510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

I Background Information:

A 510(k) Number

K201849

B Applicant

Copan Italia S.p.A.

C Proprietary and Established Names

eNAT molecular collection and preservation medium

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 make a substantial equivalence determination for the Copan eNAT for the collection, transport and storage of viral specimens to the laboratory for downstream testing.

B Measurand:

Storage and stability of nucleic acids from influenza A virus.

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:

Copan eNAT- molecular collection and preservation medium- is intended for the stabilization, transportation and inactivation of an unprocessed upper respiratory clinical specimen suspected of containing influenza A virus RNA. eNAT- molecular collection and preservation medium- is intended for use with compatible molecular assays.

C Special Conditions for Use Statement(s):

For prescription use only.

D Special Instrument Requirements:

None

IV Device/System Characteristics:

A Device Description:

The eNAT molecular collection and preservation medium, eNAT molecular collection and preservation system consists of tube with transport media, the media appears clear and transparent and is ready to use. The tubs contain 2 mL of the stabilization media. The components of the media are intended to inactivate influenza A, lyse cells, disrupt/lyse lipid membranes, denatures proteins, inactivates enzymes, and stabilize influenza A RNA. The transport device is designed for storage of specimens between 36-39 and 77 °F (2-8 to 25 °C). The media is sold in the following three configurations:

- A plastic screw-cap tube filled with 2 ml of Molecular Preservation and Transport medium.
- A plastic screw-cap tube filled with 2 ml of Molecular Preservation and Transport medium and a regular size tip nylon flocked swab for sample collection.
- A plastic screw-cap tube filled with 2 ml of Molecular Preservation and Transport and a minitip nylon flocked swab for sample collection.

B Principle of Operation:

The device components are intended to inactivate influenza A lyse cells, disrupt/lyse lipid membranes, denatures proteins, inactivates enzymes, and stabilize influenza A RNA. The transport device is designed for storage of specimens between 36-39 and 77 °F (between 2-8°C and 25 °C) for up to 28 days.
The media contains the following reagents:

- Tris-EDTA

- Guanidine thiocyanate
- Detergent
- HEPES
- Distilled water

V Substantial Equivalence Information:

A Predicate Device Name(s):
PrimeStore MTM

B Predicate 510(k) Number(s):
DEN170029

C Comparison with Predicate(s):

Device & Predicate Device(s):	K201849	DEN170029
Device Trade Name	eNAT molecular collection and preservation medium, eNAT molecular collection and preservation system	PrimeStore MTM
Device Type	Transport device for the stabilization of microbial nucleic acids	Transport device for the stabilization of microbial nucleic acids
Intended Use/Indications For Use	Copan eNAT- molecular collection and preservation medium- is intended for the stabilization, transportation and inactivation of an unprocessed upper respiratory clinical specimen suspected of containing influenza A virus RNA. eNAT- molecular collection and preservation medium- is intended for use with compatible molecular assays.	PrimeStore MTM is intended for the stabilization, transportation and inactivation of infectious unprocessed nasal washes suspected of containing influenza A virus RNA. PrimeStore MTM is also intended for the stabilization, transportation and inactivation of infectious unprocessed sputum samples suspected of containing *Mycobacterium tuberculosis* DNA from human samples.
Inactivation tested on Flu A	>4.0 log reduction in concentration at 10 seconds	Same
Storage Temperature	2-8 ºC up to 25ºC	Same

General Device Characteristic Differences		
Specimen stability	eNAT medium preserves influenza A RNA for up to 28 days at between 2-8 ºC up to 25ºC	Primestore MTM medium preserves influenza A RNA for up to 8 days at 27°C and 29 days at 4°C
Specimen Type	Upper respiratory specimen	Nasal washes and sputum samples

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

Shelf life: The shelf life for the Copan eNAT media is 18 months after the date of manufacture. The stability of the Copan eNAT media was established using a real time stability protocl on a total of three lots.

Sterilization: The Copan eNAT molecular collection and preservation medium and eNAT molecular collection and preservation system vials are provide as sterile. Vials are single use devices. The manufacturing process includes sterilization by gamma irradiation. The 18 month sterility testing demonstrated no growth detected up to and beyond this claimed time point.

2. Detection Limit:

a) LoD testing was conducted to determine the lowest concentration of analyte that can be detected with a greater than 95% detection rate. The LoD studies for influenza A were designed using the Cepheid GeneXpert Xpress Flu/RSV assay to establish a concentration of organisms used for additional testing noted below.

LoD testing was initially performed by spiking multiple concentrations of influenza A into simulated nasal matrix; simulated nasal matrix was used because of safety concerns during the COVID-19 pandemic. Contrived nasal matrix has also been used for the clearence of other invitro dignositic device during the pandemic from which a collective knowledge has been gained indicating that contrived matrix is safe and effective for the evaluation of upper respiratory samples. The matrix was composed of Porcine mucin, whole human blood, PBS, glycerol and water. The simulated matrix was spiked with influenza A (H3N2 ATCC VR-1679 A/Hong Kong/8/68) at a concertation of $3.16x10^9$ $TCID_{50}$/mL. The final concentration of each spiked simulated matrix was then added to the eNAT media with a swab to achieve a final ratio of 1:10 specimen to eNAT for

recovery using the GeneXpert Xpress Flu/RSV assay. The samples were then diluted 1:5 to determine the LoD. The study objective was to determine the detectable concentration of influenza A (H3N2 A/Hong Kong/8/68) after spiking into simulated matrix and added to eNAT media. No detection (Ct = 40) was observed at less than 0.180 TCID50/mL concentration. All other higher concentrations demonstrated recovery of influenza A. Table 1 below shows the results of the 1:5 dilutions of influenza A.

