510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

I Background Information:

A 510(k) Number

K210440

B Applicant

Wuxi Nest Biotechnology Co., Ltd.

C Proprietary and Established Names

Disposable Sampler Inactivated Transport Media, Nest 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 NEST ITM for the collection, transport and storage of viral specimens to the laboratory for downstream testing.

B Measurand:

Storage and stability of nucleic acids from adenovirus, influenza A virus or parainfluenza virus 2

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:

NEST ITM is an enclosed system intended for the collection, inactivation, stabilization and transportation of pharyngeal and nasal swabs suspected of containing adenovirus, influenza A virus or parainfluenza virus 2 from the collection site to the testing laboratory. The specimen transported in NEST ITM can be used for molecular detection in the laboratory.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

None

IV Device/System Characteristics:

A Device Description:

NEST ITM is an enclosed system intended for the collection, inactivation stabilization and transportation of swabs suspected of containing adenovirus, influenza A virus or parainfluenza virus 2 from the collection site to the testing laboratory. The specimen transported in NEST ITM can be used for molecular detection in the laboratory.

The preservation media tubes are made of polypropylene and are filled with Inactivated Transport Media. The tubes are packaged with or without sterile swabs. The specifications for the tubes and medium are listed as follows:

- A plastic screw-cap tubes filled with 1.0, 1.5, 2.0, 3.0 or 6.0 mL of medium
- A plastic screw-cap tube filled with a range of media and package with a nasopharyngeal swab
- A plastic screw-cap tube filled with a range of media and package with an oropharyngeal swab

B Principle of Operation:

The device components are intended to inactivate influenza A virus, parainfluenza virus 2, lyse cells, disrupt/lyse lipid membranes, denatures proteins, inactivates enzymes, and stabilize viral RNA. The transport device is designed for storage of specimens between 2-8°C and 25 °C (for up to 15 days.

The media contains the following reagents:

- Guanidine Isothiocyanate
- TCEP
- Sodium Acetate
- PEG-8000
- Tris HCl
- purified 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):	K210440	DEN170029
Device Trade Name	Disposable sampler inactivated transport media, Nest ITM	PrimeStore MTM
General Device Characteristic Similarities		
Intended Use/Indications For Use	NEST ITM is an enclosed system intended for the collection, inactivation, stabilization and transportation of pharyngeal and nasal swabs suspected of containing adenovirus, influenza A virus or parainfluenza virus 2 from the collection site to the testing laboratory. The specimen transported in NEST ITM can be used for molecular detection in the laboratory.	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	>4.0 log reduction in concentration at 10 seconds	Same
Storage temperatures	2-8 ºC up to 25ºC	Same
General Device Characteristic Differences		
Specimen stability	NEST ITM preserves Adenovirus, Influenza	Primestore MTM medium preserves influenza A

	A virus or Parainfluenza virus 2 for 15 days at 2-8 ºC and 25ºC	RNA for up to 8 days at 27°C and 29 days at 4°C
Specimen Type	Nasopharyngeal or Oropharyngeal Swab	Nasal washes and sputum samples
Analyte	Nasopharyngeal or Oropharyngeal swab suspected of containing Adenovirus, Influenza A virus or Parainfluenza virus 2.	Nasal wash suspected of containing Influenza A virus. Sputum samples suspected of containing MTB.

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:

   N/A

2. Linearity:

   N/A

3. Analytical Specificity/Interference:

   N/A

4. Assay Reportable Range:

   N/A

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

   Shelf life: The shelf life for the NEST ITM is 12 months after the date of manufacture. The stability of the NEST ITM was performed using Realtime and Accelerated stability on a total of three (3) lots. Stability looked for bacterial and fungal growth in the media along with properties of the media, appearance, pH, and then confirmed with viral stabilization at room

temperature to the claimed 15 days demonstrating the stability of nucleic acids was not diminished with the age of media.

Sterilization: The DNA/RNA Shield Collection tube with media are not sold as sterile nor are they intended to be sterilized by the user. These vials are single use devices that do not require cleaning by the operator. The Swabs are individually packages and sold as sterile.

6. 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 Adenovirus, Influenza A, and Parainfluenza virus 2 were designed using validated assays to establish a concentration of organisms used for additional testing noted below.

