510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K221547

B Applicant

FertiPro NV

C Proprietary and Established Names

InActiv Blue

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 InActiv Blue device for the stabilization, transport, and inactivation of upper respiratory specimens suspected of containing SARS-CoV-2 virus.

B Measurand:

Storage and stability of nucleic acids from SARS-CoV-2 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:

InActiv Blue is intended for the stabilization, transportation and inactivation of upper respiratory specimens suspected of containing SARS-CoV-2. These devices can be used for collection transport and storage of specimens at refrigerated (2-8°C) or ambient temperatures (20-25°C). SARS-CoV-2 RNA in the specimens is stabilized for 7 days at these specified temperatures. Specimens collected and stored in an InActiv Blue 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:

InActiv Blue is a virus-inactivating and lysis solution that inactivates SARS-CoV-2 viral infectious particles there by removing the infectious potential of the collected material for the target virus without affecting the integrity of the RNA. Two milliliters of the InActiv Blue solution are provided in a polypropylene flat bottom collection tube with a HDPE (high density polyethylene) cap (IB_TUB). Secondary Packaging for the collection tube configuration includes racks of 50 collection tubes and cardboard boxes with either 400 or 1200 collection tubes. Package Inserts are included with all secondary packaging configurations.

Sample types intended for collection with the device include upper respiratory swab samples. Swabs are not provided with the InActiv Blue collection tubes.

B Principle of Operation:

The active components of the InActiv Blue solution are guanidine thiocyanate, N-lauroylsarcosine sodium salt and EDTA. These components are formulated in a sodium phosphate buffered solution containing methylene blue.

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: K221547	Predicate: K202641
Device Trade Name	InActiv Blue	DNA/RNA Shield Collection Tube
General Device Characteristic Similarities		
Intended Use/Indications For Use	InActiv Blue is intended for the stabilization, transportation and inactivation of upper respiratory specimens suspected of containing SARS-CoV-2. These devices can be used for collection transport and storage of specimens at refrigerated (2-8°C) or ambient temperatures (20-25°C). SARS-CoV-2 RNA in the specimens is stabilized for 7 days at these specified temperatures. Specimens collected and stored in an InActiv Blue collection tube are suitable for use with legally marketed molecular diagnostic devices.	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 molecular diagnostic devices.
Inactivation	RNA virus particles	Same
Analyte	RNA	Same
General Device Characteristic Differences		
Sample source	Human upper respiratory	Human lower and upper respiratory
Sample storage temperature	7 days at refrigerated (2-8°C) or ambient temperatures (20-25°C)	28 days at 20-25˚C

VI Standards/Guidance Documents Referenced:

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

   Not Applicable (N/A)

2. Linearity:

   Not Applicable (N/A)

3. Analytical Specificity/Interference:

   Not Applicable (N/A)

4. Assay Reportable Range:

   Not Applicable (N/A)

5. Shelf-Life Stability:

   Shelf-life stability of InActiv Blue was evaluated to ensure product integrity over the complete shelf-life of the product. Completed real-time stability studies included at least eight lots of InActiv Blue which have been stored at room temperature for different periods of time, with performance testing conducted at regularly spaced timepoints to support a 21-month shelf life stability claim.

   At each time points, appearance by visual inspection pH (6+/- 0.6), osmolarity **(**mOsm/kg 4200+/-420) and evaporation were assessed. The product met all stability specifications for the duration of the study, supporting 21 months of shelf life.

6. Detection Limit:

   The limit of detection (LOD) of SARS-CoV-2 in InActiv Blue was established through preliminary and confirmatory LOD studies. The LOD was determined for InActiv Blue with nasopharyngeal swab samples, using the Roche cobas SARS-CoV-2 assay (EUA200009) on the cobas 6800 system.

The preliminary LOD was determined by spiking InActiv Blue with nasopharyngeal clinical matrix and SARS-CoV-2 at concentrations of 5, 15, 45, 135, and 405 particles/mL, with each determination performed in triplicate. The LOD was found to be within the range of 5-5 particles/mL.

Table 1. Preliminary LOD Study Results

SARS-CoV-2 spike (particles/mL)	Number of Positive Replicates	Number of Negative Replicates	% Positive
5	1	2	33%
15	3	0	100%
45	3	0	100%
135	3	0	100%
405	3	0	100%

The confirmatory LOD was established by spiking a swab with negative clinical nasopharyngeal matrix containing either 15 or 45 SARS-CoV-2 particles/mL, into InActiv Blue media. A total of 20 replicates were evaluated for each concentration. The nasopharyngeal swabs in InActiv Blue, containing 45 particles/mL, showed that 100% of the replicates were positive, and at a concentration of 15 particles/mL 90% of the replicates were positive.

Table 2. Confirmatory LOD Study Results

SARS-CoV-2 (particles/mL)	Number of Positive Samples	Number of Negative Samples	% Positive
15	19	2	90%
45	21	0	100%

The LOD for nasopharyngeal swab specimen in InActiv Blue was determined to be 45 particles/mL of SARS-CoV-2.

