

Verification Report for Virus Samples Preserved in Sample Preservative Fluid

Experimental Purpose: Verification of different types virus preserved in Bioer's sample preservative fluid (Inactivate Transport Medium) and the adaptability of the extraction reagents and the stability of nucleic acid in sample preservative fluid.

Experimental Equipment: centrifuge, incubator, mixing block, nucleic acid purification system NPA-32P, fluorescence quantitative PCR instrument FQD-96C / FQD-96A

Experimental Reagents: sample preservative fluid (BSC82), MagaBio plus Virus DNA/RNA purification kit II (BSC71), Biospin Virus DNA/RNA Extraction Kit (BSC77), virus fluorescence quantitative detection kit

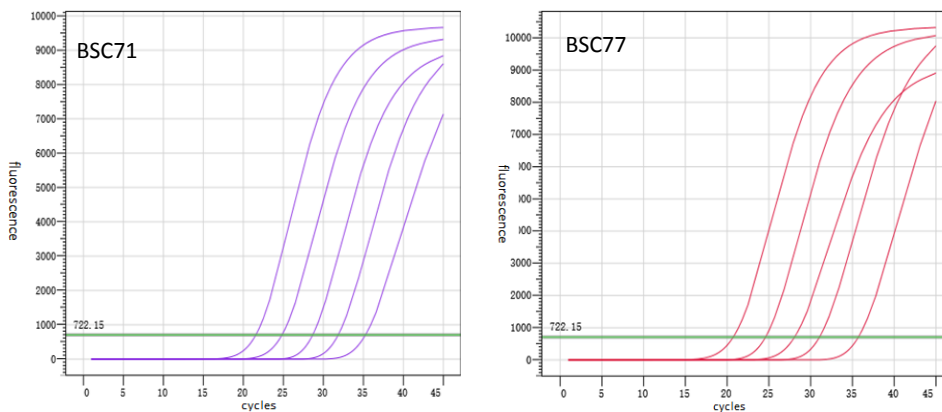
Virus and samples types for experimental verification:

Virus	Porcine Epidemic Diarrhea Virus(PEDV)	African Swine Fever Virus (ASFV)	Hepatitis B Virus (HBV)	Influenza A Virus (FLU)	Feline Coronavirus (Fcov)
Sample type	Tissue Homogenate	Whole Blood, Saliva of Swine	Serum	Swabs	Feces

Experimental Data:

1. Adaptability verification of sample preservative fluid and different extraction reagents

The positive tissue homogenate of porcine epidemic diarrhea virus (PEDV) was diluted gradiently with the sample preservative fluid, and extract the gradient diluent with MagaBio plus Virus DNA/RNA purification kit II (BSC71) and Biospin Virus DNA/RNA Extraction Kit (BSC77). Then do Real-time RT-PCR detection:

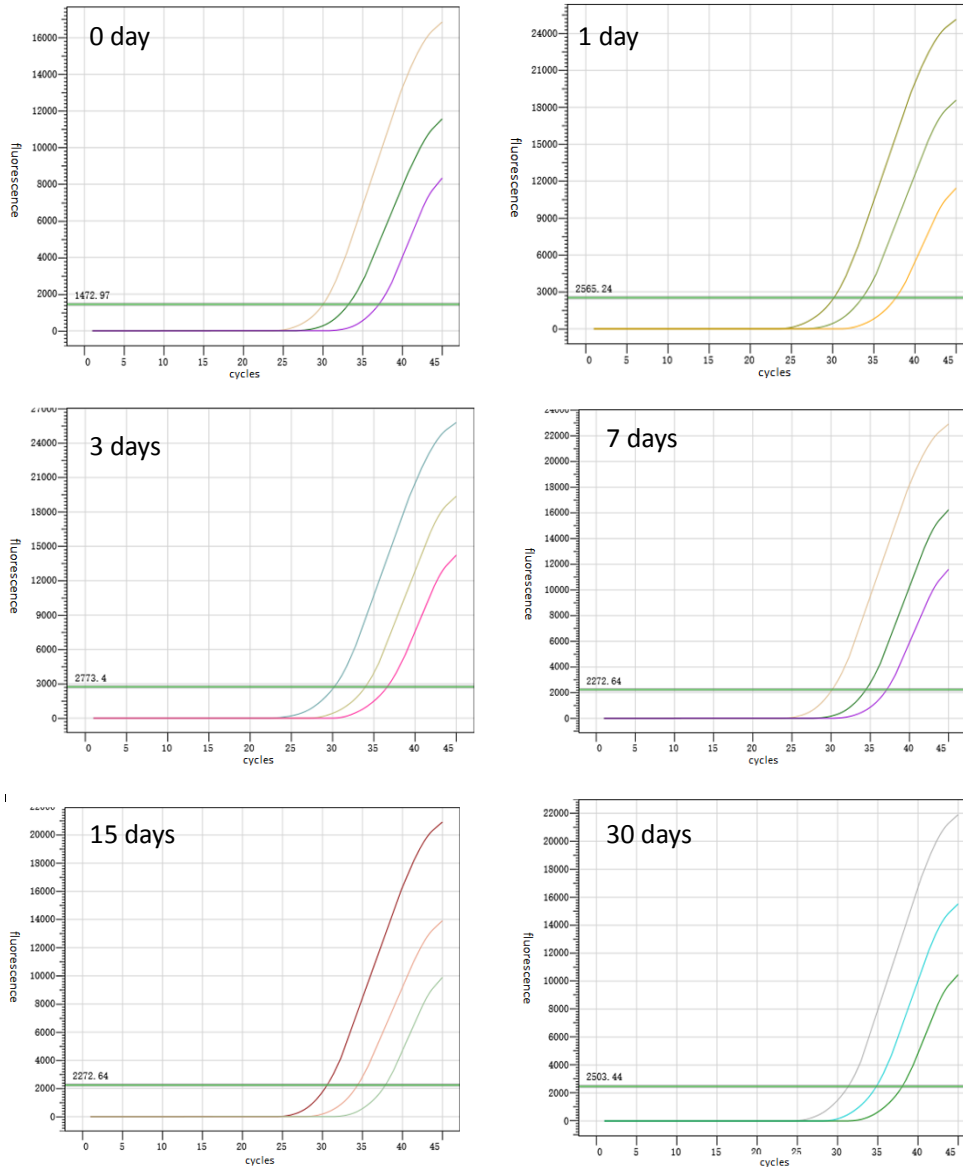


Con.	reagent	BSC71	BSC77
		Sample preservative fluid	Sample preservative fluid
	10 ⁻¹	21.71	20.86
	10 ⁻²	24.96	24.75
	10 ⁻³	28.82	28.21
	10 ⁻⁴	31.98	31.2
	10 ⁻⁵	35.41	35.85

Conclusion: The sample preservative fluid has good adaptability with different extraction reagents.

2. Stability verification of DNA virus preserved in sample preservative fluid

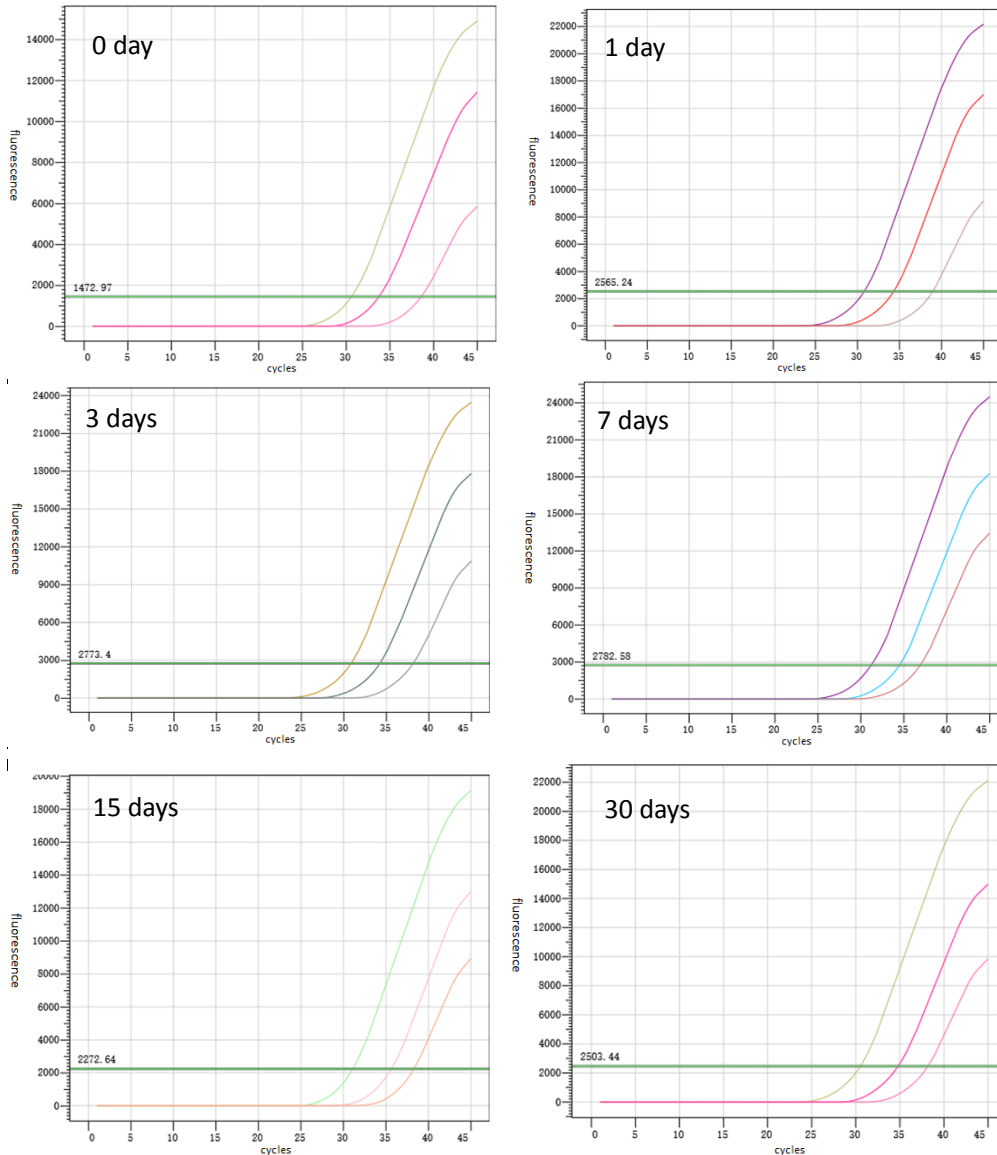
- 1) Positive swine whole blood with African swine fever virus (ASFV) was diluted gradiently with negative swine whole blood and the gradient diluents were preserved in sample preservative fluid. After storing at 37 ° C for 30 days, utilize BIOER MagaBio plus Virus DNA/RNA purification kit II to extract ASFV-DNA and verify its preservative stability:



37°C sample	0 day	1 day	3 day	7 day	15 day	30 day
++	30.14	30.28	30.32	30.25	30.69	31.94
+	33.33	33.73	34.09	34.57	34.51	35.26
+/-	37.15	37.81	36.75	37.23	37.97	38.22

Positive swine saliva with African swine fever virus (ASFV) was diluted gradiently with negative swine saliva and the gradient diluents were preserved in sample preservative fluid. After storing at

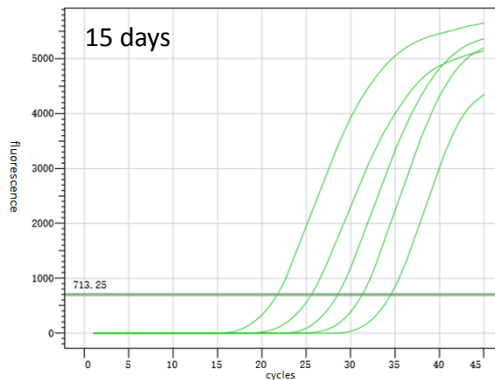
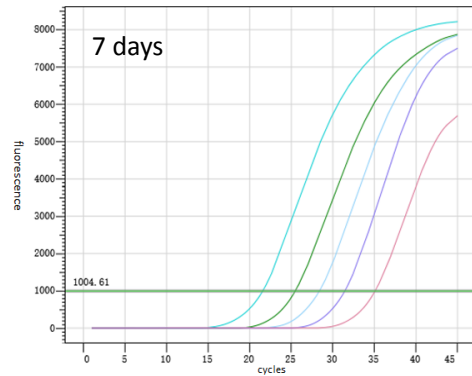
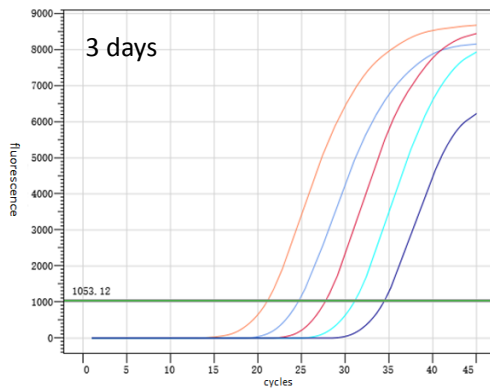
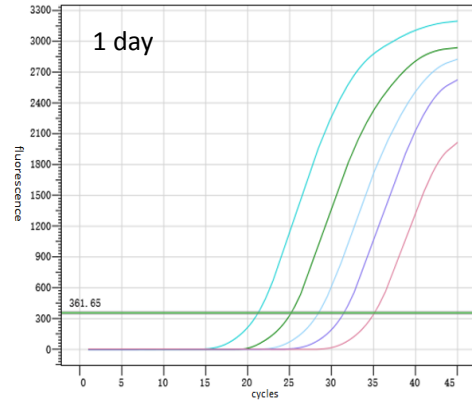
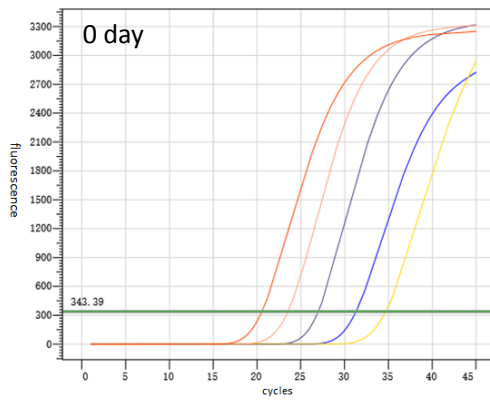
37 ° C for 30 days, utilize BIOER MagaBio plus Virus DNA/RNA purification kit II to extract ASFV-DNA and verify its preservative stability:



sample \ 37°C	0 day	1 day	3 day	7 day	15 day	30 day
++	30.72	30.90	31.17	31.30	31.21	30.64
+	33.87	34.42	34.60	34.62	35.76	34.92
+/-	38.71	39.06	38.59	37.03	38.41	38.31

Conclusion: The above experiments prove that the African swine fever virus (ASFV) can be stably preserved in the sample preservative fluid for more than 15 days.

- 2) The human hepatitis B virus positive serum (known concentration) was diluted gradiently with negative serum, and the gradient diluents were preserved in sample preservative fluid. After storing at 37°C for 15 days, utilize BIOER MagaBio plus Virus DNA/RNA purification kit II to extract HBV-DNA and verify its preservative stability:



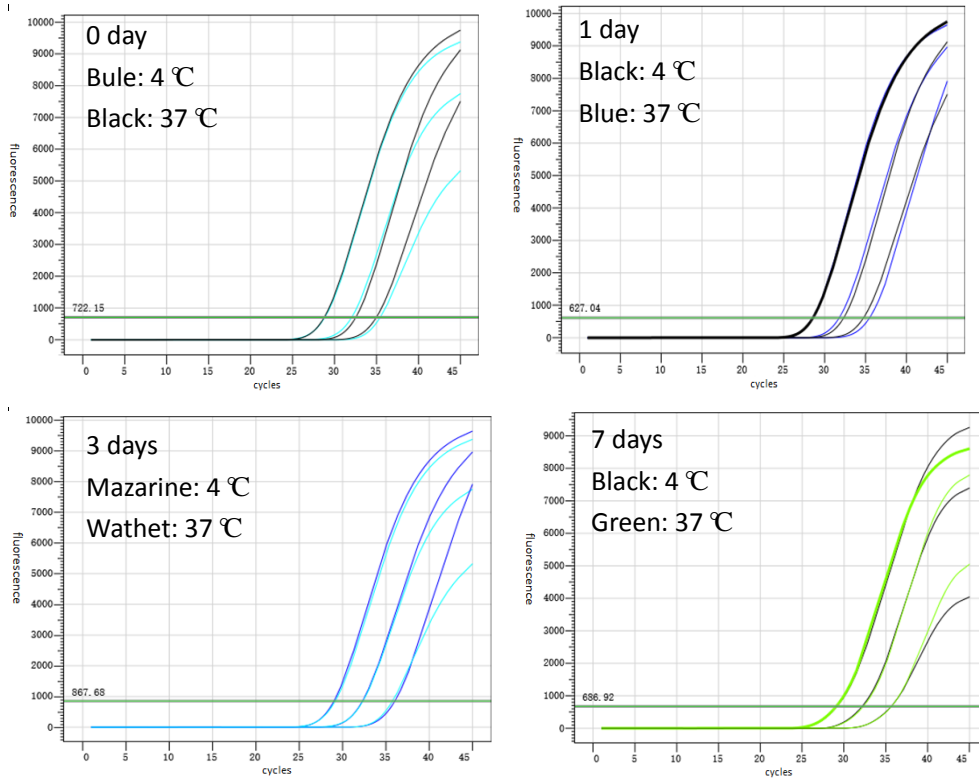
sample \ time	0 day	1 day	3 day	7 day	15 day
E5 IU/mL	21.19	21.36	21.21	21.66	21.84
E4 IU/mL	24.41	25.29	24.79	25.57	25.68
E3 IU/mL	28.63	28.55	27.86	28.48	28.66
E2 IU/mL	31.25	31.57	31.20	31.54	31.40
10 IU/mL	34.45	35.21	34.59	35.17	34.63

Conclusion: The above experiments prove that human hepatitis B virus (HBV) can be stably preserved in the sample preservative fluid for 15 days.

