-10 times to allow adequate mixing.

B Principle of Operation:

The components of the inactivating media for the PPH Saliva Collection Kit are intended to inactivate SARS CoV-2 capsids, disrupt/lyse membranes, denature proteins, inactivate enzymes, and stabilize SARS CoV-2 RNA. The inactivating solution includes following:

- Guanidine Thiocyanate
- Sarkosyl
- Sodium Acetate, pH 5
- Nuclease-free Water

V Substantial Equivalence Information:

A Predicate Device Name(s):

Spectrum Saliva Collection Device

B Predicate 510(k) Number(s):
K223497

C Comparison with Predicate(s):

Device & Predicate Device(s):	<u>Device: K240797</u>	<u>Predicate: K223497</u>
Device Trade Name	PPH Saliva Collection Device	Spectrum Saliva Collection Device
General Device Characteristic Similarities		
Intended Use/Indications For Use	The PPH Saliva Collection Kit is designed for use in the non-invasive collection, inactivation, and stabilization of viral nucleic acids for in vitro diagnostic testing of saliva samples. The device is intended to inactivate and stabilize human clinical saliva samples from the collection site to the laboratory at room temperature. The device is intended to be used by a health care provider for the collection of saliva samples suspected of containing SARS-CoV-2. The saliva sample is stabilized and suitable for use with legally marketed molecular diagnostic devices.	The Spectrum Solutions Saliva Collection Device is designed for use in the non-invasive collection, inactivation, and stabilization of viral nucleic acids for in vitro diagnostic testing of saliva samples. The device is intended to inactivate and stabilize human clinical saliva samples from the collection site to the laboratory at room temperature. The saliva sample is stabilized and suitable for use with legally marketed molecular diagnostic devices. The device is intended to be used by a health care provider for samples suspected of containing SARS-CoV-2.
Analyte	Same	RNA from SARS-CoV-2
General Device Characteristic Differences		
Specimen Stability	7 days at ambient temperature (20-25°C)	28 days at 20-25°C
Shelf life	9 months at room temperature (15-30°C)	24 months at room temperature (15-30°C)
Buffer	PPH Saliva Collection	Spectrum's Nucleic

	Kit buffer	Acid Stabilization Solution
Physical container	Plastic polypropylene collection tube	Tube – Polypropylene Funnel

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 Stability: The shelf life for the PPH Saliva Collection Kit is nine (9) months at room temperature (15-30°C) after the date of manufacture. The shelf-life stability of the PPH Saliva Collection Kit was established in a real-time stability study that evaluated several time points (T = 0, 1, 3, 6, 9, and 12 months) for both temperature extremes. In this study, shelf-life stability also included evaluation of physical stability (appearance), color, volume loss, and pH stability during the period of storage. At each time point, the appearance of the product was inspected visually as a clear liquid without precipitation and with no observed volume loss, change in buffer PH, or color change when stored under the specified storage conditions.

Sterilization: The PPH Saliva Collection Kits 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 or sterilization by the operator.

6. Detection Limit:

A limit of detection (LoD) study was conducted to determine the lowest concentration.

that contains measurable nucleic acids that can be repeatedly detected in samples collected in the PPH Saliva Collection Kit with greater than 95% accuracy. RNA extraction was performed using magnetic bead purification on a Thermo Fisher Kingfisher Apex Purification system. Viral RNA was subsequently amplified using the Triplex CII-SARS-CoV-2 rRT-PCR for SARS-CoV-2 detection assay (RUCGL.SCV2.2 assay). The primers and probes used in this assay allow simultaneous measurements of SARS-CoV-2 nucleocapsid RNA (N1 and/or N2), and human Ribonuclease P (RP) transcripts. RT-qPCR was performed with RP primers as positive control to detect the human RNase P gene.

A preliminary LoD was established by evaluating three replicates of several serial dilutions of saliva spiked with SARS-CoV-2 and collected in the PPH Saliva Collection device. These samples were evaluated on the RUCGL.SCV2.2 assay for SARS-CoV-2 detection. For this assay, Ct values <40 were considered positive for SARS-CoV-2.

Results from the preliminary LoD study are summarized in Table 1 below.

Table 1. Preliminary LoD Estimation Results

Copies/ μ L	N1 Detection Rate	N1 Mean Ct	N2 Detection Rate	N2 Mean Ct
8	3/3	31.03	3/3	30.92
4	3/3	32.13	3/3	31.97
2	3/3	33.06	3/3	32.62
1	3/3	34.37	3/3	33.90
0.5	3/3	35.23	3/3	35.20
0.25	3/3	37.85	2/3	-
0.125	3/3	39.53	2/3	-
0.06125	2/3	-	1/3	-
0	0/3	-	0/3	-

The estimated LoD based on the preliminary results was identified as 0.125 copies/ μ L.

