510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K222771

B Applicant

Hangzhou Bioer Technology Co., Ltd

C Proprietary and Established Names

Sample Preservative Fluid

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 Hangzhou Bioer Technology Co., Ltd Sample Preservative Fluid for the collection, transport, and storage of infectious, unprocessed, upper respiratory specimens suspected of containing Influenza A for downstream testing with nucleic acid-based assay.

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):
Refer to Indications for Use below.

B Indication(s) for Use:

The Sample Preservative Fluid is intended for the stabilization, transportation, and inactivation of infectious, unprocessed, upper respiratory specimens suspected of containing Influenza A virus. Specimens transported in the Sample Preservative Fluid are stable refrigerated (2-8°C) and at room temperature (20-25°C). The Sample Preservative Fluid is suitable for use with compatible legally marketed molecular diagnostic devices.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements:
Not applicable

IV Device/System Characteristics:

A Device Description:
Sample Preservative Fluid is a medium for stabilization of Influenza A RNA during sample transport/storage. The fluid is composed of guanidine thiocyanate, Triton X-100, and nuclease-free water. Sample Preservative Fluid is provided in a labeled screw-cap tube.

Sample Preservative Fluid configuration:

- BSC82X1-A1: a screw-cap tube filled with 2 ml of Sample Preservative Fluid liquid and a prepackaged nasopharyngeal swab for sample collection
- Nasopharyngeal swab: regular size, sterile disposable sample swab (80mm breakpoint)

B Principle of Operation:
Sample Preservative Fluid consists of detergent Triton X-100 and denaturant. The denaturant and detergent denature and/or separate the proteins and nucleases of the virus, then inactivate the virus. The nucleic acid is isolated and preserved in the liquid and can be stored or transported for testing at a laboratory.

V Substantial Equivalence Information:

A Predicate Device Name(s):
PrimeStore MTM

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

C Comparison with Predicate(s):

	Device: K222771	Predicate: DEN170029
Device Trade Name	Sample Preservative Fluid	PrimeStore MTM
General Device Characteristic Similarities		
Intended Use / Indications For Use	The Sample Preservative Fluid is intended for the stabilization, transportation, and inactivation of infectious, unprocessed, upper respiratory specimens suspected of containing Influenza A virus. Specimens transported in the Sample Preservative Fluid are stable refrigerated (2-8°C) and at room temperature (20-25°C). The Sample Preservative Fluid is suitable for use with compatible legally marketed molecular diagnostic devices.	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.
Specimen storage temperature	2-25ºC	2-25ºC
Shelf-life	24 months	24 months
General Device Characteristic Differences		
Microorganism nucleic acids preserved	Influenza A virus	Influenza A virus and *Mycobacterium tuberculosis*
Specimen type	Upper respiratory specimens	Nasal washes and sputum samples
Analyte	RNA	DNA, RNA
Specimen stability	2-25°C ≤ 35 days	For Influenza A virus: 4°C ≤ 29 days 27°C ≤ 8 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:
   Not applicable

2. Linearity:
   Not applicable

3. Analytical Specificity/Interference:
   Not applicable

4. Assay Reportable Range:
   Not applicable

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

   Shelf Life:
   Three lots of Sample Preservative Fluid stored at 2-8°C and 25°C were assessed at 10 timepoints (0, 3, 6, 12, 13, 15, 18, 21, and 24 months). At each timepoint, each kit was evaluated for bacterial or fungal growth, changes in appearance, the tightness of the cap to ensure no leakage, and density of the liquid.

   The study acceptance criteria included the following: there should be no bacterial or fungal growth, no obvious change in appearance, there should be no tube leakage at -0.08 MPa for ten minutes, and the media density should remain 1.06 ± 0.04 g/mL.

   All three lots stored at 2-8°C and 25°C met physical and chemical property evaluation acceptance criteria for all timepoints. There was no bacterial or fungal growth, change in appearance, no leakage, and no change in density over time. The shelf-life of the reagent when stored at 2-25°C is 24 months.

   The Sample Preservative Fluid is not claimed to be sterile nor is it intended to be sterilized by the end user. These vials are single-use devices. The products are packaged in sterile PE bags to ensure the media is not contaminated during shipping.

6. Detection Limit:
   Limit of detection (LoD) testing was conducted to evaluate the lowest concentration of analyte that can be detected at greater than or equal to 95% detection rate.

   Preliminary Limit of Detection (LoD) Study:

   The cleared assay used for the LoD study when testing samples collected in the subject device was the Cepheid Xpert Xpress Flu/RSV Assay (K180218). The LoD of the Xpert Xpress Flu/RSV Assay for Influenza A H3N2 is:
   - Influenza A/Perth/16/2009: 0.01 $TCID_{50}$/mL
   - Influenza A/Victoria/361/2011: 0.75 $TCID_{50}$/mL

   Because the two viral strains used in the Cepheid LoD study were no longer available, another Influenza A H3N2 strain, A/California/2/2014 VR-1938, was used.