3. Stability verification of RNA virus preserved in sample preservative fluid

- 1) Two sets of positive swabs with different concentrations of influenza A virus were stored in a sample preservative fluid at 4 °C and 37 °C respectively for 7 days, utilize BIOER MagaBio plus Virus DNA/RNA purification kit II to extract RNA , do the RT-PCR detection and verify its

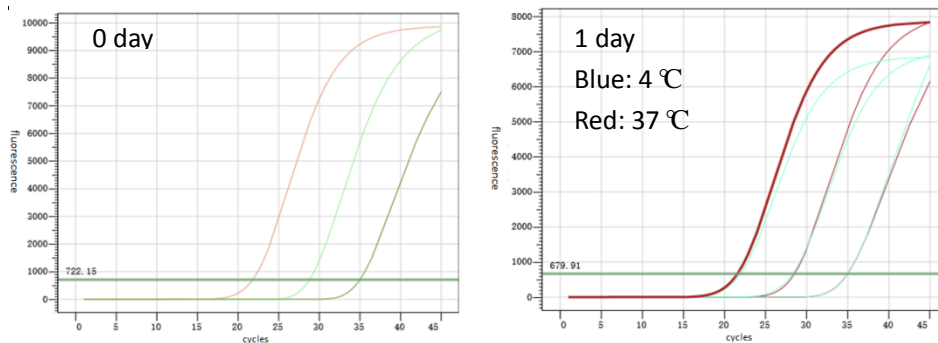
preservative stability, (due to the small sample size, the time of this stability test is short):

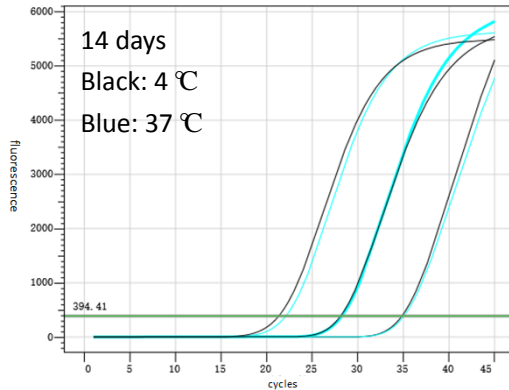
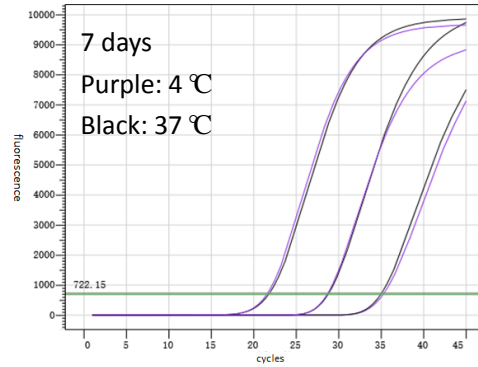
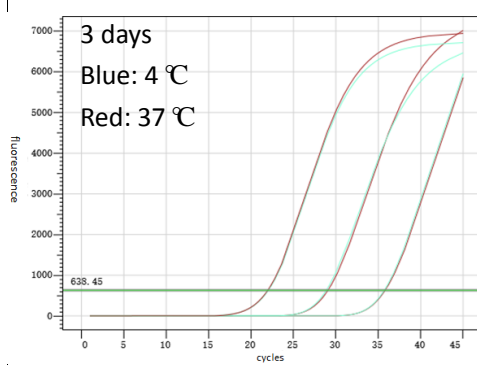


Sample	Time	Temp.	0 day	1 day	3 day	7 day
			++	4 °C	28.43	28.51
		37 °C	28.39	28.61	28.41	28.54
+		4 °C	31.22	32.73	32.56	32.39
		37 °C	31.38	32.65	32.78	32.81
+/-		4 °C	35.67	35.45	36.59	36.84
		37 °C	35.09	35.87	36.37	36.53

Conclusion: The above experiments prove that influenza A virus can be stably stored in the sample preservative fluid for 7 days.