7. Sample Stability Study:
Stability of SARS-CoV-2 in nasopharyngeal specimens stored InActiv Blue was evaluated using product lots of different ages to ensure that there is no decrease in the performance of the product over its claimed shelf-life. Lots of InActiv Blue from different ages (newly manufactured, middle aged and near/recently expired lots), spanning the claimed shelf-life were evaluated. Six replicate samples containing nasopharyngeal matrix were collected and stored in each of the lots of InActiv Blue. Each replicate was spiked with 135 particles/mL (= 3x LOD). Three of the replicates from each lot were stored at 2-8°C while the other 3

replicates were stored at 20-25°C for at least 7 days. Each sample was evaluated using the cobas SARS-CoV-2 assay (EUA200009) on the 6800 system at day 0, day 4 and day 7. All samples collected in the four lots and stored at both temperatures remained within 3 Ct of the initial reading, supporting the sample stability claim for InActiv Blue of 7 days at a temperature between 2-8 and 20-25°C. The following table summarizes the results obtained.

Table 3. SARS-CoV-2 RNA stability after 4 and 7 days of incubation at 2-8°C and 20-25°C

Storage Temperature	Lot manufacturing age	Lot #	Target 1 (ORF 1Ab) Delta Ct's		Target 2 (E gene) Delta Ct's	
			Ct Day4 - Ct Day 0	Ct Day7 - Ct Day 0	Ct Day4 - Ct Day 0	Ct Day7 - Ct Day 0
4°C	new	FP23IB01	-0.12	0.00	0.74	0.80
	middle aged	FP21IB04	0.09	0.20	0.80	0.37
	after expiry date	FP21IB01	-0.36	0.05	0.13	0.12
	stressed	FP21IB01	-0.30	0.84	0.42	0.97
20°C	new	FP23IB01	-0.55	-0.22	-0.87	-0.49
	middle aged	FP21IB04	-0.23	0.47	0.33	0.45
	after expiry date	FP21IB01	0.02	-0.41	1.10	-0.42
	stressed	FP21IB01	-0.27	-0.37	0.63	-0.05

8. Viral Inactivation:

Inactivation was evaluated by measuring the presence of residual infective SARS-CoV-2 viral particles spiked into InActiv Blue. Inactivation studies were conducted in two phases. First a 1:1000 dilution of InActiv Blue was established to eliminate the cytotoxicity of the medium. Higher concentrations of medium were found to render the cultured cells nonviable and unusable for the viral inactivation study. The second phase of viral inactivation evaluated cytotoxicity of SARS-COV-2 spiked into a 1:1000 dilution of the transport media for 1, 2.5 and 5 minutes after inoculation.

The second phase of the study used a high concentration of infectious viral particles ($TCID_{50}$/mL=$10^{7.5}$ or $10^{7.39}$). Briefly, 100 µL of virus stock was mixed 1:1 with InActiv Blue and incubated for 1, 2.5 and 5 minutes. Negative and positive controls were prepared similarly. The virus and InActiv Blue (as well as positive and negative control samples) were then diluted 1:1000 before each replicate was added to cell culture plates containing adherent Vero E6 cells to determine the minimal incubation time required to inactivate virus. Cultures were incubated for several minutes and observed for presence or absence of cytopathic effect and $TCID_{50}$ was calculated by the Reed and Muench method. Virus inactivation was evaluated using 3 lots near the expiration of InActiv Blue (approximately 21 months old). Inactivation performance of InActiv Blue after 1 minute exposure was comparable to 2.5 and 5 minutes of exposure for each of the three batches of InActiv Blue evaluated.

Since the starting viral concentration was 7.5 logs ($TCID_{50}/mL=10^{7.5}$) for two of the lots examined and 7.39 logs ($TCID_{50}/mL=10^{7.39}$) for the third lot, the log infectivity reduction factor was calculated to be at least 3.89 logs for the virus exposed to InActiv Blue medium. Negative control (without virus) demonstrated minimal cellular toxicity and positive control (virus in DMEM) showed no meaningful loss of viral recovery (data not shown). The study results are summarized in Table 4 below.

Table 4: SARS-CoV-2 Viral Inactivation when exposed to InActiv Blue

Lot	exposure time	log (TCID50 control) - log (TCID50 test)	virus log reduction factor
1	1 min	7.50-3.50	4
	2.5 min	7.50-3.50	4
	5 min	7.50-3.50	4
2	1 min	7.50-3.50	4
	2.5 min	7.50-3.50	4
	5 min	7.50-3.50	4
3	1 min	7.39-3.50	3.89
	2.5 min	7.39-3.50	3.89
	5 min	7.39-3.50	3.89

9. Assay Cut-Off:

   Not Applicable (N/A)

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Not Applicable (N/A)

2. Matrix Comparison:
   Not Applicable (N/A)

C Clinical Studies:

1. Clinical Sensitivity:

   Not Applicable (N/A)

2. Clinical Specificity:

   Not Applicable (N/A)

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

   Not Applicable (N/A)

D Clinical Cut-Off:

Not Applicable (N/A)

E Expected Values/Reference Range:
Not Applicable (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.