Performance Evaluation Report
AutoMolec 3000

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1. General Description

1.1 Manufacturer

1) Autobio Labtec Instruments Co.,Ltd.

2) No.199 15th Ave, National Eco & Tech Area, Zhengzhou, 450016 China

2. A description of the product

1) Product Name: Automated Nucleic Acid Purification and Real Time PCR System

2) Model: AutoMolec 3000

3) Basic UDI: 697304909Model59UX

4) Intended use:

AutoMolec 3000 is an in vitro molecular examination instrument integrating sample extraction and amplification analysis. It is based on the principle of polymerase chain reaction (PCR) and real-time fluorescence monitoring technology, automatically completes a series of operating steps from nucleic acid extraction and purification to amplification and examination. AutoMolec 3000 is intended for quantitative and qualitative examination of analytes in serum, plasma, whole blood, swab and sputum nucleic acid samples (DNA/RNA) from human body, such as pathogens and human gene polymorphism items, etc.

Authorization: The user manual and the operation of the AutoMolec 160 are to be only used by authorized or professionally trained personnel.

5) Structural composition

AutoMolec 3000 is composed of instrument, computer (optional), external barcode reader, control machine software (release version V1) and client software (release version V1.0). The instrument is composed of purification unit, ultraviolet unit, reagent transfer unit, PCR unit, discard unit and electric control box. The purification unit is composed of sampling probe unit, basket, magnetic incubation unit and consumables management unit.

3. Performance evaluation report

3.1 Scientific validity study report

1) There are three technical literature related with the Automated Nucleic Acid Purification and Real Time PCR System, the general information of the literature are described in the table below.

SN	Author	Literature Title	Website	Abstract	Originate	Issue time
1	Huimin Deng 1, Zhiqiang Gao 2	Bioanalytical applications of isothermal nucleic acid amplification techniques	https://pubmed.ncbi.nlm.nih.gov/25467448/	The most popular in vitro nucleic acid amplification techniques like polymerase chain reaction (PCR) including real-time PCR are costly and require thermocycling, rendering them unsuitable for uses at point-of-care. Highly efficient in vitro nucleic acid amplification techniques using simple, portable and low-cost instruments are crucial in disease diagnosis, mutation detection and biodefense. Toward this goal, isothermal amplification techniques that represent a group of attractive in vitro nucleic acid amplification techniques for bioanalysis have been developed. Unlike PCR where polymerases are easily deactivated by thermally labile constituents in a sample, some of the isothermal nucleic acid amplification techniques, such as helicase-dependent amplification and nucleic acid sequence-based amplification, enable the detection of bioanalytes with much simplified protocols and with minimal sample preparations since the entire amplification processes are performed isothermally. This review focuses on the isothermal nucleic acid amplification techniques and their applications in bioanalytical chemistry. Starting off from their amplification mechanisms and significant properties, the adoption of isothermal amplification techniques in bioanalytical chemistry and their future perspectives are discussed. Representative examples illustrating the performance and advantages of each isothermal amplification technique are discussed along with some discussion on the advantages and disadvantages of each technique.	Analytica Chimica Acta Volume 853, 1 January 2015, Pages 30-45	In the year 2014
2	Da-Sheng Lee ,	A novel real-time PCR machine with a	https://www.sciencedirect.com/science/artic	This study sets up a prototype of real-time polymerase chain reaction (RT-PCR) machine that employs a miniature spectrometer for	Sensors and Actuators B:	In the year

	Meng-HsunWu	miniature spectrometer for fluorescence sensing in a micro liter volume glass capillary	le/abs/pii/S0925400504000504?via%3Dihub	detecting the emission of fluorescence intensity from RT-PCR mix in a micro liter volume glass capillary. The RT-PCR machine is one of the major instruments for SARS virus test during the outbreak in Asia in early 2003. Comparing with traditional RT-PCR machine with discrete channels fluorescence wavelength detection, the prototype can provide continuous wavelength detection and can be employed for multiplex DNA quantification. However, only one HBV SC 11 DNA template with the SYBR Green I labeling dye were used in this study to compare DNA quantification accuracy and reproducibility of the present system and the commercial system. The two machines have different optical engine arrangement and so two separate analytical models were proposed to predict the fluorescence intensity from the RT-PCR mix during thermal cycling for the machines. Predicted results agree well with the measured data for both machines. From the predicted results, different approaches should be adopted to determine the initial DNA concentration for quantification from time recorded history of the fluorescence intensity. Measured results illustrate that the RT-PCR prototype has the same accuracy for DNA quantification and reproducibility within five intra-assay samples as compared with the commercial machine.	Chemical Volume 100, Issue 3, 15 May 2004, Pages 401-410	2004
3	Maxime Dooms, Abalo Chango, Afif Abdel-Nour 2	Quantitative PCR (qPCR) and the guide to good practices MIQE: adapting and relevance in the clinical biology context	https://pubmed.ncbi.nlm.nih.gov/24876137/	The qPCR has been introduced in clinical and biomedical research for over 10 years from now. Its use in trials and diagnostics is continuously increasing. Due to this heavy use, the question of relyability and relevance of qPCR results has to be asked. This review proposes a documented and evidence based answer to this question, thanks to the MIQE (minimum information for publication of quantitative real-time PCR experiments) guideline. The whole analysis process is addressed, from nucleic acids extraction to data management. Simple answers are given, taking into account the technical constraints from clinical research in order to allow a	Ann Biol Clin (Paris) May-Jun 2014;72(3):265-9.doi: 10.1684/abc.2014.0955.	In the year 2014

