一次性使用采样器（ITM）性能研究资料

Performance Study of Disposable Sampler (ITM)

产品描述

灭活型病毒转运培养基（ITM）中包含胍盐和离子去垢剂等，可将病毒和细胞裂解，释放蛋白质和核酸，其中蛋白质，如 DNA 酶和 RNA 酶，会迅速的被裂解变性，另一方面，保存液中的还原剂和保护剂等可保护释放的核酸在常温下不被降解。ITM 可用于临床样品的保存和灭活，如鼻拭子、咽拭子等，有效地防止样品中微生物种类和相对丰度的变化。微生物中的核酸经病毒采样管保存后具有较高的完整性，可用于酶解、PCR、新一代测序等多种生物实验。广泛用于医院、科研机构和家庭的标本采集和保存。

该 ITM 设计保存条件为 2-37℃。

NEST ITM contains guanidine salts and ionic detergents which can lyse viruses and cells to release proteins and nucleic acids. The released proteins, such as DNases and RNases, will be quickly cleaved and denatured, whereas the reducing agent and protective agent in the preservation solution can protect the released nucleic acids from being degraded at normal temperature. ITM can be used for the preservation and inactivation of clinical specimens, such as nasal and pharyngeal swabs. It can effectively prevent changes of microorganisms in such specimens in the types and relative abundance. After being preserved in the virus sampling tube, the nucleic acids in microorganisms can maintain high integrity and can be used for various biological experiments such as enzymatic hydrolysis, PCR and next-generation sequencing. ITM is widely used in the collection and preservation of specimens in hospitals, scientific research institutions, and households.

NEST ITM is designed to be stored at 2-37℃.

Indications for Use

NEST ITM is an enclosed system intended for the collection, stabilization and transportation of pharyngeal and nasal swabs from the collection site to the testing laboratory. the specimen transported in NEST ITM can be used for molecular detection in the laboratory.

Performance Characteristics

1 保存效果 Preservation

1.1 最低检出限 Limit of detection

最低检出限用于确定 ITM 中可测量的含有最低浓度的核酸。本研究采用腺病毒、流感病毒 A 型和副流感病毒 2 型为研究材料，通过特定的提取和扩增平台建立一套评价体系。在不同条件下在最

低检出限附近进行测试以研究 ITM 防止核酸降解的能力。The detection limit refers to the lowest concentration of nucleic acids that can be measured in the ITM. In this study, parainfluenza virus type 2, adenovirus and Influenza A were used as research materials and a set of evaluation systems was established through a specific extraction and amplification platform. Tests were conducted under different conditions near the detection limit to study the ability of the ITM to prevent nucleic acids from being degraded.

腺病毒最低检出限 Adenovirus Limit of Detection

以宫颈细胞培养液梯度稀释腺病毒（邦德盛，浓度：1.57E+5 copies/mL），稀释液以 1：10 的比例加入 ITM 中，然后采用无锡科智达科技有限公司核酸（DNA/RNA）提取纯化试剂盒（离心柱法）（SC903-50）提取，扩增试剂和仪器分别是无锡科智达科技有限公司腺病毒核酸检测试剂盒（恒温荧光法）和宏石 SLAN-96S 仪器。其中 1.0E+5 copies/mL-1.0E+4 copies/mL 浓度重复检测 6 个，5.0E+3 copies/mL 浓度重复 20 次。1.0E+6 copies/mL-1.0E+4 copies/mL 浓度下重复 6 次检出率应 100%，5.0E+3 copies/mL 浓度下重复 20 次检测率应不小于 95%。

Dilute the adenovirus (Guangzhou BDS Biotech Co., Ltd., concentration: 1.57E+5 copies/mL) with HELA cell in a 0.1-fold gradient, add the dilute solution into the ITM at a ratio of 1:10 and then perform extraction with nucleic acid (DNA/RNA) extraction and purification kits (spin column) (SC903-50) (Wuxi Techstar Technology Co., Ltd.). Use the adenovirus nucleic acid test kits (fluorescent isothermal amplification) of Techstar and the Hongshi SLAN-96S device for amplification. Perform the test repeatedly under the concentration of 1.0E + 5copies/mL-1.0E + 4copies/mL for 6 times, and that of 5.0E + 3copies/mL for 20 times. The detection rate should be 100% after 6 repetitions under the concentration of 1.0E+5copies/mL-1.0E+4copies/mL and be at least 95% after 20 repetitions under the concentration of 5.0E+3 copies/mL.