2) Porcine epidemic diarrhea virus (PEDV) positive tissue homogenate was diluted gradiently with PBS, and the gradient diluents were preserved in sample preservative fluid at 4 °C and 37 °C respectively for 14 days. Utilize BIOER MagaBio plus Virus DNA/RNA purification kit II to extract PEDV-RNA , do the RT-PCR detection and verify its preservative stability:

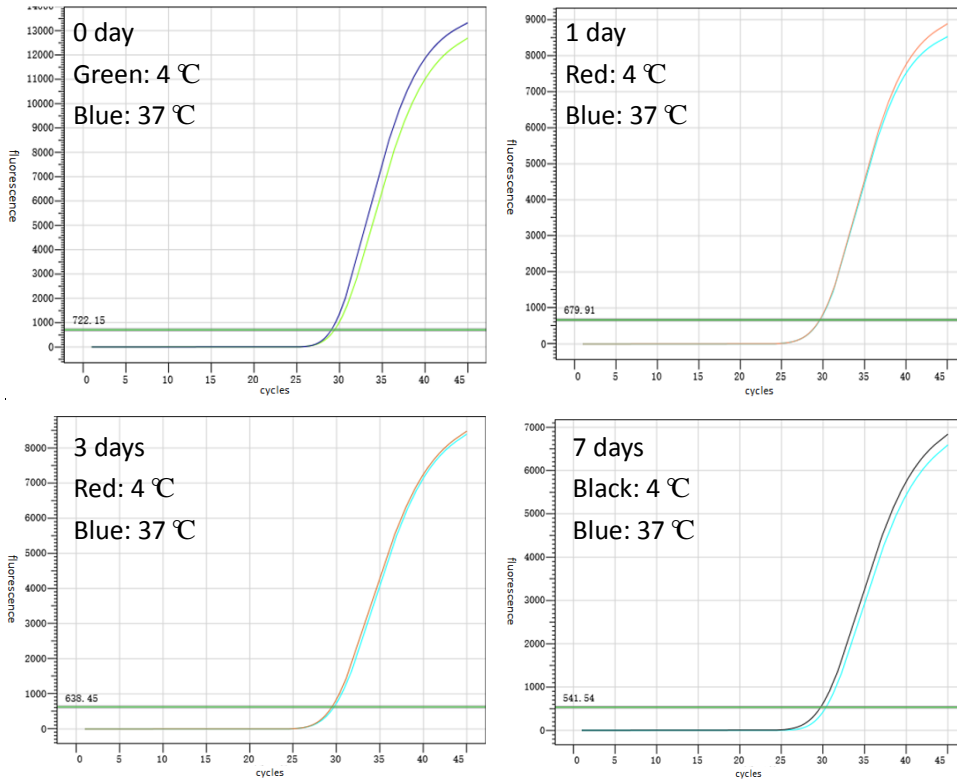




Time \ Sample	Temp.	0 day	1 day	3 day	7 day	14 day
++	4 °C	21.91	22.05	22.16	22.07	22.18
	37 °C		21.69	22.08	22.19	22.36
+	4 °C	28.91	28.42	28.97	28.83	28.79
	37 °C		28.64	29.18	28.76	28.55
+/-	4 °C	35.11	35.09	35.82	35.85	35.73
	37 °C		35.07	35.91	35.67	35.49

Conclusion: The above experiments prove that the Porcine Epidemic Diarrhea Virus (PEDV) can be stably stored in the sample preservative fluid for 14 days.

3) Feline coronavirus (Fcov) positive feces samples preserved in sample preservative fluid according to the ratio of 1:10 (1g feces stored in 10mL sample preservative fluid) at 4 ° C and 37 ° C respectively for 7 days. Utilize BIOER MagaBio plus Virus DNA/RNA purification kit II to extract Fcov-RNA , do the RT-PCR detection and verify its preservative stability:



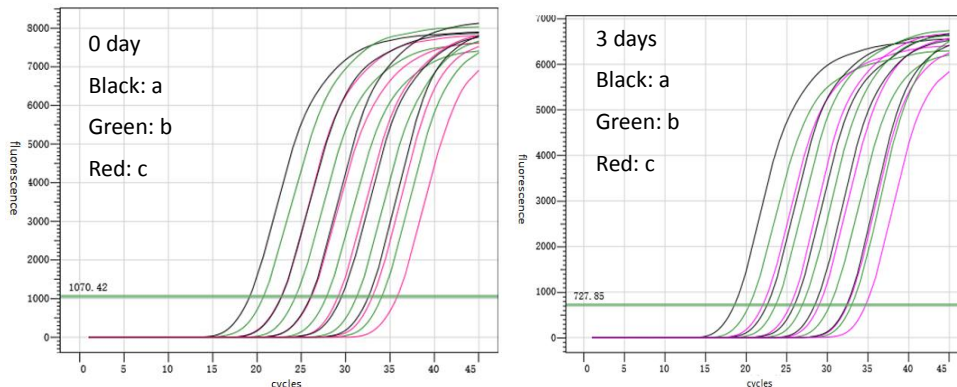
Sample	0 day	1 day (24h)	3 day (72h)	7 day (168h)
(4 °C)	29.56	29.71	29.6	29.51
(37 °C)	29.47	29.75	29.83	30.44