   Influenza A H3N2 (A/California/2/2014, VR-1938) was diluted with negative nasal matrix to 0.32 $TCID_{50}$/mL. Serial two-fold dilutions were performed to create samples at 0.16, 0.08, 0.04, 0.02 and 0.01 $TCID_{50}$/mL. Five replicates of each sample were tested for Influenza A with the Xpert Express Flu/RSV Assay. The lowest concentration that yielded a greater than or equal to 80% detection rate was further tested to confirm LoD, table 1 below.

Table 1. Preliminary LoD test results:

Influenza A Concentration ($TCID_{50}$/mL)	Rep 1 (Ct)	Rep 2 (Ct)	Rep 3 (Ct)	Rep 4 (Ct)	Rep 5 (Ct)	Avg (Ct)	SD (Ct)	CV (%)	Detection Rate (%)
0.32	33.4	33.1	34.2	33.2	33.4	33.5	0.39	1.16%	100%
0.16	34.3	34.5	36.9	34.2	34.3	34.8	1.03	2.97%	100%
0.08	35.0	36.1	35.3	35.2	34.5	35.2	0.519	1.47%	100%
0.04	37.3	36.2	39.2	36.8	No Ct	37.4	1.12	3.01%	80%
0.02	38.5	38.7	38.3	No Ct	No Ct	38.5	0.163	0.424%	60%
0.01	37.9	37.3	No Ct	No Ct	No Ct	37.6	0.300	0.798%	40%

Confirmatory Limit of Detection (LoD):
To confirm LoD, additional testing of 20 replicates at 0.04 $TCID_{50}$/mL was performed. When testing of 8 replicates at 0.04 $TCID_{50}$/mL yielded >2 negative results, testing was terminated, and a retest was performed at 0.08 $TCID_{50}$/mL. 100% of replicates at 0.08 $TCID_{50}$/mL were positive confirming the assay LoD as 0.08 $TCID_{50}$/mL, table 2 below.

Table 2. Confirmatory LoD test results:

Replicate	0.08 $TCID_{50}$/mL Influenza A (Ct)
1	34.5
2	35.2
3	36.2
4	35.2
5	35.0
6	35.1
7	36.5
8	35.4
9	35.6
10	35.1
11	35.2
12	35.8
13	34.8
14	35.2
15	35.3
16	36.4
17	35.1
18	35.2
19	35.6
20	34.7
Average	35.4
SD	0.517
CV (%)	1.46%
Detection Rate (%)	100%

7. Specimen Stability:
A specimen stability study was conducted to evaluate the stability of Influenza A virus RNA spiked into negative matrix and stored in Sample Preservative Fluid at both a refrigerated temperature (2-4°C) and at room temperature (20-25°C) for 35 days. Influenza A H3N2

(A/California/2/2014, VR-1938) was diluted to 3x the LoD (0.24 $TCID_{50}$/mL) with negative nasal matrix collected with three reagent lots. Samples collected with each of the three lots were tested in replicates of four with the Xpert Xpress Flu/RSV Assay on Day 0. Samples were stored at refrigerated (2-4°C, table 3 below) and room temperature (20-25°C, table 4 below) and tested in replicates of four on Days 1, 8, 15, 22, and 35.

Table 3. Influenza A stability 2-4°C:

Specimens	Lot	Rep	Day0 (Ct)	Day1 (Ct)	Day8 (Ct)	Day15 (Ct)	Day22 (Ct)	Day35 (Ct)
0.24 $TCID_{50}$/mL Influenza A in nasal matrix	1	1	33.5	33.1	33.4	33.9	33.6	35.6
		2	33.5	34.0	33.7	34.0	33.9	34.3
		3	33.0	33.4	33.4	33.5	34.3	33.9
		4	33.4	34.8	33.9	33.3	33.9	34.0
	2	1	33.4	33.2	33.5	33.8	33.5	34.3
		2	34.0	33.4	33.4	33.9	34.0	34.4
		3	33.5	33.5	33.9	33.7	33.5	34.1
		4	33.9	33.3	33.8	33.5	34.0	33.7
	3	1	33.7	33.6	33.8	33.5	33.8	34.2
		2	33.7	33.3	33.7	33.6	33.8	33.7
		3	33.5	33.2	34.0	33.6	33.7	34.2
		4	33.3	33.5	33.7	33.7	33.7	34.4
	Average		33.5	33.5	33.7	33.7	33.8	34.2
	SD		0.256	0.446	0.203	0.197	0.222	0.473
	CV (%)		0.764%	1.33%	0.604%	0.586%	0.655%	1.38%

Table 4. Influenza A stability 20-25°C:

Specimens	Lot	Rep	Day0 (Ct)	Day1 (Ct)	Day8 (Ct)	Day15 (Ct)	Day22 (Ct)	Day35 (Ct)
0.24 $TCID_{50}$/mL Influenza A in nasal matrix	1	1	34.0	34.1	34.3	33.6	33.4	33.9
		2	34.0	33.9	33.3	33.9	33.9	34.7
		3	33.7	33.3	34.0	33.9	33.9	34.1
		4	33.5	33.9	34.3	34.2	33.7	34.4
	2	1	33.8	33.3	33.6	33.5	33.6	34.0
		2	33.9	33.2	33.4	34.0	34.0	33.4
		3	33.4	33.4	34.5	33.4	33.9	33.7
		4	33.7	33.5	33.4	33.6	33.4	34.1
	3	1	34.0	33.4	33.6	33.3	33.7	34.1
		2	33.5	33.0	33.7	34.3	34.0	35.2
		3	34.0	33.5	33.8	34.2	33.6	34.2
		4	34.3	33.1	33.6	34.1	33.7	34.0
	Average		33.8	33.5	33.8	33.8	33.7	34.2
	SD		0.254	0.325	0.379	0.180	0.201	0.443
	CV (%)		0.752%	0.971%	1.12%	0.534%	0.597%	1.30%

At each timepoint, Influenza A spiked into nasal matrix and preserved at refrigerated temperature (2-4°C) or room temperature (20-25°C) yielded positive results, which showed the Influenza A H3N2 nucleic acid was detected.

All the Ct values fell within a 3.0 Ct range of results from the Ct values generated for time point zero, which met the pre-defined acceptance criteria. The Influenza A H3N2 virus nucleic acid was preserved in the Sample Preservative Fluid without degradation at refrigeration (2-4°C) and room (20-25°C) temperatures for 35 days.

8. Viral Inactivation Study:
An inactivation study was performed to determine the rate the Sample Preservative Fluid inactivates Influenza A.

Cytotoxicity Study:
A cytotoxicity study was performed to determine at what dilution ratio the Sample Preservative Fluid would not be toxic to a cell monolayer.

A preliminary test was performed by diluting the Sample Preservative Fluid with complete cell culture medium at multiple dilutions (from 1:10 up to 1:8000) and adding it to a monolayer of cells. When the Sample Preservative Fluid dilution ratio exceeded 1:4000 both the test group and the control group showed normal cell morphology and growth, demonstrating that there was no significant cytotoxicity for the Sample Preservative Fluid with high dilution ratios.

A confirmatory test was performed by diluting the Sample Preservative Fluid with complete cell culture medium at multiple dilution (from 1:1000 to 1:10000) and adding it to a monolayer of cells. When the dilution ratio reached or exceeded 1:3500, both the test group and the control group showed normal cell morphology and growth status, and no significant difference between the test group and the control group were observed.

Conclusion: When the dilution ratio reached or exceeded 1:3500, both the test group and the control group showed normal cell morphology and growth status, and no significant difference between the test group and the control group were observed. There was no toxicity to the MDCK cell monolayer when the Sample Preservative Fluid was diluted to equal to or greater than 3500 times.

Inactivation Study:
To evaluate successful inactivation of Influenza A virus, Influenza A H3N2 virus was combined at a ratio of 1:1 with negative nasal matrix mixed with Sample Preservative Fluid and incubated for 30 and 60 seconds. The initial concentration of the Influenza A H3N2 was $>1x10^7$ $TCID_{50}$/mL. Each mixture was then diluted 3500x (as determined in the cytotoxicity study) with complete cell culture medium. Each dilution was plated into 8 wells of a 96-well cell culture plate in triplicate and incubated for 3-4 hours. The medium was removed and replaced with 100µL of complete cell culture medium. The plate was incubated at 34°C and 5% CO_2 atmosphere for 4-7 days. The plate was observed for cytopathic effects and virus titers were calculated.

Additionally, positive controls containing Influenza A H3N2 virus and sterile saline were mixed at a ratio of 1:1 and incubated for 30 and 60 seconds. Each mixture was then diluted to

3500x (as determined in the cytotoxicity study) with complete cell culture medium. Each dilution was plated into 8 wells of a 96-well cell culture plate in triplicate and incubated for 3-4 hours. The medium was removed and replaced with 100µL of complete cell culture medium. The plate was incubated at 34°C and 5% CO_2 atmosphere for 4-7 days. The plate was observed for cytopathic effects and virus titers were calculated.

Negative controls were also evaluated as part of this study in which MDCK cells were incubated with complete cell culture medium 34°C and 5% CO_2 atmosphere for 4-7 days. The plate was observed for cell growth and culture contamination.

No cytotoxicity was observed in the cell monolayer after exposure to Influenza A virus samples incubated in Sample Preservative Fluid for 30 or 60 seconds. The result was a greater than a ≥4.7 logarithmic reduction in Influenza A after a 30 and 60 seconds in the Sample Preservative Fluid. Therefore, the Sample Preservative Fluid Inactivated Influenza A H3N2 in MDCK cells and the device demonstrated a viral inactivation ration of >99.99%. Cytotoxicity was observed in the positive control group, and no viral infection was observed in the negative control group.

9. Assay Cut-Off:
   Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:
   Not applicable

2. Matrix Comparison:
   Not applicable

C Clinical Studies:

1. Clinical Sensitivity:
   Not applicable

2. Clinical Specificity:
   Not applicable

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

D Clinical Cut-Off:
Not applicable

E Expected Values/Reference Range:
Not applicable

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.