表 1 1.0E+5 copies/mL-1.0E+4 copies/mL 浓度腺病毒最低检出限检测结果

Table 1 Limit test results under the concentration of 1.0E + 5copies/mL-1.0E + 4copies/mL of adenovirus

concentration （copies/mL）	Test1 （Ct）	Test2 （Ct）	Test3 （Ct）	Test4 （Ct）	Test5 （Ct）	Test6 （Ct）	AVERAGE （Ct）	CV
1.0E+5	12.65	12.64	12.85	12.14	12.48	12.95	12.62	2.28%
1.0E+4	15.96	16.81	14.58	15.34	14.96	15.71	15.56	5.07%

表 2 5.0E+3 copies/mL 浓度腺病毒最低检出限检测结果

Table 2 Limit test results under the concentration of 5.0E + 3copies/mL of adenovirus

5.0E+3 copies/mL	
Times of repetition	Ct
1	17.58
2	17.28
3	18.05
4	17.62
5	19.34
6	18.09
7	18.39
8	17.39

9	19.25
10	20.39
11	21.85
12	24.39
13	21.36
14	19.27
15	19.57
16	18.46
17	17.69
18	19.2
19	18.08
20	19.36
AVERAGE	19.13
CV	9.25%

流感病毒 A 型最低检出限 Influenza A Limit of Detection

以宫颈细胞培养液梯度稀释流感病毒 A 型（邦德盛，浓度：1.82E+5 copies/mL），稀释液以 1：10 的比例加入 ITM 中，然后采用无锡科智达科技有限公司核酸（DNA/RNA）提取纯化试剂盒（离心柱法）（SC903-50）提取，扩增试剂和仪器分别是无锡科智达科技有限公司流感病毒 A 型核酸检测试剂盒（恒温荧光法）和宏石 SLAN-96S 仪器。其中 1.0E+5 copies/mL-1.0E+4 copies/mL 浓度重复检测 6 个，5.0E+3 copies/mL 浓度重复 20 次。1.0E+5 copies/mL-1.0E+4 copies/mL 浓度下重复 6 次检出率应 100%，5.0E+3 copies/mL 浓度下重复 20 次检测率应不小于 95%。

Dilute the Influenza A (Guangzhou BDS Biotech Co., Ltd., concentration: 1.82E+5 copies/mL) with HELA cell in a 0.1-fold gradient, add the dilute solution into the ITM at a ratio of 1:10 and then perform extraction with nucleic acid (DNA/RNA) extraction and purification kits (spin column) (SC903-50) (Wuxi Techstar Technology Co., Ltd.). Use the Influenza A nucleic acid test kits (fluorescent isothermal amplification) of Techstar and the Hongshi SLAN-96S device for amplification. Perform the test repeatedly under the concentration of 1.0E + 5copies/mL-1.0E + 4copies/mL for 6 times, and that of 5.0E + 3copies/mL for 20 times. The detection rate should be 100% after 6 repetitions under the concentration of 1.0E+5copies/mL-1.0E+4copies/mL and be at least 95% after 20 repetitions under the concentration of 5.0E+3 copies/mL.

表 3 1.0E+5 copies/mL-1.0E+4 copies/mL 浓度流感病毒 A 型最低检出限检测结果

Table 3 Limit test results under the concentration of 1.0E + 5copies/mL-1.0E + 4copies/mL of Influenza A

concentration （copies/mL）	Test1 （Ct）	Test2 （Ct）	Test3 （Ct）	Test4 （Ct）	Test5 （Ct）	Test6 （Ct）	平均值 （Ct）	CV
1.0E+5	15.39	16.34	16.31	15.96	15.48	16.05	15.92	2.55%
1.0E+4	19.36	18.39	20.05	19.37	19.81	18.84	19.3	3.17%

表 4 5.0E+3 copies/mL 浓度流感病毒 A 型最低检出限检测结果

Table 4 Limit test results under the concentration of 5.0E + 3copies/mL of Influenza A

5.0E+3 copies/mL	
Times of	Ct

repetition	
1	23.54
2	30.07
3	26.28
4	29.31
5	30.61
6	27.04
7	26.85
8	25.2
9	28.36
10	25.14
11	29.35
12	26.2
13	25.84
14	24.09
15	22.24
16	25.39
17	26.07
18	30.54
19	28.25
20	32.14
AVERAGE	27.13
CV	9.69%

副流感病毒 2 型最低检出限 Parainfluenza virus type 2 Limit of Detection

以宫颈细胞培养液梯度稀释副流感病毒 2 型培养物（邦德盛，浓度：1.0E+5 copises/mL），稀释液以 1：10 的比例加入 ITM 中，然后采用无锡科智达科技有限公司核酸（DNA/RNA）提取纯化试剂盒（离心柱法）（SC903-50）提取，扩增试剂和仪器分别是无锡科智达科技有限公司副流感病毒 2 型核酸检测试剂盒（恒温荧光法）和宏石 SLAN-96S 仪器。其中 1.0E+5 copises/mL 和 1.0E+4 copises/mL 浓度重复检测 6 个，5.0E+3 copises/mL 浓度重复 20 次。1.0E+5 copises/mL 和 1.0E+4 copises/mL 浓度下重复 6 次检出率应 100%，5.0E+3copises /mL 浓度下重复 20 次检测率应不小于 95%。