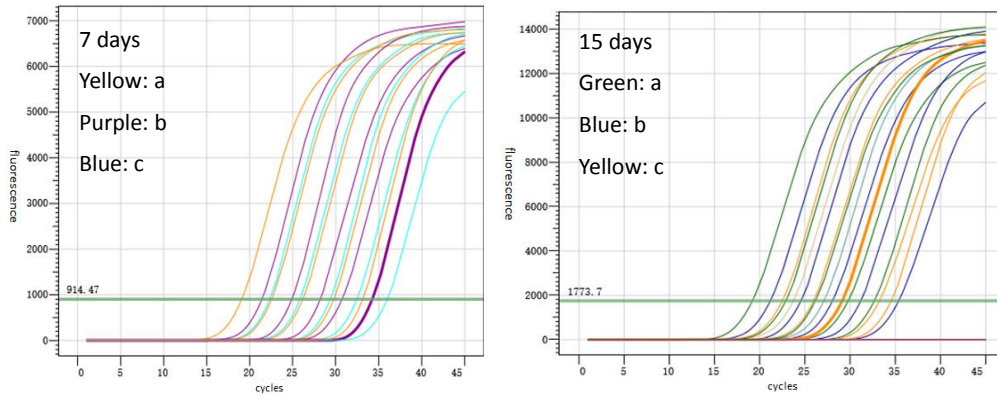
Conclusion: This experiment proves that feline coronavirus (Fcov) can be stably stored in the sample preservative fluid for 7 days.

4. Verification of the stability of different proportions of sample in sample preservative fluid

1) RNA virus

Porcine epidemic diarrhea virus (PEDV) positive tissue homogenate was processed into samples as follows: a. PEDV positive tissue homogenate was diluted gradiently with sample preservative fluid; b. PEDV positive tissue homogenate was diluted gradiently with PBS, the gradient diluent mixed with sample preservative fluid at the ratio of 1:2; c. PEDV positive tissue homogenate was diluted gradiently with PBS, the gradient diluent mixed with sample preservative fluid at the ratio of 1:10. Store all samples at 37 °C for 15 days, utilize BIOER MagaBio plus Virus DNA/RNA purification kit II to extract PEDV-RNA, do the RT-PCR detection and verify its preservative stability:





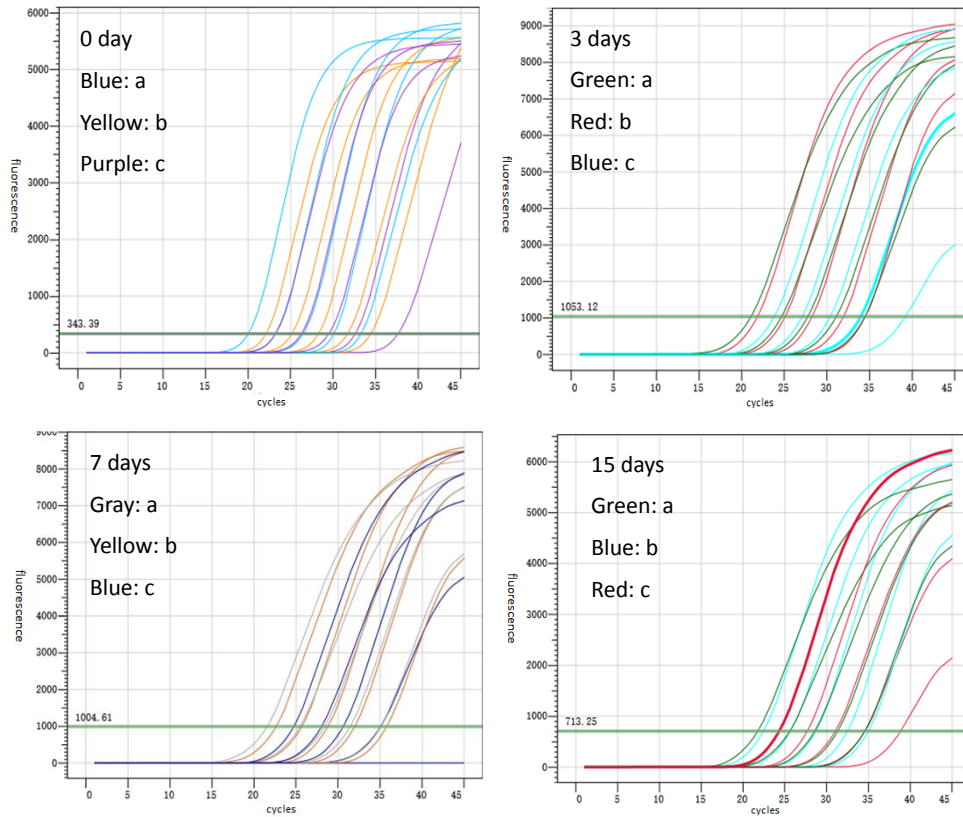
Time \ Method	Con.	(a)	(b)	(c)
0 day	10 ⁻¹	19.29	20.83	22.83
	10 ⁻²	22.88	24.46	26.26
	10 ⁻³	26.16	27.95	29.25
	10 ⁻⁴	29.65	31.37	33.11
	10 ⁻⁵	32.69	34.17	35.92
3 day	10 ⁻¹	18.68	20.53	22.16
	10 ⁻²	22.70	23.74	25.57
	10 ⁻³	26.03	27.01	29.09
	10 ⁻⁴	28.56	30.44	32.60
	10 ⁻⁵	32.48	33.10	34.82
7 day	10 ⁻¹	19.18	21.54	22.43
	10 ⁻²	22.66	25.00	25.93
	10 ⁻³	26.28	28.30	29.48
	10 ⁻⁴	29.87	30.83	32.56
	10 ⁻⁵	33.24	34.35	36.10
15 day	10 ⁻¹	19.30	21.29	22.62
	10 ⁻²	23.01	24.69	26.05
	10 ⁻³	26.26	28.16	29.25
	10 ⁻⁴	29.90	31.30	33.37
	10 ⁻⁵	32.79	35.50	34.68