LoD testing was initially performed by spiking multiple concentrations of Adenovirus, Influenza A, and Parainfluenza virus 2 into contrived matrix and spiking it onto a swab. Adenovirus, Influenza A, and Parainfluenza virus 2 were spike at a final concertation range of $1.0x10^4$, $1.0x10^3$, $5.0x10^2$, and $1.0x10^2$ copies/mL into the NEST ITM with a swab. A validated PCR assay was use to detemine the LoD to be $5.0x10^2$ copies/mL for each of the three viruses. Table 1 below shows the results of preliminary LoD for Adenovirus, influenza A, and Parainfluenza virus 2.

Table 1. Preliminary Limit of Detection

Concentration (copies/mL)	Adenovirus, 5 Reps Average (C_t)	SD (C_t)	Influenza A, 5 Rep Average (C_t)	SD (C_t)	Parainfluenza virus 2, 5 Rep Average (C_t)	SD (C_t)
$1.0x10^4$	29.3	0.18	32.04	1.08%	31.07	1.08%
$1.0x10^3$	32.0	0.26	34.75	2.11%	34.28	2.18%
$5.0x10^2$	34.3	0.95	35.48	1.97%	36.89	2.98%
$1.0x10^2$	>40	-	>40	-	>40	-

Confirmatory LoD testing was provided at a concentration of $5.0x10^2$ copies/mL with 20 replicates. The validated assay had an LoD with an acceptance criteria of virus detection at a concentration range $5.0x10^2$ copies/mL. The same detection range was replicated with the NEST ITM and further determine by the concentration that yielded at least a 95% of the replicates were recoverable within this range. At a concentration of $5.0x10^2$ copies/mL, 20 of 20 replicates had recoverable concentrations. Viral nuclic acids were extracted using a nucleic acid (DNA/RNA) extraction and purification kits (spin column) (SC903-50) (Wuxi TechstarTechnology Co., Ltd.) and amplified using the respective kits on the ABI 7500. The average C_t values for each virus are listed below in Table 2.

Table 2. Limit of detection C_t values for each virus

	C_t Value		
Replicates	Adenovirus	Influenza A	Parainfluenza virus 2
1	36.2	32.0	38.0
2	37.0	32.4	38.2
3	38.1	35.4	37.8
4	38.1	35.2	36.7
5	36.8	35.4	36.4
6	36.9	37.0	38.4
7	35.5	35.8	37.3
8	36.1	35.0	35.2
9	37.2	35.4	35.9
10	35.8	35.7	36.5
11	37.7	35.8	36.2
12	36.3	35.8	36.2
13	36.4	35.3	34.2
14	37.5	36.2	35.3
15	36.7	34.1	36.1
16	35.7	35.6	35.1
17	37.0	35.9	36.6
18	37.0	35.7	35.8
19	36.8	36.2	37.5
20	37.1	35.0	36.3
AVG:	36.8	35.2	36.3
SD:	0.73	1.2	1.1

LoD testing at $5.0x10^2$ copies/mL resulted in all 20 replicates for the concentration meeting the pre-defined acceptance criteria.

b) Viral Stability

The stability of Adenovirus, Influenza A, and Parainfluenza virus 2 at 1 x LoD ($5.0x10^2$ copies/mL) was evalutaed by spiking virus into simulated matrix incubated in the NEST ITM at refridgerated temperature (4°C, 39°F) for 15 days (see Table 3), and ambient temperature (25°C, 77° F) for 15 days (see Table 4). Validated PCR assays was used to determine stability of Adenovirus, Influenza A, and Parainfluenza virus 2 in the NEST ITM. The stability study analyzed a total of three lots near the manufacturer claimed 12-month stability. Testing used at least 20 replicates for each virus, time point and stoage condition. An initial time point designated as Day 0 was included as the initial C_t average for each of the two temperature ranges tested. Testing at three time points was performed at Day 0, 9 and 15 for refrigerated temperature (2-8°C, 36-39°F), and three time points, Day 0, 9, and 15, for ambient temperature (25°C, 77°F).

The validated PCR assays were run with all applicalbe controls to valid and confirm the detection of the target virus, Adenovirus, Influenza A, and Parainfluenza virus 2. A pre-defined acceptance criteria of (+/-) 3.0 C_t from the initial time zero value was the acceptance criteria.