Dilute the HPIV-2 virus (Guangzhou BDS Biotech Co., Ltd., concentration: 2.0E+4 copises/mL) with HELA cell in a 0.1-fold gradient, add the dilute solution into the ITM at a ratio of 1:10 and then perform extraction with nucleic acid (DNA/RNA) extraction and purification kits (spin column) (SC903-50) (Wuxi Techstar Technology Co., Ltd.). Use the HPIV-2 virus nucleic acid test kits (fluorescent isothermal amplification) of Techstar and the Hongshi SLAN-96S device for amplification. Perform the test repeatedly under the concentration of 1.0E + 5copies/mL-1.0E + 4copies/mL for 6 times, and that of 5.0E + 3copies/mL for 20 times. The detection rate should be 100% after 6 repetitions under the concentration of 1.0E+5copies/mL-1.0E+4copies/mL and be at least 95% after 20 repetitions under the concentration of 5.0E+3copies/mL.

表 5 1.0E+5 copises/mL 和 1.0E+4 copises/mL 浓度副流感病毒 2 型最低检出限检测结果

Table 5 Limit test results under the concentration of 1.0E + 5copies/mL-1.0E + 4copies/mL of HPIV-2 virus

concentration （copises/mL）	Test1 （Ct）	Test2 （Ct）	Test3 （Ct）	Test4 （Ct）	Test5 （Ct）	Test6 （Ct）	平均值 （Ct）	CV
1.0E+5	14.39	14.85	14.3	15.2	14.07	14.67	14.58	2.81%
1.0E+4	16.34	16.86	16.53	16.38	16.11	16.04	16.38	1.82%

表 6 5.0E+3copises/mL 浓度副流感病毒 2 型最低检出限检测结果

Table 6 Limit test results under the concentration of 5.0E + 3copies/mL of HPIV-2 virus

5.0E+3 copises/mL	
Times of repetition	Ct
1	17.45
2	18.4
3	17.6
4	18.1
5	18.23
6	18.15
7	17.94
8	18.04
9	17.36
10	20.5
11	18.64
12	17.93
13	17.37
14	17.62
15	18.05
16	18.54
17	17.33
18	17.11
19	17.95
20	18.12
AVERAGE	18.02
CV	3.9%

以上实验结果表明，ITM 在保存腺病毒、流感病毒 A 型和副流感病毒 2 型时，在 5.0E+3 copies/mL 浓度下都具有较好一致性。

The above test results show that ITM is well consistent when preserving parainfluenza virus type 2, adenovirus and Influenza A under the concentration of 5.0E + 3copises/mL.

1.2 保存稳定性实验 Stability

保存稳定性实验用于确定 ITM 对 DNA 和 RNA 的保存能力，本研究采用腺病毒、流感病毒 A 型和副流感病毒 2 型为研究材料，通过不同保存环境和保存时间确定 ITM 的保存能力。The stability experiment was used to determine the capacity of ITM to preserve DNA and RNA. In this study,

parainfluenza virus type 2, adenovirus and Influenza A were used as research materials and the preservation capacity of ITM was determined by different preservation conditions and duration.

腺病毒保存稳定实验 Stability of adenovirus

以宫颈细胞培养液稀释至 5.0E+3 copies/mL 浓度的腺病毒按 1：10 的比例加入 ITM 中，分别保存于 4℃和 37℃环境下。在保存第 0 天、3 天、9 天、15 天、21 天、27 天后采用无锡科智达科技有限公司核酸（DNA/RNA）提取纯化试剂盒（离心柱法）（SC903-50）提取，扩增试剂和仪器分别是无锡科智达科技有限公司腺病毒核酸检测试剂盒（恒温荧光法）和宏石 SLAN-96S 仪器。每种保存环境下每个检测点重复提取 20 次，以 Ct 值的差异程度代表保存稳定性。

Dilute the adenovirus to the concentration of 5.0E + 3copises/mL with HELA cell, add the dilute solution to the ITM at a ratio of 1:10 and store the solution at the temperature of 4 ℃ and 37 ℃ respectively. On Day 0, 3, 9, 15, 21, and 27 after storage, perform extraction with nucleic acid (DNA/RNA) extraction and purification kits (spin column) (SC903-50) (Wuxi Techstar Technology Co., Ltd.) and use the adenovirus nucleic acid test kits (fluorescent isothermal amplification) of Techstar and the Hongshi SLAN-96S device for amplification. Perform the extraction 20 times repeatedly for each detection point under each storage condition, and the degree of difference in Ct values represents the preservation stability.