To further confirm this preliminary LoD assessment, four concentrations of SARS-CoV-2 (0, 0.0625, 0.125, and 0.25 copies/ μ L) in saliva were evaluated in 20 replicates using the PPH Saliva Collection Kit. Results from the LoD confirmation study are summarized in Table 2 below.

Table 2. Confirmatory LoD Study Results

Copies/ μ L	N1 Detection Rate	N1 Mean Ct	N2 Detection Rate	N2 Mean Ct
0.25	20/20	35.99	19/20	37.57
0.125	17/20	-	4/20	-
0.06125	18/20	-	5/20	-
0	0/20	-	-	-

In summary, the results of the LoD study indicate an LoD of 0.25 copies/μL with observed detection of 100% (20/20) for the N1 target and 95% (19/20) for N2 replicate.

7. **Sample Stability:**

A real-time sample stability study was conducted to evaluate the stability of samples containing SARS-CoV-2 when stored for up to 8 days at ambient temperature (20-25°C). Inactivated SARS-CoV-2 (Zeptomatrix, Cat # NATSARS(COV2)-ST, 1.21x10⁶ copies/ μl) was used to prepare contrived samples including 20 low positive samples (2X LoD) and 10 negative samples for each device lot. Briefly, saliva matrix and SARS-CoV-2 were combined at a ratio of 1:1 and then added to each collection tube containing 300 μl of PPH Saliva Collection Kit buffer.

Sample stability was assessed at baseline (day 0), day 7, and day 8 after being placed in PPH Saliva Collection Kit buffer and stored at ambient temperature (20-25°C). The difference in Ct over this time course is less than 1. The largest change in Ct observed was 0.40 at 8 days.

These test results fall within the established acceptance criteria and indicate that samples are stable in the PPH Saliva Collection Kits inactivating media for up to 7 days. Results for sample stability testing are summarized in Table 3 below.

Table 3. Sample Stability Study Results

Samples Evaluated	Mean Ct & SD	Day 0	Day 7	Day 8
Low positive (60 samples)	N1 Mean Ct	35.9	36.14	36.2 7
	SD	0.43	0.5	0.523
	N2 Mean Ct	35.49	35.63	35.7
	SD	0.4	0.47	0.4
Negative (30 samples)	-	-	-	-

SARS-CoV-2 Inactivation

An inactivation study was conducted to verify that the PPH Saliva Collection device inactivates SARS-CoV-2 as efficiently as the predicate device.

Cytotoxicity: A cytotoxicity study was performed to first determine at what dilution ratio the PPH Saliva Collection Kit buffer would not be toxic to a cell monolayer.

To determine the cytotoxic effect of the buffer on test cells, Vero E6 cells that were seeded at a concentration of 2500 cells/well in each well of a 96 well plate was exposed to serially diluted PPH Saliva Collection Kit buffer prepared in Vero E6 DMEM culture medium. Viability of the test cells was assessed using the CellTiterGlo assay (Promega #G7570) after 4 days of exposure and normalized to untreated cells. After the incubation period, a concentration of 1:5,000 PPH Saliva Collection Kit buffer was determined not to have a cytopathic effect to the host cells.

Therefore, to conduct the viral inactivation study the PPH Saliva Collection Kit buffer was diluted to 1:5,000 with DMEM media.

Inactivation Assay:

Viral inactivation was assessed using a stock of SARS-CoV-2 (USA WA1/2020) at 5.65×10^6 PFU/ml. To determine the amount of contact time required for viral inactivation of SARS-CoV-2 with PPH Saliva Collection Kit buffer, the samples were prepared as follows:

1. Mixed virus stock with saliva at a 1:1 ratio.
2. Mixed virus and saliva with PPH Saliva Collection Kit buffer at a 1:1 ratio.
3. Incubated for 15, 30, 45, 60, 90, 120, 180, or 240 seconds.
4. Diluted virus, saliva and buffer 1:5,000 with Dulbecco's modified Eagle's Medium (DMEM), a concentration previously determined not to be cytotoxic.

100 μ L of each sample containing ~ 113 PFU of SARS-CoV-2 was added to a 96-well plate confluent with Vero E6 cells after media was removed. Six replicates were plated for each time point. The controls included and plated in triplicate included only media, Saliva and PPH Saliva Collection Kit buffer (no virus), and Virus only. No PFUs were obtained after 60, 90, 120, 180 and 240 seconds of exposure of the cells to the media in the PPH Saliva Collection Kit buffer. Acceptance criteria were met for no detectable virus (zero positive plaques) after 4 days growth.

These study results support SARS-CoV-2 inactivation after 90 seconds of exposure to PPH Saliva Collection Kit buffer.

8. Assay Cut-Off:

N/A

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.