Scientific and Social Research, 2026, Volume 8, Issue 3
https://ojs.bbwpublisher.com/index.php/SSR
Online ISSN: 2981-9946
Print ISSN: 2661-4332

Discussion on the Validation of a Fully-Automated Nucleic Acid Processing System

Yang Cheng, Dandan Xu, Yu Cao, Yuanyuan Shi*

Lianyungang Central Blood Station, Lianyungang 222000, Jiangsu, China

**Author to whom correspondence should be addressed.*

Copyright: © 2026 Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), permitting distribution and reproduction in any medium, provided the original work is cited.

Abstract: *Objective:* The newly purchased automated nucleic acid assembly line must be verified to ensure compliance with ISO 15189 standards for medical laboratories before use. *Methods:* Multiple departments collaborate to complete installation qualification; operational qualification is conducted for five functional modules; performance verification is carried out using system performance verification panels, analytical sensitivity verification panels, reference materials, and specimens from voluntary blood donors. *Results:* Hardware and software installations meet the usage requirements of the detection system. The ATM-style self-service sample handover module, post-handover automatic sample refrigeration module, sample processing module, nucleic acid mixing and extraction module, and post-processing automatic sample refrigeration module are all operating normally. The concordance rates for HBV DNA, HCV RNA, and HIV-1 RNA were 100%. The detection rates for both pooled and individual testing modes were 100%, meeting the standard requirement of ≥ 95%. Neither a triglyceride concentration of 33 g/L nor a hemoglobin concentration of 5 g/L affected the detection of weakly positive samples at 3 times the limit of detection (LOD). In 20 repeated tests, the within-run coefficients of variation (CV) for HBV DNA, HCV RNA, and HIV RNA were 2.88%, 2.78%, and 2.49%, respectively, while the between-run CVs were 3.45%, 3.20%, and 2.42%. All results met the requirements of within-run CV < 10% and between-run CV < 15%.The results for negative controls were all non-reactive in 15 tests, while the detection results for HBV, HCV, and HIV at 3 times the limit of detection were all reactive, with coefficients of variation (CV) of 1.58%, 1.50%, and 1.08%, respectively, meeting the requirements for repeatability verification. Cross-contamination resistance was validated using a system performance panel. With positive samples evenly interspersed among negative samples, the detection of positive samples did not affect the results of the negative samples. A total of 34 specimens from voluntary blood donors were tested across 12 batches, and the results were fully consistent, meeting the requirements for parallel testing verification. *Conclusion:* The fully-automated nucleic acid processing system, after installation qualification, operational qualification, and performance qualification, complies with the requirements of ISO 15189 and meets the standards for nucleic acid testing in blood screening laboratories.

Keywords: Installation qualification; Operational qualification; Performance qualification; ISO 15189

Online publication: April 15, 2026

1. Introduction

The Kehua NS2000 Fully Automated Nucleic Acid Testing (NAT) Assembly Line is an integrated nucleic acid testing instrument that encompasses functions such as specimen handover, nucleic acid testing, and post-testing specimen handling. The system includes the fully automated sample processing system Aurora SPS1200 and the fully automated nucleic acid extraction instrument Aurora Flexi96, with nucleic acid amplification equipped with the ABI7500 fluorescent PCR instrument. With the application of nucleic acid testing in blood station blood screening, newly purchased instruments must be confirmed as required before being put into use. To further enhance the testing capabilities of laboratory personnel and advance the ISO 15189 accreditation process for medical laboratories, it is necessary to conduct installation confirmation, performance verification, and operational verification of the Kehua NAT assembly line system before use to ensure it meets the laboratory's intended use requirements. Based on the requirements of the "Technical Operating Procedures for Blood Stations" (2019 edition), CNAS-CL02-A001:2023, CNAS-GL039, and CNAS-GL050, the following discussion will be conducted on the installation and operational confirmation of each functional module of the Kehua NAT assembly line system, as well as its compliance rate, sensitivity, specificity, limit of detection, anti-interference capability, method precision, anti-cross-contamination, and parallel experiment results [1–3].

