510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K212878

B Applicant

Zhejiang GENE SCIENCE Co., Ltd.

C Proprietary and Established Names

Sample preservation solution

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 Zhejiang GENE SCIENCE Co., Ltd.'s Sample Preservation Solution for the collection, inactivation, preservation/ stabilization, and transport of unprocessed upper respiratory clinical specimens containing influenza A virus.

B Measurand:

Inactivation of viral particles and stabilization of nucleic acids from influenza A virus upon storage in the subject device.

C Type of Test:

Microbial Inactivation and Nucleic Acid Stability assays

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Zhejiang GENE SCIENCE Co., Ltd.'s Sample Preservation Solution is intended for the collection, inactivation, preservation/stabilization, and transport of unprocessed upper respiratory clinical specimens from individuals suspected of influenza A infection by their healthcare provider. Specimens collected with the Sample Preservation Solution can be stored and transported at 4 ºC or at room temperature (20-25ºC) for preservation of microbial nucleic acid until the sample is processed and used with compatible molecular diagnostic assays.

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:

The Zhejiang GENE SCIENCE Co., Ltd.'s Sample Preservation Solution consists of a plastic, polypropylene screw-cap collection tube filled with non-sterile sample preservation medium. The tubes are pre-filled with either 2 mL (suitable for swabs with flocking length of 20-24 mm) or 3 mL (suitable for swabs with flocking length of 24-30 mm) of solution. The device is non-sterile, singe use. Swabs are not included.

The Zhejiang GENE SCIENCE Co., Ltd.'s Sample Preservation Solution is offered in the following configurations:

SKU or Catalog Number	Device Description	Pack Size
YA-02	2.0 mL/tube	100pcs/Tray, 15 Trays/Box
YA-03	3.0 mL/tube	100pcs/Tray, 15 Trays/Box

B Principle of Operation:

The Zhejiang GENE SCIENCE Co., Ltd.'s Sample Preservation Solution consists of the following: Guanidine isothiocyanate, EDTA, Tris-HCl, Yeast RNA, TWEEN-20, Phenol red, and *in vitro* diagnostic grade water. The guanidine isothiocyanate lyses cells, denatures proteins, and inhibits nuclease activity, thereby stabilizing nucleic acids. EDTA is a chelating agent that deactivates RNAse and stabilizes RNA. Tris-HCl is a pH buffer system to maintain reagent pH. Yeast RNA serves as blocking agent, protects RNA, helps coprecipitating microbial nucleic acid. TWEEN-20 is a detergent that disrupts membranes and denatures proteins. Phenol red is a pH

indicator which serves as a visual quality control mechanism. The *in vitro* diagnostic grade water serves as a diluent to adjust molarity of the buffer/preservation medium.

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):	Subject Device: K212878	Predicate: K201849
Device Trade Name	Sample Preservation Solution	Copan eNAT Molecular Collection and Preservation Medium
General Device Characteristic Similarities		
Intended Use/Indications For Use	The Zhejiang GENE SCIENCE Co., Ltd.'s Sample Preservation Solution is intended for the collection, inactivation, preservation/stabilization, and transport of unprocessed upper respiratory clinical specimens from individuals suspected of influenza A infection by their healthcare provider. Specimens collected with the Sample Preservation Solution can be stored and transported at 4 ºC or at room temperature (20-25ºC) for preservation of microbial nucleic acid until the sample is processed and used with compatible molecular diagnostic assays.	Copan eNAT is intended for the collection, inactivation and transport of clinical specimens containing influenza A viruses from the collection site to the testing laboratory. eNAT can be processed and used with compatible molecular assays that require stabilization of nucleic acids from influenza A viruses.
Specimen Type	Upper respiratory specimens	Same

Analyte tested	Influenza A	Same
Single Use Device	Yes	Same
General Device Characteristic Differences		
Ingredients	Guanidine isothiocyanate EDTA Tris-HCl Yeast RNA TWEEN-20 Phenol red *In Vitro* Diagnostic grade water	Tris-EDTA Guanidine thiocyanate Detergent HEPES Distilled water
Specimen stability	Preserves influenza A RNA for up to 6 days at 4-25 ºC	Preserves influenza A RNA for up to 28 days at 2-25°C
Inactivation of influenza A	>6.2 log reduction in concentration at 5 minutes	>4.0 log reduction in concentration at 10 seconds
Shelf-life	24 months	18 months

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 3 mL specification of the Zhejiang GENE SCIENCE Co., Ltd.'s Sample Preservation Solution device were used to conduct the following studies to evaluate physical characteristics. The product was assessed qualitatively at each time point to determine agreement with specifications for appearance (packaging has good seal, no damage, leakage, or deformation; the label is clean, clear, and complete; the color of the reagent is consistent, no precipitation, no impurities). Additionally, pH and evaporation were assessed to confirm the pH of (7.3+ 0.2) and a limit of evaporation up to ≥97% of the loading volume. The product met these acceptance criteria under all conditions.

