510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K220059

B Applicant

Shenzhen Dakewe Bio-engineering Co., Ltd.

C Proprietary and Established Names

Biosci Inactivated Transport Medium, Biosci ITM

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 Biosci Inactivated Transport Medium for the collection, inactivation, stabilization, and transport of upper respiratory specimens to the laboratory for downstream testing using molecular assays.

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:

Biosci Inactivated Transport Medium (Biosci ITM) is intended for the collection, inactivation, stabilization and transportation of an unprocessed upper respiratory clinical specimen suspected of containing influenza A virus RNA from the collection site to the testing laboratory. The specimen collected in Biosci ITM is suitable for use with compatible molecular assays.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

None

IV Device/System Characteristics:

A Device Description:

Biosci ITM contains a detergent and a protein denaturant to inactivate influenza A, lyse cells, disrupt lipid membranes, denature proteins and enzymes, and preserve and stabilize influenza A RNA. Therefore, Biosci ITM is not intended to be used for culture-based techniques. A specimen bag is also provided for safe transportation of specimens, as well as providing appropriate biosafety warning.

Biosci ITM has different configurations:

- A screw-cap tube filled with 1.0, 2.0, or 3.0 mL of Biosci ITM.
- A screw-cap tube filled with a range of media and package with a nasopharyngeal swab for nasopharyngeal specimen collection.
- A screw-cap tube filled with a range of media and package with an oropharyngeal swab for oropharyngeal specimen collection.
- A screw-cap tube filled with a range of media and package with a mid-turbinate swab for mid-turbinate specimen 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 14 days. The media contains the following reagents:

- Guanidine hydrochloride
- EDTA disodium salt dihydrate
- Trisodium citrate dihydrate
- Tris
- TCEP
- HCl
- Antifoam A Concentrate
- NP-40
- Distilled water

V Substantial Equivalence Information:

A Predicate Device Name(s):

eNAT molecular collection and preservation medium

B Predicate 510(k) Number(s):

K201849

C Comparison with Predicate(s):

Device & Predicate Device(s):	Device: K220059	Predicate: K201849
Device Trade Name	Biosci Inactivated Transport Medium	eNAT molecular collection and preservation medium
Intended Use/Indications For Use	Biosci Inactivated Transport Medium (Biosci ITM) is intended for the collection, inactivation, stabilization and transportation of an unprocessed upper respiratory clinical specimen suspected of containing influenza A virus RNA from the collection site to the testing laboratory. The specimen collected in Biosci ITM is suitable for use with compatible molecular assays.	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.
General Device Characteristic Similarities		

Single use device	Yes	Same
Specimen types	Upper respiratory specimens	Same
Microorganism nucleic acids preserved	Influenza A virus	Same
Container	Tube; plastic; conical bottom self-standing with a screw cap	Same
Shelf-life	18 months	Same
General Device Characteristic Differences		
Media formulation	-Guanidine hydrochloride -EDTA disodium salt dihydrate -Trisodium citrate dihydrate -Tris -TCEP -HCl -Antifoam A Concentrate -NP-40 -Distilled water	-Tris-EDTA -Guanidine thiocyanate -Detergent -HEPES -Distilled water
Media volume	1, 2, and 3 mL	2 mL
Swabs	Nylon flocked swabs (oropharyngeal, nasopharyngeal, mid-turbinate)	Nylon flocked swabs (oropharyngeal and mid-turbinate)
Specimen stability	Up to 14 days at 2-25°C	Up to 28 days at 2-25°C
Storage temperature	18-25°C	2-25°C

VI Standards/Guidance Documents Referenced:

1. ISO 11737-1 Third edition 2018-01 *Sterilization of health care products - Microbiological methods - Part 1: Determination of a population of microorganisms on product*
2. ISO 11137-2 Third edition 2013-06-01 *Sterilization of health care products - Radiation - Part 2: Establishing the sterilization dose*

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

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

Shelf-life:

a. The shelf life for the Biosci ITM is 18 months, after the date of manufacture, when stored at room temperature (18-25ºC). The stability of the Biosci ITM was performed using Realtime stability on a total of three (3) lots. Stability studies included assessment of the bacteriostatic effect in the media, media properties including, appearance, and pH.
b. The shelf life for the nasopharyngeal, oropharyngeal, and mid-turbinate Disposable Sterile Swab is 18 months after the date of manufacture. The stability of the Disposable Sterile Swab was performed using ongoing Realtime stability on a total of three (3) lots for each swab type stored at room temperature (18-25ºC). Stability looked for microbial barrier system, appearance, mechanical properties and sterility.

Sterilization: The Biosci ITM tube and media are not sold as sterile nor are they intended to be sterilized by the user. These vials are single use devices. The appropriate Disposable Sterile Swab type (which may be included with the media) are sterilized according to ISO 11737-1 and ISO 11137-2 via e-beam irradiation to achieve a Sterility Assurance Level of 10^{-6}. Therefore, the Disposable Sterile Swabs are individually packaged and sold as sterile.