2. Materials and Methods

2.1. Sample Sources

2.1.1. Performance verification panel

Performance Verification Panel for Hepatitis B Virus Nucleic Acid (HBV DNA)/Hepatitis C Virus Nucleic Acid (HCV RNA)/Human Immunodeficiency Virus Nucleic Acid (HIV-1 RNA) (Manufacturer: Beijing Kangche Sitan Biotechnology Co., Ltd., Batch Number: 202404001): A total of 240 samples, including 11 vials of HBV DNA (1.5ml each), 10 vials of HCV RNA (1.5ml each), 7 vials of HIV-1 RNA (1.5ml each), 2 vials of HIV-1 RNA+HBV DNA (1.5ml each), 1 vial of HIV-1 RNA+HCV DNA (1.5ml each), 9 vials of NHP (nucleic acid negative) (1.5ml each), and 200 vials of nucleic acid negative (1.0ml each).

2.1.2. Analytical sensitivity verification panel

Analytical Sensitivity Verification Panel for Hepatitis B Virus Nucleic Acid (HBV DNA)/Hepatitis C Virus Nucleic Acid (HCV RNA)/Human Immunodeficiency Virus Nucleic Acid (HIV-1 RNA) (Manufacturer: Beijing Kangche Sitan Biotechnology Co., Ltd., Batch Number: 20240805), as shown in **Table 1**.

Table 1. Sample composition of the analytical sensitivity verification panel for Hepatitis B Virus Nucleic Acid (HBV DNA)/Hepatitis C Virus Nucleic Acid (HCV RNA)/Human Immunodeficiency Virus Nucleic Acid (HIV-1 RNA)

Name	Packaging Specification	Concentration (IU/ml)	Composition
HBV DNA	2.0 ml/vial	7.5 / 2.5 / 1	20 vials of each / set
HCV RNA	2.0 ml/vial	30 / 10 / 3	20 vials of each / set
HIV RNA	2.0 ml/vial	50 / 20 / 10	20 vials of each / set

2.1.3. The concentrations of the reference materials

HBV DNA/HCV RNA/HIV-1 RNA are 50 IU/ml, 200 IU/ml, and 1000 IU/ml, respectively (manufacturer: Beijing

Kangche Sitan Biotechnology Co., Ltd., batch number: KHB20240806).

2.1.4. Negative plasma, non-reactive lipemic specimens, and non-reactive hemolytic specimens

Negative plasma, non-reactive lipemic specimens, and non-reactive hemolytic specimens are all derived from voluntary blood donor specimens and have tested negative for HBV-DNA/HCV-RNA/HIV-RNA using a nucleic acid detection system.

2.1.5. Nucleic acid negative (liquid) non-valued quality control material

Nucleic acid negative (liquid) non-valued quality control material (manufacturer: Beijing Kangche Sitan Biotechnology Co., Ltd., batch number: 20240806).

2.1.6. Voluntary blood donor specimens

These specimens have tested negative for HBV-DNA/HCV-RNA/HIV-RNA using a nucleic acid detection system consisting of a Hamilton Star automated sample processor and an ABI7500 fluorescent PCR instrument.

2.2. Specimen preparation

2.2.1. Concordance, sensitivity, and specificity

A performance verification panel for hepatitis B virus nucleic acid (HBV DNA)/hepatitis C virus nucleic acid (HCV RNA)/human immunodeficiency virus nucleic acid (HIV-1 RNA) systems.

2.2.2. Limit of detection

Use reference materials HBV DNA (50 IU/ml), HCV RNA (50 IU/ml), and HIV-1 RNA (1000 IU/ml) as defined specimens, with nucleic acid-negative plasma as the dilution matrix, diluted to the concentration specified in the reagent instructions as the minimum limit of detection: for pooled testing mode, HBV DNA 15 IU/ml, HCV RNA 54 IU/ml, HIV-1 RNA 120 IU/ml. Use samples from the analytical sensitivity verification panel for hepatitis B virus nucleic acid (HBV DNA)/hepatitis C virus nucleic acid (HCV RNA)/human immunodeficiency virus nucleic acid (HIV-1 RNA), where samples numbered HBV21-40 and HIV141-160 have concentrations of 2.5 IU/ml and 20 IU/ml, respectively, meeting the minimum limit of detection for single testing mode. Samples numbered HCV81-10 have a concentration of 10 IU/ml, and each is diluted to 9 IU/ml by adding 222 ul of nucleic acid-negative plasma for testing. Each sample is tested 20 times repeatedly to calculate the detection rate.