表 7 腺病毒保存稳定实验

Table 7 Preservation Stability Experiment for adenovirus

Days		0	3	9	15	21	27
4℃	AVERAGE（Ct）	20.24	19.39	22.24	19.25	19.37	20.92
	CV	10.25%	9.28%	10.52%	8.25%	9.3%	12.28%
37℃	AVERAGE（Ct）	21.28	22.92	22.84	19.67	19.6	20.85
	CV	12.2%	10.51%	8.52%	9.71%	9.34%	10.43%

流感病毒 A 型保存稳定实验 Stability of Influenza A

以宫颈细胞培养液稀释至 5.0E+3 copies/mL 浓度的流感病毒 A 型按 1：10 的比例加入 ITM 中，分别保存于 4℃和 37℃环境下。在保存第 0 天、3 天、9 天、15 天、21 天、27 天后采用无锡科智达科技有限公司核酸（DNA/RNA）提取纯化试剂盒（离心柱法）（SC903-50）提取，扩增试剂和仪器分别是无锡科智达科技有限公司流感病毒 A 型核酸检测试剂盒（恒温荧光法）和宏石 SLAN-96S 仪器。每种保存环境下每个检测点重复提取 20 次，以 Ct 值的差异程度代表保存稳定性。

Dilute the Influenza A to the concentration of 5.0E + 3copises/mL with HELA cell, add the dilute solution to the ITM at a ratio of 1:10 and store the solution at the temperature of 4 ℃ and 37 ℃ respectively. On Day 0, 3, 9, 15, 21, and 27 after storage, perform extraction with nucleic acid (DNA/RNA) extraction and purification kits (spin column) (SC903-50) (Wuxi Techstar Technology Co., Ltd.) and use the Influenza A nucleic acid test kits (fluorescent isothermal amplification) of Techstar and the Hongshi SLAN-96S device for amplification. Perform the extraction 20 times repeatedly for each detection point under each storage condition, and the degree of difference in Ct values represents the preservation stability.

表 8 流感病毒 A 型保存稳定实验

Table 8 Preservation Stability Experiment for Influenza A

Days		0	3	9	15	21	27
4℃	AVERAGE（Ct）	28.49	29.34	31.51	30.51	29.74	30.4

	CV	7.64%	9.25%	10.2%	10.62%	9.71%	9.15%
37℃	AVERAGE（Ct）	30.52	29.84	31.52	31.84	29.54	30.48
	CV	10.25%.	12.52%	12.57%	10.23%	9.4%	10.84%

副流感病毒 2 型保存稳定实验 Stability of HPIV-2 virus

以宫颈细胞培养液稀释至 5.0E+3 copies/mL 浓度的副流感病毒 2 型培养物（邦德盛）按 1：10 的比例加入 ITM 中，分别保存于 4℃和 37℃环境下。在保存第 0 天、3 天、9 天、15 天、21 天、27 天后采用无锡科智达科技有限公司核酸（DNA/RNA）提取纯化试剂盒（离心柱法）（SC903-50）提取，扩增试剂和仪器分别是无锡科智达科技有限公司副流感病毒 2 型核酸检测试剂盒（恒温荧光法）和宏石 SLAN-96S 仪器。每种保存环境下每个检测点重复提取 20 次，以 Ct 值的差异程度代表保存稳定性。

Dilute the HPIV-2 virus to the concentration of 5.0E + 3copises/mL with HELA cell, add the dilute solution to the ITM at a ratio of 1:10 and store the solution at the temperature of 4 ℃ and 37 ℃ respectively. On Day 0, 3, 9, 15, 21, and 27 after storage, perform extraction with nucleic acid (DNA/RNA) extraction and purification kits (spin column) (SC903-50) (Wuxi Techstar Technology Co., Ltd.) and use the HPIV-2 virus nucleic acid test kits (fluorescent isothermal amplification) of Techstar and the Hongshi SLAN-96S device for amplification. Perform the extraction 20 times repeatedly for each detection point under each storage condition, and the degree of difference in Ct values represents the preservation stability.

表 9 副流感病毒 2 型保存稳定实验

Table 9 Preservation Stability Experiment for HPIV-2 virus

Days		0	3	9	15	21	27
4℃	AVERAGE（Ct）	19.04	20.43	19.2	18.83	19.43	20.34
	CV	6.94%	7.54%	6.93%	7.16%	5.39%	7.94%
37℃	AVERAGE（Ct）	22.54	21.56	19.54	20.57	22.63	23.79
	CV	7.93%	6.03%	6.5%	6.15%	5.93%	5.83%

以上实验结果表明，ITM 在保存检测限附近的腺病毒、流感病毒 A 型和副流感病毒 2 型时，具有良好的稳定性。

The above experimental results showed that ITM had good stability in the preservation of adenovirus, influenza A and parainfluenza virus type 2 near the detection limit.