Note: a. The actual sample volume is 300μL; b. The actual sample volume is 100μL; c. The actual sample volume is 30μL.

2) DNA virus

The human hepatitis B virus (HBV) positive serum (known concentration) was processed into samples as follows: a. HBV positive serum was diluted gradiently with sample preservative fluid; b. HBV positive serum was diluted gradiently with PBS, the gradient diluent mixed with sample

preservative fluid at the ratio of 1:2; c. HBV positive serum was diluted gradiently with PBS, the gradient diluent mixed with sample preservative fluid at the ratio of 1:10. Store all samples at 37 °C for 15 days, utilize BIOER MagaBio plus Virus DNA/RNA purification kit II to extract HBV-DNA, do the Real Time PCR detection and verify its preservative stability:



Time \ Method	Con.	(a)	(b)	(c)
0 day	E5 IU/mL	21.19	22.27	23.38
	E4 IU/ mL	24.41	25.31	26.43
	E3 IU/ mL	28.63	28.44	29.65
	E2 IU/ mL	31.25	31.83	32.75
	10 IU/ mL	34.45	34.58	37.76
3 day	E5 IU/mL	21.21	21.96	23.93
	E4 IU/ mL	24.79	25.20	27.20
	E3 IU/ mL	27.86	28.36	30.49
	E2 IU/ mL	31.20	32.04	34.06
	10 IU/ mL	34.59	34.59	39.28
7 day	E5 IU/mL	21.66	22.73	24.82
	E4 IU/ mL	25.57	25.78	28.13
	E3 IU/ mL	28.48	28.87	30.70

	E2 IU/ mL	31.54	32.07	35.15
	10 IU/ mL	35.17	35.87	-
15 day	E5 IU/mL	21.84	22.53	24.41
	E4 IU/ mL	25.68	25.75	27.77
	E3 IU/ mL	28.66	28.79	31.12
	E2 IU/ mL	31.40	32.57	34.68
	10 IU/ mL	34.63	35.04	38.91

Note: a. The actual sample volume is 300 μ L; b. The actual sample volume is 100 μ L; c. The actual sample volume is 30 μ L.

Conclusion: This experiment proves that the sample can be stably stored in the sample preservative fluid for 15 days; the lower concentration of sample will affect the positive detection rate. When storing the liquid sample, it is recommended to add equal volume or double volume of sample preservative fluid.

Experimental Conclusion

1. The above experiments prove that the sample preservative fluid has good adaptability among different extraction reagents and can be applied to various extraction reagents on the market;
2. The sample preservative fluid can store a variety of sample types, such as: whole blood, serum, plasma, tissue homogenate, feces, oral fluid, swabs, etc. ;
3. The sample preservative fluid perfectly inhibits DNase / RNase activity and can stably store viral nucleic acid;
4. According to the above experimental data, after collecting samples:
 - 1) It can be transported under room temperature; under the condition of 2 °C ~ 8 °C, the sample can be stored stably in the sample preservative fluid for 1 month; under the condition of -20 °C, it can be stored for a long time;
 - 2) When storing liquid samples, it is recommended to add equal volume or double volume of sample preservative fluid; when storing swab samples, it is generally recommended to add 1ml-2ml of sample preservative fluid to immerse the swab.