2.2.3. Anti-interference capability

Specimens are divided into Group A, Group B, and Group C. Group A (lipemic): Mix Beijing Kangche Sitan reference materials (with HBV DNA/HCV RNA/HIV-1 RNA concentrations of 50 IU/ml/200 IU/ml/1000 IU/ml, respectively), negative plasma, and non-reactive lipemic specimens to prepare a final sample that is a weakly positive sample at 3 times the limit of detection (LOD), with a triglyceride concentration of 33 g/L; Group B (hemolytic): Mix Beijing Kangche Sitan reference materials (with HBV DNA/HCV RNA/HIV-1 RNA concentrations of 50 IU/ml/200 IU/ml/1000 IU/ml, respectively), negative plasma, and non-reactive hemolytic specimens to prepare a final sample that is a weakly positive sample at 3 times the LOD, with a hemoglobin concentration of 5 g/L; Group C (negative control): Mix Beijing Kangche Sitan reference materials (with HBV DNA/HCV RNA/HIV-1 RNA concentrations of 50 IU/ml/200 IU/ml/1000 IU/ml, respectively) and negative plasma to prepare a final sample that is a weakly positive sample at 3 times the LOD.

Specific dilution protocols are shown in **Table 2**.

Table 2. Specimen dilution protocols for anti-interference capability (Lipemic Group, Hemolytic Group)

Item		Single-Test Mode		
		HBV DNA	HCV RNA	HIV RNA
Nucleic acid concentration of the reference standard (IU/ml)		50	200	1000
Lower limit of detection claimed in the instructions (IU/ml)		2.5	9	20
3 times LOD (IU/ml)		7.5	27	60
Sample volume required for one test (ml)		1	1	1
Sample volume required for three tests (ml)		3	3	3
Lipemic Group	TG concentration in lipemic plasma (g/L)	42	42	42
	Volume of the reference standard (ml)	0.45	0.405	0.18
	Volume of lipemic plasma (ml)	2.4	2.4	2.4
	Volume of non-reactive negative plasma (ml)	0.15	0.195	0.42
	Concentration of the reference standard after dilution (IU/ml)	7.5	27	60
	TG concentration after dilution in lipemic sample (g/L)	33	33	33
Hemolytic Group	Hb concentration in hemolyzed sample (g/L)	9	9	9
	Volume of the reference standard (ml)	0.45	0.405	0.18
	Volume of hemolyzed plasma (ml)	1.7	1.7	1.7
	Volume of non-reactive negative plasma (ml)	0.85	0.895	1.12
	Concentration of the reference standard after dilution (IU/ml)	7.5	27	60
	Hb concentration after dilution in hemolyzed sample (g/L)	5	5	5
Control Group	Volume of the reference standard (ml)	0.45	0.405	0.18
	Volume of non-reactive negative plasma (ml)	2.55	2.595	2.82
	Concentration of the reference standard after dilution (IU/ml)	7.5	27	60

Note: The volumes configured in the table represent the total volume for three repeated tests, with each configuration distributed into individual test tubes for testing.

2.2.4. Method precision experiment

Beijing Kangchestan reference materials (with concentrations of HBV DNA/HCV RNA/HIV-1 RNA at 50 IU/ml/200 IU/ml/1000 IU/ml, respectively).

2.2.5. Repeatability experiment

Add nucleic acid-negative (liquid) non-quantitative quality control samples to the reference materials to achieve a concentration of 3 times the limit of detection (LOD). Under mixed testing mode, the concentrations of 3 times LOD for HBV DNA, HCV RNA, and HIV-1 RNA are 45 IU/ml, 162 IU/ml, and 360 IU/ml, respectively.

2.2.6. Specimen preparation for anti-cross-contamination experiment

System performance verification panel for hepatitis B virus nucleic acid (HBV DNA)/hepatitis C virus nucleic

acid (HCV RNA)/human immunodeficiency virus nucleic acid (HIV-1 RNA).

2.2.7. Specimen preparation for parallel experiment

34 specimens from voluntary blood donors.

2.3. Reagents and instruments

Nucleic acid test kits for hepatitis B virus, hepatitis C virus, and human immunodeficiency virus (types 1 and 2) (Shanghai Kehua Bio-engineering Co., Ltd., Batch No. 202402031); HBV DNA reference material S6 (50 IU/ml)/ HCV RNA reference material S8 (200 IU/ml)/HIV RNA reference material S7 (1000 IU/ml) (Beijing Kangchestan Biotechnology Co., Ltd., KHB20240806). Fully automated nucleic acid blood screening assembly line KHB Aurora SPS1200, ABI7500 gene amplification detector (Serial No. 275052881), vortex mixer (Haimen Qilinbel Instrument Manufacturing Co., Ltd., Model XW-80A), pipettes (eppendorf 200–1000 μl/2–20 μl/20–200 μl), palm-type centrifuge (Haimen Qilinbel Instrument Manufacturing Co., Ltd., Model LX-100), biosafety cabinet (Sujing Antai, Model BSC-1004IIA2), fully automated blood analyzer (Sysmex, Model XS-500i), electronic digital thermometer (Jingchuang).