2 灭活

三种病毒细胞培养物，包括腺病毒、甲型流感病毒和副流感病毒 2 型（浓度为 10^7TCID50/ml），分别与 ITM 混匀 10s、30s 和 60s。只有病毒和 ITM 的作为对照同时进行，培养 4 天后进行细胞染色，没有染色代表出现细胞病变。

灭活率：

实验结果表明 ITM 在 1：100 的稀释倍数下仍对 MDCK、MRC-5 和 HELA 细胞均具有毒性作用，但在 1：1000 的稀释倍数下其对细胞的毒性明显下降，病毒细胞培养物与 ITM 混匀 10s、30s 和 60s 后，细胞下降超过 4log，单独的 ITM 实验结果一致。

Three kinds of virus, including adenovirus, Influenza A and parainfluenza virus 2 (10^7TCID50/ml), were incubated with ITM for 10, 30 and 60 seconds respectively. virus only and ITM only were also incubated accordingly to serve as internal controls. Four days after inoculation, the cells were fixed and stained with 0.06% crystal violet in 1%

glutaraldehyde. Wells that were not stained demonstrated the cytopathic effect (CPE) of the virus. The titer of the virus was recorded as the TCID50.

Inactivation rate:

ITM showed cytotoxicity on MDCK cells (Influenza A), MRC-5 cells (adenovirus) and HELA cells (parainfluenza virus 2) when diluted 1:100 but not cytotoxicity at 1:1,000. The mixture of ITM and virus had similar profiles as ITM alone with 10, 30 and 60 second incubation, ITM rapidly inactivated virus with a >4.0 log reduction in concentration at 10 seconds.

表 10 ITM 灭活病毒的结果 Virus inactivation in ITM

	10s incubation	30s incubation	60s incubation
Adenovirus only	6.14	6.39	6.45
Adenovirus and ITM	＜ 2	＜ 2	＜ 2
ITM only	＜ 2	＜ 2	＜ 2
	10s incubation	30s incubation	60s incubation
Influenza A only	6.75	6.28	6.69
Influenza A and ITM	＜ 2	＜ 2	＜ 2
ITM only	＜ 2	＜ 2	＜ 2
	10s incubation	30s incubation	60s incubation
Parainfluenza virus 2 only	6.63	6.58	6.1
Parainfluenza virus 2 and ITM	＜ 2	＜ 2	＜ 2
ITM only	＜ 2	＜ 2	＜ 2

3 临床对比研究 Clinical Comparison

3.1 提取试剂和检测系统兼容性 Extraction platform and amplification instrument compatibility

腺病毒 Adenovirus

以宫颈细胞培养液梯度稀释腺病毒（邦德盛，浓度：1.57E+5 copies/mL），使最终浓度在 5.0E+3 copies/mL-1.0E+5 copies/mL 之间。稀释液以 1：10 的比例加入 ITM 中，采用以下商业化核酸提取试剂处理以确定 ITM 培养基与核酸分离试剂兼容性。

- 核酸（DNA/RNA）提取纯化试剂盒（离心柱法）（无锡科智达科技有限公司，SC903-50）
- 核酸提取或纯化试剂盒（中山大学达安基因股份有限公司）
- EasyPure® Viral DNA/ RNA Kit（北京全式金生物技术有限公司）
- AxyPrep Body Fluid Viral DNA/RNA Miniprep Kit（康宁生命科学（吴江）有限公司）

200μL 加入稀释液后的保存液采用以上提取试剂提取，100μL 洗脱液洗脱后采用无锡科智达科技有限公司腺病毒核酸检测试剂盒（恒温荧光法）进行扩增，检测仪器采用 ABI 7500 和上海宏石 SLAN-96S。每个浓度重复 20 次。

Dilute the adenovirus to the concentration between 1.0E+5 copies/mL-5.0E + 3copises/mL with HELA cell, add the dilute solution to the ITM at a ratio of 1:10. The following commercial nucleic acid extraction reagents were used to determine the compatibility of ITM with nucleic acid extraction reagents.

- Nucleic acid (DNA/RNA) Extraction Kit (Wuxi Techstar Technology Co., LTD.)
- Nucleic acid (DNA/RNA) Extraction Kit（Da An Gene Co.,Ltd. Of Sun Yat-sen University）
- EasyPure® Viral DNA/ RNA Kit（Beijing TransGen Biotech Co., LTD）
- AxyPrep Body Fluid Viral DNA/RNA Miniprep Kit（Corning Life Sciences(Wujiang)Co. LTD）

The 200μL solution was extracted with the above extraction reagent, and eluent with 100μL elution solution and amplified with the adenovirus nucleic acid detection kit (thermostatic fluorescence method) of Wuxi Techstar Technology Co., LTD. ABI 7500 and Shanghai Hongzhi SIAN-96s were used as detection instruments. Each concentration is repeated 20 times.