The shelf-life of the Zhejiang GENE SCIENCE Co., Ltd.'s Sample Preservation Solution was assessed by storing three lots of the product at room temperature (20-25 ºC) for 0, 1, 3, 6, 12, 15, 18, 21, and 24 months in their Finished Product warehouse to test the real storage environment. At each time point, appearance, pH and loading volume were assessed for each product lot in triplicate and test results met the quality requirements. The Zhejiang GENE SCIENCE Co., Ltd.'s Sample Preservation Solution results after 24 months of storage showed that the actual volume was no less than 97% of the theoretical volume, the appearance was acceptable, and the pH was 7.3+ 0.2. There was no leakage or deformation, showing that the product met the predefined quality standards.

6. Detection Limit:
To determine the Limit of Detection (LoD) of H1N1 influenza A virus stored in the Sample preservation solution, an LoD study was conducted using a validated RT-PCR assay. In brief, a serial dilution of contrived H1N1 influenza A virus samples were prepared by spiking the virus into human upper respiratory matrix and Sample Preservation Solution into five groups of final concentration (10^5, 10^4, 10^3, 10^2 and 10 copies/mL). Each concentration was tested in 4 replicates to establish the pre-liminary LoD. Samples were extracted with the Qiagen RNeasy Mini Kit and RT-PCR was performed using the Invitrogen SuperScript III Platinum One-Step Quantitative Kit on the ThermoFisher ABI 7500 Instrument. The preliminary LoD was determined to be 10^2 copies/mL and confirmed by performing 20 replicates with a 100% positivity agreement, average Ct value was 30.9 with a SD of 0.94. (**Table 1-2**).

Table 1: Preliminary LoD Determination

Replicates	**Concentration (copies/mL) (Ct <40)**				
	10^5	10^4	10^3	10^2	10^1
1	27	28	31	33	40
2	24	28	31	32	40
3	25	27	32	37	42
4	24	29	31	35	39
Total Positive	4/4	4/4	4/4	4/4	1/4

Table 2: Confirmation of LoD

ThermoFisher ABI 7500 Instrument (10^2 copies/mL)	Influenza A (Ct<40)	Interpretation	Total Positive/Tested
1	31	Positive	20/20
2	32	Positive	
3	31	Positive	
4	31	Positive	
5	31	Positive	
6	30	Positive	
7	31	Positive	
8	30	Positive	
9	31	Positive	
10	30	Positive	
11	31	Positive	
12	32	Positive	
13	31	Positive	
14	31	Positive	
15	32	Positive	
16	31	Positive	
17	31	Positive	
18	29	Positive	
19	29	Positive	
20	33	Positive	
Mean	30.9		
SD	0.94		

7. Nucleic Acid Sample Stability:

To determine nucleic acid stability of the Zhejiang GENE SCIENCE Co., Ltd.'s Sample Preservation Solution, three lots of Sample Preservation Solution of varying ages (one month, and two various age lots near expiration) were tested. Contrived swabs samples were prepared by spiking with 3 x LoD influenza A in negative upper respiratory swab matrix. Triplicate contrived swab samples were immersed into the Sample Preservation Solution and incubated for time 0, 3 day and 6 days at 4±1°C and room temperature (20-25°C) for each lot of Sample Preservation Solution. At the end of each time point samples were extracted with the Qiagen RNeasy Mini Kit and RT-PCR testing was performed using the Invitrogen SuperScript III Platinum One-Step Quantitative Kit on the ThermoFisher ABI 7500 Instrument.

Average Ct values were calculated per lot at each time point. Comparison of values obtained at each time point to the average T_0 values indicated similar results regardless of the lot age. Overall, when results of all lots were combined a decrease in the Ct values of 0.43 and of 2.76 Ct was observed for the samples stored at 4±1°C for 3 and 6 days, respectively (**Table 3**). For the samples stored at room temperature (20-25°C), an increase in the Ct values of 0.57 and 0.44 was observed for the samples stored for 3 and 6 days, respectively, meeting the pre-defined acceptance criteria of (+/-) 3.0 Ct from the initial time zero value (**Table 3**).