2.4. Methods

2.4.1. Installation confirmation

After the equipment arrives at the laboratory, laboratory personnel, the equipment management department, and the manufacturer's equipment installation engineer jointly unpack the equipment and check the accessories against the packing list; inspect the equipment's appearance for any damage (no dents, scratches, or defects); and confirm that the installation conditions meet the requirements. The engineer installs the equipment hardware and software, performs test programming, and sets parameters according to the equipment manual.

2.4.2. Operational confirmation

Test Method: The Kehua NS2000 fully automated nucleic acid testing assembly line system includes five functional modules: an "ATM"-style self-service sample handover module, an automatic post-handover sample refrigeration module, a sample processing module, a nucleic acid pooling and extraction module, and an automatic post-processing sample refrigeration module. Test method for the "ATM"-style self-service sample handover module: Conduct specimen handover for 7 days, simultaneously loading two or more sample racks containing multiple blood sample tubes of varying quantities to be handed over, testing equipment performance with ≥20 specimens per day. Test method for the automatic refrigeration module (pre-refrigeration, post-refrigeration): The quality control department tests the refrigerator temperature, the refrigerator temperature performance, and the accurate positioning of the refrigerator manipulator for specimen grasping during specimen handover. Test method for the sample processing module: Test the specimen tube opening and closing, barcode scanner identification of specimen tube label information, track operation, extraction process incubation module, refrigeration module, and sample addition module. Nucleic acid pooling and extraction module: Conduct a blank water test to simulate the nucleic acid extraction process, testing whether the liquid aspiration volume, liquid disposal volume, incubation temperature, vibration frequency, and time meet the nucleic acid extraction standard requirements.

Acceptance Criteria: All equipment modules are complete, the instrument operates normally, the software operates normally, specimens can be scanned normally, the mechanical gripper operates and grasps normally,

specimen scanning is normal, and the temperature meets the requirements. The sample transfer track operates normally, meeting the requirements for blood testing equipment, and specimens can undergo nucleic acid extraction normally.

2.5. Performance confirmation

2.5.1. Compliance rate, sensitivity, and specificity

Test all specimens in the system performance verification panel and compare the test results with the serum panel results to calculate the compliance rate.

Calculation Method: Sensitivity = [a/(a+c)] * 100%, Specificity = [d/(b+d)] * 100%, Compliance Rate = [(a+d)/(a+b+c+d)] * 100%.

Acceptance Criteria: Positive specimens test positive, negative specimens test negative; sensitivity, specificity, and overall compliance rate are all 100%.

2.5.2. Limit of detection

Experimental Method: Repeat the test 20 times for specimens at each concentration (mixed and single testing at the lowest limit of detection) for 5 consecutive days, with 4 tests per day.

Acceptance Criteria: For the manufacturer-declared limit of detection concentrations of HBV DNA, HCV RNA, and HIV RNA, if each is tested 20 times and the positive detection rate is ≥95%, the verification passes.

2.5.3. Anti-interference capability (lipemia and hemolysis)

Experimental Method: Test the configured specimens in each group and repeat the test 3 times.

Acceptance Criteria: If the manufacturer-declared 3 times LOD weakly positive lipemic specimens with triglycerides below 33 g/L and 3 times LOD weakly positive hemolytic specimens with hemoglobin below 5 g/L still test positive, the verification passes.

2.5.4. Method precision experiment

Experimental Method: Repeatedly test HBV-DNA, HCV-RNA, and HIV-RNA quality control samples in mixed testing mode, with 20 test wells for intra-batch testing and at least 20 consecutive days for inter-batch testing.

Acceptance Criteria: The coefficient of variation (CV) of the test results should be <10% for intra-batch testing and <15% for inter-batch testing [4].

2.5.5. Repeatability experiment

Experimental Method: Combine 3 negative quality control samples and 3 positive plasma samples each for HBV, HCV, and HIV nucleic acids at a concentration of 3 times the lowest limit of detection into a sample panel. Use the same operator on the same equipment to conduct mixed testing for 5 consecutive days and statistically analyze the sample test results.