表 11 提取试剂和检测系统兼容性实验结果

Table 11 Experimental results of extraction platform and amplification instrument compatibility

Concentration of adenovirus	Instrument	Nucleic acid (DNA/RNA) Extraction Kit (Wuxi Techstar Technology Co., LTD.) **Positive Results/Total Tests**	Nucleic acid (DNA/RNA) Extraction Kit（Da An Gene Co.,Ltd. Of Sun Yat-sen University） **Positive Results/Total Tests**	EasyPure® Viral DNA/ RNA Kit （Beijing TransGen Biotech Co., LTD） **Positive Results/Total Tests**	AxyPrep Body Fluid Viral DNA/RNA Miniprep Kit （Corning Life Sciences(Wujiang)Co. LTD） **Positive Results/Total Tests**
1.0E+5 copies/mL	ABI 7500	20/20	20/20	20/20	20/20
	SLAN-96S	20/20	20/20	20/20	20/20
1.0E+4 copies/mL	ABI 7500	20/20	20/20	20/20	20/20
	SLAN-96S	20/20	20/20	20/20	20/20
5.0E+3 copies/mL	ABI 7500	19/20	19/20	19/20	19/20
	SLAN-96S	20/20	19/20	20/20	19/20

流感病毒 A 型

以宫颈细胞培养液梯度稀释流感病毒 A 型（邦德盛，浓度：1.82E+5 copies/mL），使最终浓度在 5.0E+3 copies/mL-1.0E+5 copies/mL 之间。稀释液以 1：10 的比例加入 ITM 中，采用以下商业化核酸提取试剂处理以确定 ITM 培养基与核酸分离试剂兼容性。

- 核酸（DNA/RNA）提取纯化试剂盒（离心柱法）（无锡科智达科技有限公司，SC903-50）
- 核酸提取或纯化试剂盒（中山大学达安基因股份有限公司）
- EasyPure® Viral DNA/ RNA Kit（北京全式金生物技术有限公司）
- AxyPrep Body Fluid Viral DNA/RNA Miniprep Kit（康宁生命科学（吴江）有限公司）

200μL 加入稀释液后的保存液采用以上提取试剂提取，100μL 洗脱液洗脱后采用无锡科智达科技有限公司流感病毒 A 型核酸检测试剂盒（恒温荧光法）进行扩增，检测仪器采用 ABI 7500 和上海宏石 SLAN-96S。每个浓度重复 20 次。

Dilute the Influenza A to the concentration between 1.0E+5 copies/mL-5.0E + 3copises/mL with HELA cell, add the dilute solution to the ITM at a ratio of 1:10. The following commercial nucleic acid extraction

reagents were used to determine the compatibility of ITM with nucleic acid extraction reagents.

- Nucleic acid (DNA/RNA) Extraction Kit (Wuxi Techstar Technology Co., LTD.)
- Nucleic acid (DNA/RNA) Extraction Kit（Da An Gene Co.,Ltd. Of Sun Yat-sen University）
- EasyPure® Viral DNA/ RNA Kit（Beijing TransGen Biotech Co., LTD）
- AxyPrep Body Fluid Viral DNA/RNA Miniprep Kit（Corning Life Sciences(Wujiang)Co. LTD）

The 200μL solution was extracted with the above extraction reagent, and eluent with 100μL elution solution and amplified with the Influenza A nucleic acid detection kit (thermostatic fluorescence method) of Wuxi Techstar Technology Co., LTD. ABI 7500 and Shanghai Hongzhi SlAN-96s were used as detection instruments. Each concentration is repeated 20 times.

表 12 提取试剂和检测系统兼容性实验结果

Table 12 Experimental results of extraction platform and amplification instrument compatibility

Concentration of Influenza A	Instrument	Nucleic acid (DNA/RNA) Extraction Kit (Wuxi Techstar Technology Co., LTD.) **Positive Results/Total Tests**	Nucleic acid (DNA/RNA) Extraction Kit（Da An Gene Co.,Ltd. Of Sun Yat-sen University） **Positive Results/Total Tests**	EasyPure® Viral DNA/ RNA Kit （Beijing TransGen Biotech Co., LTD） **Positive Results/Total Tests**	AxyPrep Body Fluid Viral DNA/RNA Miniprep Kit （Corning Life Sciences(Wujiang)Co. LTD） **Positive Results/Total Tests**
1.0E+5 copies/mL	ABI 7500	20/20	20/20	20/20	20/20
	SLAN-96S	20/20	20/20	20/20	20/20
1.0E+4 copies/mL	ABI 7500	20/20	20/20	20/20	20/20
	SLAN-96S	20/20	20/20	20/20	20/20
5.0E+3 copies/mL	ABI 7500	19/20	19/20	19/20	19/20
	SLAN-96S	19/20	19/20	19/20	19/20

副流感病毒 2 型

以宫颈细胞培养液梯度稀释副流感病毒 2 型（邦德盛，浓度：1.0E+5 copises/mL），使最终浓度在 5.0E+3 copises/mL -1.0E+5 copises/mL 之间。稀释液以 1：10 的比例加入 ITM 中，采用以下商业化核酸提取试剂处理以确定 ITM 培养基与核酸分离试剂兼容性。