2. Detection Limit:

a. Limit of Detection Study:

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 a validated RT-PCR assay to establish a concentration of Influenza A used for additional testing noted below.

LoD testing was initially performed by spiking Influenza A (Flu A, H1N1 ATCC VR-1736 A/Virginia/ATCC1/2009; original concentration is 1.2×10^8 $TCID_{50}$/mL) into negative pooled nasopharyngeal clinical matrix. A 50 µL volume of the Flu A/matrix mixtures, of various concentrations, were individually added to each sterile swab that were then eluted into 450 µL Biosci ITM to yield concentrations of 5.0, 1.0, 0.20, 0.04 and 0.008 $TCID_{50}$/mL. A negative control was created by adding 50 µL negative nasopharyngeal matrix onto a sterile swab that was then eluted into 450 µL Biosci ITM to obtain a Flu A-free sample. Each concentration was analyzed in 4 replicates. A validated RT-PCR assay was used to determine the preliminary LoD to be 0.2 $TCID_{50}$/mL for Flu A. No detection ($Ct \geq 40$) was observed at less than 0.2 $TCID_{50}$/mL concentration. All other higher concentrations demonstrated recovery of Flu A. Table 1 below shows the results of preliminary LoD testing for Flu A.

Table 1: Preliminary LoD summary for Flu A

Influenza A Concentration ($TCID_{50}$/mL)	Biosci ITM 4 Reps Mean (Ct)	SD (Ct)	% Detection
5.0	29.18	0.19	100
1.0	32.54	0.53	100
0.20	35.64	0.48	100

0.04	>40	0	0
0.008	>40	0	0
0	>40	0	0

Confirmatory LoD testing was then performed using Flu A samples prepared as described above to final concentrations of 1.0, 0.2, 0.04, and 0.008 $TCID_{50}$/mL. A total of 24 replicates were analyzed for each concentration and 100% of replicates were detected for Flu A at 1.0 and 0.2 $TCID_{50}$/mL and met the pre-defined acceptance criteria. The LoD was confirmed as 0.2 $TCID_{50}$/mL and is considered the LoD of the assay. The data is presented in table 2 below.

Table 2: Confirmatory LoD summary for Flu A

	Ct Values*			
	1.0 $TCID_{50}$/mL	0.2 $TCID_{50}$/mL	0.04 $TCID_{50}$/mL	0.008 $TCID_{50}$/mL
24 Replicates	32.42	36.2	>40	>40
	32.08	35.84	>40	>40
	32.15	34.26	>40	>40
	31.99	35.70	>40	>40
	32.27	36.28	>40	>40
	32.32	35.79	>40	>40
	32.15	36.68	>40	>40
	32.31	35.68	>40	>40
	32.27	34.73	>40	>40
	32.11	35.16	>40	>40
	32.20	35.12	>40	>40
	32.01	35.09	>40	>40
	32.35	36.30	>40	>40
	32.09	35.84	>40	>40
	32.21	36.29	38.68	>40
	31.81	36.20	>40	>40
	31.99	36.02	>40	>40
	31.99	36.50	>40	>40
	32.08	35.52	>40	>40
	32.16	36.35	>40	>40
	32.25	36.16	>40	>40
	32.06	34.83	>40	>40
	31.79	36.99	>40	>40
	32.33	35.49	>40	>40
AVG (Ct)	32.14	35.79	>40	>40
SD (Ct)	0.16	0.67	N/A	N/A
% Detection	100	100	0	0

*Ct value of <37 is defined as positive result and > 40 is defined as no detection in this assay.

b. Viral Nucleic Acid Stability Study:

The stability of influenza A virus (Flu A, H1N1 ATCC VR-1736) at 5X the LoD of 0.2 $TCID_{50}$/mL (i.e., test concentration at 1.0 $TCID_{50}$/mL) was evaluated by spiking virus into negative clinical nasopharyngeal matrix. A 50 µL volume of the virus/matrix mixture was then inoculated onto sterile swabs that were then incubated in 450 µL Biosci ITM at

ambient temperature (25°C) for 28 days, and refrigerated temperature (2-8°C) for 28 days. A negative control was created by adding 50 µL negative nasopharyngeal matrix onto a sterile swab that was also incubated in 450 µL Biosci ITM to obtain a Flu A-free sample. A validated RT-PCR assay was used to determine the ability of the media to stabilize Flu A nucleic acids in Biosci ITM. The stability study analyzed a total of six lots; two lots five months post expiration date, two lots within the validity period (Biosci ITM has a claimed 18-month stability), and two newly manufactured lots. Four tubes from each lot were tested at each time point 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 four time points was performed at Day 0, 7, 14 and 28 for refrigerated temperature (2-8°C) and ambient temperature (25°C) to support a claim of Flu A nucleic acid stability for 14 days. A pre-defined acceptance criteria of (+/-) 3.0 Ct from the initial time zero value and SD <1 were the acceptance criteria. The data for the 14 days stability claim is shown below for ambient temperature (25ºC; Table 3) and refrigerated temperature (2-8ºC; Table 4).