Acceptance Criteria: The negative quality control samples should yield non-reactive results, and the positive samples should yield reactive results. Statistical analysis of the signal values should show a variation of less than 15% of the inter-batch variation of the reagent [5].

2.5.6. Anti-cross-contamination experiment

Experimental Method: Arrange the negative and positive specimens from the serum panel alternately in the sample

racks, with positive specimens evenly distributed among negative specimens.

Acceptance Criteria: All positive specimens should be detected, and the test results for negative specimens should all be negative for acceptance.

2.5.7. Parallel Experiment

Experimental Method: Test at least 30 specimens from voluntary blood donors in mixed testing mode and 4 specimens from voluntary blood donors in single testing mode for at least 10 consecutive days using the same mode.

Acceptance Criteria: The test results should be consistent with the previously completed test results.

3. Results

3.1. Installation confirmation results

All met the requirements

3.2. Operational confirmation results

3.2.1. "ATM"-style Self-service sample handover module

The automatic scanning of specimen barcodes was normal during specimen handover for seven consecutive days. After testing multiple groups of specimens with varying quantities under the corresponding set programs, the specimen handover module operated normally and met the expected equipment requirements.

3.2.2. Automatic refrigeration module (pre-refrigeration, post-refrigeration)

The quality control department's test results for the refrigerator temperature showed that the temperature fluctuation range met the requirements. The refrigerator temperature remained stable during specimen handover, with a temperature fluctuation range between 2°C and 8°C. The mechanical gripper could automatically adjust to the number of specimens in the adapter, grasping accurately and operating stably.

3.2.3. Sample processing module

The grippers rotated flexibly and operated stably, capable of normally opening and closing lids and scanning barcodes. The barcode scanner could automatically scan the barcode information on the specimen tubes, accurately identifying the labels on the specimen tubes. The track operated smoothly, and each incubation module, refrigeration module, and sample addition module during the extraction process met the requirements, with no dripping phenomena, and the sample addition volume conformed to requirements.

3.3. Performance confirmation results

3.3.1. Compliance rate, sensitivity, and specificity

The positive compliance rate (sensitivity), negative compliance rate (specificity), and overall compliance rate for HBV DNA, HCV RNA, and HIV-1 RNA were all 100%. Specific results are shown in **Table 3**.

Table 3. Verification results of method compliance rates for HBV DNA, HCV RNA, and HIV-1 RNA

		HBV DNA			HCV RNA			HIV-1 RNA		
		Reference Method		Total	Reference Method		Total	Reference Method		Total
		Positive	Negative		Positive	Negative		Positive	Negative	
Candidate Method	Positive	a(13)	b(0)	a+b(13)	a(11)	b(0)	a+b(11)	a(10)	b(0)	a+b(10)
	Negative	c(0)	d(9)	c+d(9)	c(0)	d(9)	c+d(9)	c(0)	d(9)	c+d(9)
Total		a+c(13)	b+d(9)	a+b+c+d(22)	a+c(11)	b+d(9)	a+b+c+d(20)	a+c(10)	b+d(9)	a+b+c+d(19)

3.3.2. Experimental results for limit of detection

Experimental results for the limit of detection are shown in **Table 4**.

Table 4. CT values and detection rates for nucleic acid detection at the limit of detection in pooled and single testing modes

Testing Mode	Single-sample Testing Mode			Pooled Testing Mode		
Item	HBV-DNA	HCV-RNA	HIV-RNA	HBV-DNA	HCV-RNA	HIV-RNA
Detection Limit	2.5 IU/ml	9 IU/ml	20 IU/ml	15 IU/ml	54 IU/ml	120 IU/ml
Number of Tests	20	20	20	20	20	20
Detection Rate	100%	100%	100%	100%	100%	100%

3.3.3. Experimental results for anti-interference capability (lipemia and hemolysis)

Experimental results for anti-interference capability (lipemia and hemolysis) are shown in **Table 5**.

Table 5. Results of the anti-interference ability (lipemia and hemolysis) experiment

Sample ID	Test Item		
	HBV-DNA (CT Value)	HCV-RNA (CT Value)	HIV-RNA (CT Value)
ZX-1	29.72	28.01	30.85
ZX-2	30.16	28.16	30.86
ZX-3	29.61	28.54	30.83
RX-1	27.36	26.61	30.13
RX-2	29.73	27.90	30.33
RX-3	29.88	28.31	30.81
DZ-1	28.86	29.28	30.83
DZ-2	29.35	26.60	30.26
DZ-3	29.21	27.27	30.26

Note: ZX, RX, and DZ represent the lipemic group, hemolytic group, and control group, respectively.