- 核酸（DNA/RNA）提取纯化试剂盒（离心柱法）（无锡科智达科技有限公司，SC903-50）
- 核酸提取或纯化试剂盒（中山大学达安基因股份有限公司）
- EasyPure® Viral DNA/ RNA Kit（北京全式金生物技术有限公司）
- AxyPrep Body Fluid Viral DNA/RNA Miniprep Kit（康宁生命科学（吴江）有限公司）

200μL 加入稀释液后的保存液采用以上提取试剂提取，100μL 洗脱液洗脱后采用无锡科智达科技有限公司副流感病毒 2 型核酸检测试剂盒（恒温荧光法）进行扩增，检测仪器采用 ABI 7500 和上海宏石 SLAN-96S。每个浓度重复 20 次。

Dilute the parainfluenza virus 2 to the concentration between 1.0E+5 copies/mL-5.0E + 3copises/mL with HELA cell, add the dilute solution to the ITM at a ratio of 1:10. The following commercial nucleic acid extraction reagents were used to determine the compatibility of ITM with nucleic acid extraction reagents.

- Nucleic acid (DNA/RNA) Extraction Kit (Wuxi Techstar Technology Co., LTD.)
- Nucleic acid (DNA/RNA) Extraction Kit（Da An Gene Co.,Ltd. Of Sun Yat-sen University）
- EasyPure® Viral DNA/ RNA Kit（Beijing TransGen Biotech Co., LTD）
- AxyPrep Body Fluid Viral DNA/RNA Miniprep Kit（Corning Life Sciences(Wujiang)Co. LTD）

The 200μL solution was extracted with the above extraction reagent, and eluent with 100μL elution solution and amplified with the parainfluenza virus 2 nucleic acid detection kit (thermostatic fluorescence method) of Wuxi Techstar Technology Co., LTD. ABI 7500 and Shanghai Hongzhi SlAN-96s were used as detection instruments. Each concentration is repeated 20 times.

表 13 提取试剂和检测系统兼容性实验结果

Table 13 Experimental results of extraction platform and amplification instrument compatibility

Concentration of Influenza A	Instrument	Nucleic acid (DNA/RNA) Extraction Kit (Wuxi Techstar Technology Co., LTD.) **Positive Results/Total Tests**	Nucleic acid (DNA/RNA) Extraction Kit（Da An Gene Co.,Ltd. Of Sun Yat-sen University） **Positive Results/Total Tests**	EasyPure® Viral DNA/ RNA Kit（Beijing TransGen Biotech Co., LTD） **Positive Results/Total Tests**	AxyPrep Body Fluid Viral DNA/RNA Miniprep Kit （Corning Life Sciences(Wujiang)Co. LTD） **Positive Results/Total Tests**
1.0E+5 copises/mL	ABI 7500	20/20	20/20	20/20	20/20
	SLAN-96S	20/20	20/20	20/20	20/20
1.0E+4 copises/mL	ABI 7500	20/20	20/20	20/20	20/20
	SLAN-96S	20/20	20/20	20/20	20/20
5.0E+3 copises/mL	ABI 7500	19/20	20/20	19/20	19/20
	SLAN-96S	19/20	19/20	20/20	20/20

以上试验结果表明，提取方法和扩增仪器的使用可能不会影响下游试验的 LoD。以上列出的提取方法都表明它们与 NEST ITM 兼容。当 ABI - 7500 和 SLAN-96S 进行比较时，也观察到类似的结果。但提取试剂和检测平台多种多样，使用其他提取平台或扩增仪器可能需要额外的验证，以确保提供可靠的结果。

The above test results indicate that the extraction method and amplification instruments used may not affect the LoD of the downstream assay. The extraction methods listed above all indicate they are compatible with NEST ITM. A similar result is observed when the ABI 7500 and SLAN-96S are compared to each other. But there are many kinds of extracting reagents and testing platforms, Other extraction platform or amplification instrument be used may need additional validation to ensure robust results are provided.

3.2 保存效果对比 Comparison of preservation performance

腺病毒 Adenovirus

以宫颈细胞培养液稀释至 1.0E+4 copies/mL 和 5.0E+3 copies/mL 浓度的腺病毒按 1：10 的比例加

入 ITM 和竞品保存液中，保存液置于 37℃环境下保存。在保存第 20 天和 23 天后采用无锡科智达科技有限公司核酸（DNA/RNA）提取纯化试剂盒（离心柱法）（SC903-50）提取，扩增试剂和仪器分别是无锡科智达科技有限公司腺病毒核酸检测试剂盒（恒温荧光法）和宏石 SLAN-96S 仪器。每个检测点重复 20 次，以阳性检测率代表保存液性能。

The adenovirus with a concentration of 1.0E+4 copies/mL and 5.0E+3 copies/mL diluted with HELA cell was added to ITM at a ratio of 1:10, and the preservation solution was placed at 37℃ for preservation. After 20 and 23 days of storage, the nucleic acid (DNA/RNA) extraction kit of Wuxi Techstar Technology Co., LTD was used for extraction and purification (SC903-50). The amplification reagent and instrument were respectively The adenovirus nucleic acid detection kit (thermostatic fluorescence method) and Hongshi SlAN-96S instrument. Each detection point was repeated 20 times, with the positive detection rate representing the performance of the preservation solution.