Table 3: Influenza A nucleic acid Stability in Sample Preservation Solution*

Time	4 °C		Room temperature (20-25 °C)	
	Average Ct (Mean ± SD)	Average Δ Ct from T_0	Average Ct (Mean ± SD)	Average Δ Ct from T_0
0	35.43 ± 0.38	N/A	35.43 ± 0.75	N/A
3 Day	35.0 ± 0	-0.43	36.0 ± 0.73	0.57
6 Day	32.67 ± 0.35	-2.76	35.87± 1.8	0.44

*Data for all age lots were combined since no differences were noted.

Overall, the nucleic acid study results support the ability of Zhejiang GENE SCIENCE Co., Ltd.'s Sample Preservation Solution to stabilize the Influenzas A RNA up to 6 days at 4°C and room temperature (20-25°C)

8. Viral Inactivation:
A viral inactivation study was conducted to test the ability of GENE SCIENCE Co., Ltd.'s Sample Preservation Solution to inactivate Influenza A virus.

   a. **Cytotoxicity:** To determine cytotoxicity of Zhejiang GENE SCIENCE Co., Ltd. Sample Preservation Solution, three (3) lots at varying ages were examined. Each sample preservation solution was serially diluted with diluent to create 2, 4, 8, 16, 32, 64, and 128-fold dilutions. Each dilution was inoculated to cell monolayers in triplicate and examined for Cytopathic Effect (CPE). The lowest dilution to indicate normal cell growth (i.e., no cytotoxicity) was determined to be the 64-fold dilution.

   b. **Viral Inactivation:** To determine the degree of viral inactivation produced by the Zhejiang GENE SCIENCE Co., Ltd. Sample Preservation Solution, three (3) lots of varying ages were examined. Upper respiratory matrix in saline was spiked with $\geq 10^7$ copies per mL of influenza A virus (A/PR/8/34 H1N1) in a 1:1 suspension to create spiked upper respiratory swab samples for the following three groups.

      i. **Test Group (C_t):** Using a new swab, spiked upper respiratory swab samples (~0.08 mL) were inserted into the collection device with 3mL of Sample Preservation Solution. They were then exposed for 0, 5, and 10 minutes. At the end of the exposure, samples were diluted 1:64 which was determined not to be toxic to the cell culture monolayer, inoculated onto the host cell monolayers, incubated 7 days, observed daily for CPE. The remaining infectivity (log $TCID_{50}$) was calculated and corrected using a dilution factor to account for the 1:64 dilution of Sample Preservation Solution to the diluent Medium to avoid cytotoxicity (dilution factor is 1.81 log) and the initial dilution of 0.08 ml into 3.08 ml (dilution factor is 1.58 log).

      ii. **Positive Control Group (C_0):** Using a new swab, spiked upper respiratory samples (~0.08 mL) were inserted into a collection tube containing 3 mL of diluent (0.85% saline). They were then exposed for 0, 5, and 10 minutes. At the end of the exposure, samples were diluted 1:64 which was determined not to be toxic to the cell culture monolayer, inoculated onto the host cell monolayers,

incubated 7 days, and observed daily for CPE. Log $TCID_{50}$ was calculated and corrected using the dilution factors described above.

iii. **Negative Control Group (C_n)**: Upper respiratory swab matrix only samples (~0.08 mL) were inserted into the collection device with 3 mL Sample Preservation Solution and exposed for 0, 5, and 10 minutes. At the end of the exposure, samples were diluted 1:64 which was determined not to be toxic to the cell culture monolayer, inoculated onto the host cell monolayers, incubated 7 days, observed daily for CPE. No CPE was observed, confirming that he 1:64 dilution eliminated the cell toxicity produced by the Sample Preservation Solution.

Using the Inactivation (log reduction) formula below, Influenza A viral inactivation rate of Zhejiang GENE SCIENCE Co., Ltd's. Sample Preservation Solution was determined to be >6.2 log reduction at 5 minutes which was equivalent to greater than 99.99% inactivation Influenza A virus (**Table 4**).

Inactivation (log reduction): Mv = log (C0/Ct) = log(C0)-log (Ct)

Table 4: Influenza A Viral Inactivation in Sample Preservation Solution

Time (min)	Inactivation (log reduction, Mv)		
	Lot#1	Lot#2	Lot#3
0	>6.97	>6.97	3.28
5	>6.20	>6.20	>6.20
10	>6.75	>6.75	>6.75

Conclusion:
Zhejiang GENE SCIENCE Co., Ltd's. Sample Preservation Solution inactivates Influenza A virus when incubated for at least 5 minutes.

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.