3.3.4. Experimental results for method precision

Experimental results for method precision are shown in **Table 6**.

Table 6. Experimental results for inter-batch and intra-batch precision

	Between-Batch Precision Experiment Results			Within-Batch Precision Experiment Results		
Item	**HBV DNA**	**HCV RNA**	**HIV RNA**	**HBV DNA**	**HCV RNA**	**HIV RNA**
Number of detections	20	20	20	20	20	20
Mean (Ct value)	30.01	28.20	30.22	30.27	28.52	30.40
SD (Ct value)	1.03	0.90	0.73	0.87	0.79	0.76
CV	3.45%	3.20%	2.42%	2.88%	2.78%	2.49%

3.3.5. Results of the repeatability experiment

Results of the repeatability experiment are shown in **Table 7**.

Table 7. Results of the repeatability experiment

Item	Negative Quality Control	3×LOD HBV DNA	3×LOD HCV RNA	3×LOD HIV RNA
Number of Tests	15	15	15	15
Mean (CT value)	/	30.44	27.24	31.99
SD (CT value)	/	0.48	0.41	0.35
CV (CT value)	/	1.58%	1.50%	1.08%

3.3.6. Results of the anti-cross-contamination experiment

Results of the anti-cross-contamination experiment are shown in **Table 8**.

Table 8. Results of the anti-cross-contamination experiment

Mixed Mode Detection Results for Samples from Series P*** (Set 6)					
ID	**Item**	**Ct Value**	**ID**	**Item**	**Ct Value**
P01B	HBV	30.78	P21I	HIV-1	28.49
P02B	HBV	31.16	P22I	HIV-1	33.17
P03I	HIV-1	27.51	P23N	NHP	N/A
P04N	NHP	N/A	P24C	HCV	22.13
P05C	HCV	25.79	P25N	NHP	N/A
P06B	HBV	26.36	P26B	HBV	22.88
P07I	HIV-1	31.47	P27B	HBV	22.93
P08B	HBV	27.48	P28N	NHP	N/A
P09C	HCV	22.23	P29B	HBV	30.27
P10N	NHP	N/A	P30C	HCV	27.78
P11B	HBV	22.37	P31I	HIV-1	27.25
P12C	HCV	29.78	P32C	HCV	29.39

Table 1 (Continued)

Mixed Mode Detection Results for Samples from Series P*** (Set 6)					
ID	**Item**	**Ct Value**	**ID**	**Item**	**Ct Value**
P13I	HIV-1	27.14	P33C	HCV	28.53
P14B	HBV	19.72	P34N	NHP	N/A
P15N	NHP	N/A	P35C	HCV	27.90
P16B	HBV	25.65	P36I	HIV-1	33.13
P17C	HCV	31.66	P37I+B	HIV, HBV	29.76,31.84
P18B	HBV	27.00	P38C+I	HCV, HIV	31.63,29.30
P19C	HCV	25.71	P39N	NHP	N/A
P20N	NHP	N/A	P40I+B	HIV, HBV	32.48,23.94

3.3.7. Parallel experiment results

Thirty-four specimens were tested consecutively 12 times, and the results were consistent with previously obtained results.

4. Discussion

The "Technical Operating Procedures for Blood Stations (2019 Edition)" requires installation confirmation, operational confirmation, and performance confirmation for new testing systems before use, with nucleic acid testing also necessitating analytical sensitivity verification. This experiment confirmed the Kehua NS2000 fully automated nucleic acid testing line before use, ensuring that personnel met nucleic acid testing requirements, the laboratory environment complied with standards, all necessary instruments and equipment were within calibration cycles, nucleic acid reagents met quality inspection requirements, internal quality control was stable, and the laboratory's BSLMBOX-V.3.0.8.4 blood station inspection quality management system and KHB Aurora SPS1200 operating software system (V1.00.00.20) were functioning properly.

This process required the cooperation of multiple departments to ensure that the pre-use verification was authentic and effective. The laboratory environment, required voltage for equipment, system hardware, and software, all met installation requirements, enabling normal operation for testing, data transmission, result interpretation, and report printing. Operational confirmation included five modules, and through blank water testing, it was confirmed that all equipment modules functioned normally, the instrument operated correctly, the mechanical gripper operated and grabbed properly, and the specimen transport track operated normally, meeting pre-use operational confirmation requirements.