表 14 不同保存液保存性能对比（腺病毒）

Table 14 Comparison of the test device with predicate device(Adenovirus)

Times	solutions	1.0E+4 copies/mL Positive Results/Total Tests	5.0E+3 copies/mL Positive Results/Total Tests
20 days	ITM	20/20	19/20
	Predicate Device	20/20	20/20
23 days	ITM	20/20	19/20
	Predicate Device	20/20	19/20

流感病毒 A 型 Influenza A

以宫颈细胞培养液稀释至 1.0E+4 copies/mL 和 5.0E+3 copies/mL 浓度的流感病毒 A 型按 1：10 的比例加入 ITM 和竞品保存液中，保存液置于 37℃环境下保存。在保存第 20 天和 23 天后采用无锡科智达科技有限公司核酸（DNA/RNA）提取纯化试剂盒（离心柱法）（SC903-50）提取，扩增试剂和仪器分别是无锡科智达科技有限公司流感病毒 A 型核酸检测试剂盒（恒温荧光法）和宏石 SLAN-96S 仪器。每个检测点重复 20 次，以阳性检测率代表保存液性能。

The Influenza A with a concentration of 1.0E+4 copies/mL and 5.0E+3 copies/mL diluted with HELA cell was added to ITM at a ratio of 1:10, and the preservation solution was placed at 37℃ for preservation. After 20 and 23 days of storage, the nucleic acid (DNA/RNA) extraction kit of Wuxi Techstar Technology Co., LTD was used for extraction and purification (SC903-50). The amplification reagent and instrument were respectively The Influenza A nucleic acid detection kit (thermostatic fluorescence method) and Hongshi SlAN-96S instrument. Each detection point was repeated 20 times, with the positive detection rate representing the performance of the preservation solution.

表 15 不同保存液保存性能对比（流感病毒 A 型）

Table 15 Comparison of the test device with predicate device(Influenza A)

Times	solutions	1.0E+4 copies/mL Positive Results/Total Tests	5.0E+3 copies/mL Positive Results/Total Tests
20 days	ITM	20/20	19/20
	Predicate Device	19/20	20/20
23 days	ITM	19/20	19/20

	Predicate Device	19/20	19/20

副流感病毒 2 型 Parainfluenza virus 2

以宫颈细胞培养液稀释至 1.0E+4 copies/mL 和 5.0E+3 copies/mL 浓度的副流感病毒 2 型培养物（邦德盛）按 1：10 的比例加入 ITM 和竞品保存液中，保存液置于 37℃环境下保存。在保存第 20 天和 23 天后采用无锡科智达科技有限公司核酸（DNA/RNA）提取纯化试剂盒（离心柱法）（SC903-50）提取，扩增试剂和仪器分别是无锡科智达科技有限公司副流感病毒 2 型核酸检测试剂盒（恒温荧光法）和宏石 SLAN-96S 仪器。每个检测点重复 20 次，以阳性检测率代表保存液性能。

The parainfluenza virus 2 with a concentration of 1.0E+4 copies/mL and 5.0E+3 copies/mL diluted with HELA cell was added to ITM at a ratio of 1:10, and the preservation solution was placed at 37℃ for preservation. After 20 and 23 days of storage, the nucleic acid (DNA/RNA) extraction kit of Wuxi Techstar Technology Co., LTD was used for extraction and purification (SC903-50). The amplification reagent and instrument were respectively The parainfluenza virus 2 nucleic acid detection kit (thermostatic fluorescence method) and Hongshi SlAN-96S instrument. Each detection point was repeated 20 times, with the positive detection rate representing the performance of the preservation solution.

表 16 不同保存液保存性能对比（副流感病毒 2 型培养物）

Table 16 Comparison of the test device with predicate device(Parainfluenza virus 2)

Times	**solutions**	**1.0E+4 copies/mL** **Positive Results/Total Tests**	**5.0E+3 copies/mL** **Positive Results/Total Tests**
20 days	ITM	20/20	19/20
	Predicate Device	20/20	20/20
23 days	ITM	20/20	20/20
	Predicate Device	20/20	20/20

以上实验结果表明，NEST ITM 在 37℃环境下保存 RNA 23 天和竞品试剂对比没有明显差异。

The above test results indicate that compared to the predicate device, EST ITM stored RNA at 37℃ for 23 days showed no significant difference.