Table 3. Adenovirus, Influenza A, and Parainfluenza 2 virus ($5.0x10^2$ copies/mL) stability at 4°C

	Day 0	Day 9	Day 15
Adenovirus AVG (C_t):	36.0	36.7	36.9
CV (C_t):	2.15%	1.8%	2.0%
Influenza A AVG (C_t):	35.4	35.4	35.7
CV (C_t):	2.0%	2.3%	2.5%
Parainfluenza 2 AVG (C_t):	36.1	37.0	37.0
CV (C_t):	2.0%	2.0%	2.4%

Table 4. Adenovirus, Influenza A, and Parainfluenza 2 virus ($5.0x10^2$ copies/mL) stability 25°C

	Day 0	Day 9	Day 15
Adenovirus AVG (C_t):	36.1	37.0	36.9
CV (C_t):	2.0%	2.4%	2.0%
Influenza A AVG (C_t):	35.3	36.1	35.3
CV (C_t):	2.4%	2.6%	2.1%
Parainfluenza 2 AVG (C_t):	36.2	36.9	37.0
CV (C_t):	2.3%	2.6%	2.1%

Stability testing of RNA from whole Adenovirus, Influenza A, and Parainfluenza virus 2 were spiked into matrix and stored in NEST ITM, resulted in a maximum average variation of 0.8 C_t over 15 days at 25°C and a maximum variation of 0.9 C_t over 15 days at 2-8°C.

c) Inactivation

Adenovirus, Influenza A, and Parainfluenza virus 2 at a concentration of 1.0×10^7 $TCID_{50}$/ml was incubated with NEST ITM for 10 seconds. Each virus only and NEST ITM were also incubated accordingly to serve as controls. Adenovirus, Influenza A, and Parainfluenza 2 virus (virus alone), Virus and NEST ITM, or NEST ITM alone was then inculated on to cell cultures after incubation. Four days after inoculation, the cells were fixed and stained with 0.06% crystal violet in 1% glutaraldeyde. Cells that did not take up the stain were considered evidence of a viral cytopathic effect (CPE) and as a result were considered a measure of viral viability. The titer of the virus CPE was calcuated and recorded as the $TCID_{50}$.

Inactivation time:

The NEST ITM showed no cytotoxicity on MDCK cells at a 1:1,000 dilution factor and greater; therefore at least a 1:1,000 dilution factor is needed to avoid a direct cytotoxic effect of the NEST ITM. Adenovirus, Influenza A, and Parainfluenza virus 2, were then exposed to NEST ITM for 10 seconds prior to serial 10 fold dilutions and incubations

(final concentration $\leq 10^3$ $TCID_{50}$/mL), while Influenza Adenovirus, Influenza A, and Parainfluenza virus 2 samples had viral loads of greater than 1.0 x 10^6 $TCID_{50}$ of virus and NEST ITM alone was diluted 1:1000 to see no CPE. The NEST ITM rapidly inactivated all viruses tested 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 NEST ITM See Table 5 below.

Table 5 Adenovirus, Influenza A, and Parainfluenza virus 2 inactivation in NEST ITM

10s incubation	Adenovirus $TCID_{50}$ (log)	Influenza A $TCID_{50}$ (log)	Influenza A $TCID_{50}$ (log)
Virus only	6.14	6.75	6.63
Virus and NEST ITM	< 3.0	< 3.0	< 3.0
NEST ITM only*	≤ 3.0	≤ 3.0	≤ 3.0

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

NEST ITM must be used at a ratio of at least 1:10 at a minimum of 10 seconds exposure time to demonstrate inactivation of Adenovirus, Influenza A, and Parainfluenza virus 2. Measuring Adenovirus, Influenza A, and Parainfluenza virus 2 inactivation below 1 x 10^3 $TCID_{50}$ was not possible because of the cytotoxic affects NEST ITM has on the cell culture based assay.

7. Assay Cut-Off:

   N/A

B Comparison Studies:

1. Method Comparison with Predicate Device:

   N/A

2. Matrix Comparison:

   N/A

C Clinical Studies:

1. Clinical Sensitivity:

   N/A

2. Clinical Specificity:

   N/A

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

N/A

D Clinical Cut-Off:

N/A

E Expected Values/Reference Range:

N/A

F Other Supportive Instrument Performance Characteristics Data:

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.