According to the experimental results, the positive concordance rate, negative concordance rate, and overall concordance rate for HBV DNA, HCV RNA, and HIV-1 RNA were all 100%. The sensitivity and limit of detection of the second-generation Kehua nucleic acid testing reagents used with this testing system were higher than those of the first-generation reagents. The limits of detection for HBV-DNA, HCV-RNA, and HIV-RNA using the first-generation reagents were 5 IU/ml, 50 IU/ml, and 50 IU/ml in single testing mode and 30 IU/ml, 300 IU/ml, and 300 IU/ml in pooled testing mode, respectively. These were improved to 2.5 IU/ml, 9 IU/ml, and 20 IU/ml in single testing mode and 15 IU/ml, 54 IU/ml, and 120 IU/ml in pooled testing mode using the second-generation

reagents (**Table 4** results). The detection rates were all 100%, exceeding acceptable standard requirements and meeting verification criteria.

Studies have shown that interfering microorganisms, such as Epstein-Barr virus and exogenous interfering substances like interferon-2a, do not affect testing results [6]. The primary anti-interference substances affecting nucleic acid testing results come from lipemic and hemolytic specimens. According to **Table 5**, the system's anti-interference capability was analyzed, revealing that triglyceride concentrations of 33 g/L and hemoglobin concentrations of 5 g/L did not affect the detection of weakly positive specimens at 3 times the limit of detection (LOD). This verification referenced CNAS-GL039, adopting a three-test approach that met standard requirements. To ensure the accuracy of nucleic acid testing, specimens with triglyceride concentrations exceeding 33 g/L and hemoglobin concentrations exceeding 5 g/L should be identified, and it is recommended to create visual color comparison cards for lipemic and hemolytic specimens to reject non-compliant specimens [7].

The intra-batch coefficient of variation (CV) results for HBV-DNA, HCV-RNA, and HIV-RNA were 2.88%, 2.78%, and 2.49%, respectively, all within 10%. The inter-batch CV results were 3.45%, 3.20%, and 2.42%, respectively, all within 15%, meeting requirements. From **Table 7**, it can be seen that the negative quality control results were non-reactive, while the positive sample testing results were reactive. After repeating the test 15 times, the CVs were 1.58% for HBV-DNA, 1.50% for HCV-RNA, and 1.08% for HIV-RNA, all less than the inter-batch reagent variation of 15%, indicating good repeatability.

From **Table 8**, it can be analyzed that nucleic acid-negative (NHP) specimens tested negative, while positive specimens tested positive. The testing system could detect single-item HBV DNA at concentrations of 50 IU/ml, 1200 IU/ml, 12000 IU/ml, and 200000 IU/ml; single-item HCV RNA at concentrations of 300 IU/ml, 3000 IU/ml, and 30000 IU/ml; and single-item HIV-1 RNA at concentrations of 1500 IU/ml and 15000 IU/ml. For dual-item specimens, it could detect HIV+HBV (HIV concentration 10000 IU/ml, HBV 50 IU/ml), HCV+HIV (HCV concentration 300 IU/ml, HIV 10000 IU/ml), and HIV+HBV (HIV concentration 1000 IU/ml, HBV 10000 IU/ml). High-concentration sample testing did not interfere with negative specimens. After testing, attention should be paid to laboratory environment cleaning to prevent positive specimen contamination, and an emergency response plan should be developed in case of contamination [8]. This laboratory serves as a backup for another blood station laboratory, and emergency drills meet requirements. Parallel testing of 34 voluntary blood donor specimens over 12 consecutive days yielded consistent results, meeting verification requirements. To judge the validity of nucleic acid testing experiments, the following points should be noted: 1) Conduct internal quality control for each batch of specimens at least once, collecting 20 data points from the same quality control batch to plot a Levey-Jennings chart. Weakly positive quality control at 2–5 times the concentration should test reactive and comply with other validity judgment rules. Error analysis should be conducted for "out-of-control" batches, including the impact of freeze-thaw cycles on reagent kit stability, the effect of different operators on testing results, and potential causes of downward CT value trends, such as cumulative contamination, or upward CT value trends such as uneven temperature distribution in amplification instrument wells or loss of target nucleic acid [6, 9]. After implementing corrective and preventive measures, retesting should be conducted, and the results of specimens tested before the out-of-control incident should be evaluated [10]. 2) Reagent kit negative and positive controls must meet kit requirements. 3) If the internal control amplification curve CT is <40, nucleic acid extraction can be judged as effective. Reactive well internal controls may have no value due to competitive inhibition. 4) The national external quality assessment program for clinical laboratories has a compliance rate of 100%, exceeding the passing standard of 80%. The nucleic acid testing systems currently used in this laboratory include the Hamilton Star fully