Confirmatory LoD testing was provided at a concentration of 0.180 $TCID_{50}$/mL with 24 replicates in both eNAT and the media used for the original clearance of GeneXpert Xpress Flu/RSV assay. The GeneXpert Xpress Flu/RSV has an acceptance criteria of virus detection at a concentration range of 0.75 – 0.006 TCID50/ml in matrix. The same detection range was applied to the eNAT media and further determine by the concentration that yielded at least a 95% of the replicates were recoverable within this range. At a concentration of 0.180 $TCID_{50}$/mL, 24 of 24 replicates had recoverable concentrations. Influenza A RNA was extracted and amplified using the GeneXpert Xpress Flu/RSV assay with an average CT = 34.4; S.D. = 0.94.

Table 1. Influenza A Preliminary Limit of Detection

FLUA Concentration ($TCID_{50}$/mL)	eNAT 4 Reps Average (Ct)	SD (Ct)	Media used for Xpress assay 4 rep Average (Ct)	SD (Ct)
4.480	29.3	0.18	29.2	0.32
0.90	32.0	0.26	31.7	0.60
0.180	34.3	0.95	33.9	0.28
0.036	>40	0	>40	0
0.072	>40	0	>40	0

LoD testing at 0.180 $TCID_{50}$ resulted in all 24 replicates for the concentration meeting the pre-defined acceptance criteria.

b) Viral Stability

The stability of influenza A virus (H3N2 A/Hong Kong/8/68) at 10 x LoD (1.8 $TCID_{50}$/mL) was evalutaed by spiking virus into simulated matrix incubated in Copan eNAT at ambient temperature (25°C, 77° F) for 28 days (see Table 3), and refridgerated temperature (2-8°C, 36-39°F) for 28 days (see Table 4). The Cepheid GeneXpert Xpress Flu/RSV assay was used to determine stability of Flu A in Copan eNAT. The stability study analyzed a total of six lots, three lots near the manufacture date and three lots at the end the end of the claimed 18-month stability. Four tubes from each lot were tested totaling 24 replicates tested at each time point and each temperature range. An initial time point designated as Day 0 was included as the initial Ct average for each of the two temperature ranges tested. Testing at three time points was performed at Day 0, 14 and 28 for refrigerated temperature (2-8°C, 36-39°F), and four additional time points, Day 7, 14, 21 and 28, for ambient temperature (25°C, 77°F).

The Xpert Xpress Flu/RSV Assay has an SPC Negative Control SPC that, per the package insert, must be amplified for a run to be valid and confirm the absenec of pathogen. If pathogen RNA is detected (in this case Flu A) there is no need for SPC to be

detected. A pre-defined acceptance criteria of (+/-) 3.0 Ct from the initial time zero value was the acceptance criteria.

Table 3. Flu A (1.80 $TCID_{50}$ / mL) stability at 25°C

Days (4°C)	**0**	**7**	**14**	**21**	**28**
AVG (Ct):	30.8	30.6	30.7	30.8	30.7
SD (Ct):	0.4	0.24	0.2	0.44	0.28

Table 4. Flu A (1.80 $TCID_{50}$ / mL) stability at 2-8°C

Days	**0**	**14**	**28**
AVG (Ct):	30.8	30.5	30.6
SD (Ct):	0.4	0.13	0.2

Stability testing of RNA from influenza A whole virus spiked into nasal wash and stored in eNAT resulted in a maximum variation of 0.2 C_t over 28 days at 25°C and a maximum variation of 0.3 C_t over 28 days at 2-8°C.

c) Inactivation

Influenza A virus (H3N2 A/Hong Kong/8/68) at a concentration of 3.16 x 10^7 $TCID_{50}$/ml was incubated with eNAT media for 10 seconds. Influenza A only and Copan eNAT only were also incubated accordingly to serve as internal controls. Influenza A, Influenza A and eNAT or eNAT alone was then inculated on to cell cultures. Four days after inoculation, the cells were fixed and stained with 1% crystal violet in 80% acetone. Cells that did not take up the stain were considered evidence of a viral cytopathic effect (CPE) and as a result was considered a measure of viral viability. The titer of the virus CPE was calcuated and recorded as the $TCID_{50}$.

Inactivation time:

The Copan eNAT showed no cytotoxicity on MDCK cells at a 1:1,000 dilution factor; therefore at least a 1:1,000 dilution factor is needed to avoid a direct cytotoxic effect of the eNAT media. Influenza A was then exposed to Copan eNAT for 10 seconds prior to dilution and incubation (final concentration $\leq 10^3$ $TCID_{50}$/mL), while Influenza only samples had viral loads of 3.16 x 10^7 $TCID_{50}$ of virus and eNAT alone was diluted 1:1000. The eNAT media rapidly inactivated Influenza virus with a >4.0 log reduction at a 1:10 specimen to media concentration at 10 seconds. Viral CPE could not be observed at < 3.0 logs due to cellular destruction by eNAT media See Table 5 below.

Table 5 Flu A inactivation in eNAT

	10s incubation $TCID_{50}$
Flu A only	3.15×10^7
Flu A and eNAT	≤ 3.0
eNAT only*	≤ 3.0

*eNAT shows cytotoxicity on MDCK cells when diluted to 1:1,000.

Copan eNAT must be used at a ratio of at least 1:10 in Copan eNAT media at a minimum of 10 seconds exposure time to demonstrate inactivation of influenza A. Measuring Influenza inactivation below 1×10^3 $TCID_{50}$ was not possible because of the cytotoxic affects Copan eNAT has on the cell culture based

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.