Table 3: Flu A stability at 25°C

Days (25°C)	**0**	**7**	**14**
AVG (Ct):	32.20	32.03	31.55
Variation (Ct):	-	-0.17	-0.65
SD (Ct):	0.31	0.23	0.28

Table 4: Flu A stability at 2-8°C

Days (2-8°C)	**0**	**7**	**14**
AVG (Ct):	32.26	31.97	31.58
Variation (Ct):	-	-0.29	-0.68
SD (Ct):	0.22	0.29	0.31

The results indicate that the RNA from Flu A whole virus spiked into nasopharyngeal matrix and stored in Biosci ITM resulted in a maximum variation of 0.65 Ct for 14 days at 25°C and a maximum variation of 0.68 Ct for 14 days at 2-8°C, indicating no effect of media age on the ability of Biosci ITM to stabilize Flu A nucleic acid. At each time point and each temperature, all average Ct values measured resulted in a difference < 3.0 Ct and all SD values are < 1, when compared to day 0, which indicated that the results met the pre-defined acceptance criteria and supports a claim for Flu A viral RNA stability in Biosci ITM for 14 days. Additionally, data indicate that Flu A plus matrix, stored at the indicated temperatures and time points, in the absence of Biosci ITM, could not be detected post day 0. All negative control had no Flu A nucleic acid detection.

c. *Inactivation Study:*

Test group samples were created with Influenza A live virus (Flu A, H1N1 ATCC VR-1736), diluted in negative nasopharyngeal matrix, to a final concentration of 1.2×10^9, 1.2×10^8, and 1.2×10^7 $TCID_{50}$/ml. A 50 µL volume of each virus concentration from the

test group was added to individual sterile swabs that were then incubated in 450 µL Biosci ITM for 10, 20, or 30 seconds at room temperature (1:10 dilution). As negative controls, Biosci ITM and negative nasopharyngeal matrix were individually diluted 1:1000 with DMEM. As positive controls, Flu A only or Flu A added to negative nasopharyngeal matrix to reach each of the concentrations above, were each then further diluted 1:10,000 with DMEM. Negative and positive control samples were added directly to MDCK cells without incubation. After the test group was incubated at the times and temperature above, all samples were then individually diluted 1:1000 with DMEM and inoculated onto MDCK cells at three replicates each and incubated for four days at 37ºC. After incubation, 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). Therefore, CPE was considered a measure of viral viability.

The titer of the virus CPE was calculated and recorded as the $TCID_{50}$. When no CPE was observed or observed at a level below a set threshold, that was taken as an indication of effective virus inactivation.

Inactivation time:
The Biosci ITM showed no cytotoxicity on MDCK cells at a 1:1,000 dilution factor with DMEM culture media; therefore at least a 1:1,000 dilution factor is needed to avoid a direct effect on cellular integrity caused by the Biosci ITM which would prevent appropriate evaluation and interpretation. Influenza A was therefore exposed to Biosci ITM for 10, 20, and 30 seconds, at room temperature, prior to 1:1000 dilution with DMEM and incubation with MDCK cells (final concentrations of 1.2×10^5, 1.2×10^4, and 1.2×10^3 $TCID_{50}$/mL). Positive controls showed cytopathic effect (CPE) on MDCK cells and negative controls did not (Table 5). The Biosci ITM rapidly inactivated Influenza A virus with a >4.0 log reduction at a 1:10 specimen to inactivation media concentration at 10 seconds (Table 6). Viral CPE was not observed at $<10^3$ $TCID_{50}$/mL due to viral destruction by the Biosci ITM, see Table 6 below. Also as noted in the table, CPE was still present in samples with starting viral concentrations of $\geq 10^9$ $TCID_{50}$/mL. Although reduced by ≥5 logs by the Biosci ITM, CPE was observed because this high viral concentration is outside the working range of the cell culture-based assay and the effect of the inactivation media can no longer be adequately assessed. Data from the lower viral concentrations are used to assess the inactivation effect and was acceptable.

Table 5: Flu A Inactivation Study Data Summary for Control Groups

	Preincubation ($TCID_{50}$)	Presence of CPE
Flu A only	1.2×10^5	Yes
	1.2×10^4	Yes
	1.2×10^3	Yes
Flu A and matrix	1.2×10^5	Yes
	1.2×10^4	Yes
	1.2×10^3	Yes
Matrix only	-	No
Biosci ITM only *	-	No

*Biosci ITM showed no cytotoxicity on MDCK cells when diluted to 1:1,000.

Table 6: Flu A Inactivation Study Data Summary at Room Temperature

Sample	Starting Flu A concentration	10s incubation (TCID50 LogΔ）	20s incubation (TCID50 LogΔ)	30s incubation (TCID50 LogΔ)	Presence of CPE
Flu A, matrix and Biosci ITM	1.2×10^9	≥-5.0	≥-5.0	≥-5.0	Yes
	1.2×10^8	>-5.0	>-5.0	>-5.0	No
	1.2×10^7	>-4.0	>-4.0	>-4.0	No

*Biosci ITM showed no cytotoxicity on MDCK cells when diluted to 1:1,000.

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

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.