automated sample processor with ABI7500 fluorescent PCR instrument nucleic acid testing system, the Beijing Wantai nucleic acid testing system, and the Kehua NS2000 fully automated nucleic acid testing line. Compared to the other two systems, the advantages of this system are as follows: 1) It reduces manual intervention, standardizes and automates the entire process, and facilitates operator use. 2) It facilitates integrated information management, allowing traceability of each operation step from "ATM"-style self-service specimen handover to pre-processing, testing, and automatic refrigerated storage after testing through the same information management system, facilitating quality control management. 3) It shortens nucleic acid testing time. The system can develop a reasonable pooling plan based on specimen quantity, featuring automatic PCR reagent configuration, automatic sealing, and automatic sorting of specimens for further testing, improving testing efficiency. The Beijing Wantai nucleic acid testing system currently only performs single testing, while both the Hamilton Star fully automated sample processor with ABI7500 fluorescent PCR instrument nucleic acid testing system and the Kehua NS2000 fully automated nucleic acid testing line use second-generation Kehua nucleic acid testing reagents and can test a maximum batch of 552 specimens. The nucleic acid testing times from specimen pre-processing to result reporting are 6.5 hours and 5–5.5 hours, respectively, shortening the testing time by 1 to 1.5 hours in actual work.

The Kehua NS2000 fully automated nucleic acid testing line meets nucleic acid testing requirements in terms of installation confirmation, operational confirmation, and performance confirmation, complying with ISO 15189 standards. Confirmation of the testing system requires strict control over various aspects, including the nucleic acid laboratory environment, comparisons among testing personnel, design of performance verification experiments, and preparation of diluted specimens. ISO 15189 accreditation is an effective certification of laboratory testing capabilities and management systems. The laboratory continuously optimizes its existing quality system and improves verification plans during the testing process to standardize and normalize testing, effectively ensuring the safety of the blood supply and promoting the high-quality development of the blood collection and supply industry.

Disclosure statement

The authors declare no conflict of interest.

References

[1] China National Accreditation Service for Conformity Assessment (CNAS), 2023, Accreditation Criteria for the Quality and Competence of Medical Laboratories (CNAS-CL02-A001:2023).

[2] China National Accreditation Service for Conformity Assessment (CNAS), 2019, Guidelines for Performance Verification of Molecular Diagnostic Testing Procedures (CNAS-GL039).

[3] China National Accreditation Service for Conformity Assessment (CNAS), 2025, Accreditation Guidelines for the Molecular Diagnostics Field in Medical Laboratories (CNAS-GL050).

[4] National Health and Family Planning Commission of the People's Republic of China, 2017, Analytical Quality Requirements for Important Routine Items in Clinical Qualitative Immunological Testing (WS/T 494-2017).

[5] China Blood Transfusion Association, 2019, Technical Requirements for Nucleic Acid Screening of Pathogens Transmissible Through Blood Transfusion (T/CSBT008-2019).

[6] Zang CB, Wang Y, Wang L, 2023, Exploration of Performance Verification Based on T/CSBT for Nucleic Acid Testing Systems in Blood Banks. Chinese Journal of Blood Transfusion, 36(9): 830–834.

[7] Han XY, Zhang ML, Feng X, et al., 2022, Exploration of Performance Verification Based on ISO 15189 for Nucleic Acid Testing Projects in Blood Banks. Chinese Journal of Blood Transfusion, 35(1): 95–98.

[8] Chen K, Wang YH, Yuan ZF, 2022, Exploration of Performance Verification Methods for Nucleic Acid Testing Systems in Blood Banks. Journal of Clinical Hematology, 38(8): 584–591.

[9] Xie YN, Li FY, Zhao Q, et al., 2022, Establishment of Statistical Quality Control Methods for Qualitative Nucleic Acid Testing Laboratories in Blood Banks. Journal of Qiqihar Medical University, 43(3): 245–249.

[10] National Health Commission of the People's Republic of China, 2024, Guidelines for the Clinical Laboratory Application of Real-Time Fluorescent Polymerase Chain Reaction (WS/T230—2024).

Publisher's note

Bio-Byword Scientific Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.