				realistic application of this guideline.		

2） Literature research:

For the literatures searched, these 3 literature related to the AutoMolec 3000 technology, these literature are related to Nucleic Acid Amplification Real time PCR technology.

3.2 Analytic performance study report

3.2.1. Overview

This performance evaluation study was carried out in accordance with the BS EN 13612:2002 standard and was carried out in strict accordance with BS EN 13612:2002 in terms of researchers, research sites, evaluation plans, experimental design and test records, ensuring the rigor of the experiment and the reliability of the report.

3.3.2 Performance Evaluation Study

3.2.2.1 Date and Site

Research time: 2019~2020

Site: Test Lab

3.2.2.2 Qualification of personnel and training

No.	Personnel	Qualificati ons	Remarks
1	Li Zhenkun	Project manager	Medical device project management training
2	Sun Long	Mechanical design engineer	Medical device mechanical design training
3	Zhao Junhu	Electronic design engineer	EMC design training
4	Fu Kunming	Software design engineer	Software coding specification training
5	He Fazhan	Liquid path design engineer	Fluid mechanics design training

3.2.2.3 Specifications of the device

1) Instrument Information

AutoMolec 3000 is composed of instrument, computer (optional), external barcode reader, control machine software (release version V1) and client software (release version V1.0). The instrument is composed of purification unit, ultraviolet unit, reagent transfer unit, PCR unit, discard unit and electric cabinet. The purification unit is composed of sampling probe unit, basket, magnetic incubation unit and consumables management unit.

2) Performance to be Evaluated:

No.	Items	Design requirements
1	Appearance	The graphic symbols and words on the panel shall be accurate, clear, uniform, and shall not have obvious scratches;
		Fastener connection should be firm and reliable, not loose;
		Moving parts should be stable, not be stuck or jump.
2	Temperature of the thermal	Low temperature incubation: 37℃±2℃;
		High temperature incubation:80℃±3℃。
		Heating rate: a)The average heating rate shall be no less than 3.0℃/s from 50℃~90℃; b) The maximum heating rate shall be no less than 6.0℃/s from 50℃~90℃;
		Cooling rate:a)The average cooling rate shall be no less than 2.5℃/s from 90℃~50℃; b) The maximum cooling rate shall be no less than 4.0℃/s from 90℃~50℃;
		Temperature control precision of module:module control precision should be no large than 0.2℃.
		Temperature accuracy: The absolute value of the difference between the measured value and the set temperature should not be greater than 0.1℃ at 60℃; b） The absolute value of the difference between the measured value and the set temperature should not be greater than 0.3℃ at

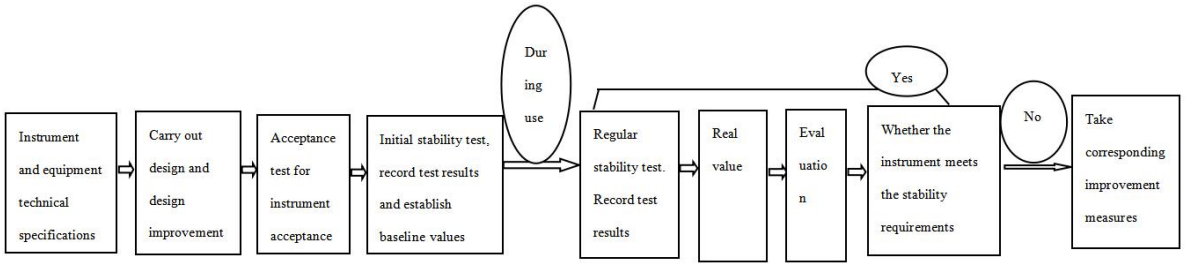
		95℃.
		Module temperature uniformity: a) Temperature uniformity at 60℃ should be within the range of ±0.2℃; b)Temperature uniformity at 95℃ should be within the range of ±0.5℃.
		Temperature duration accuracy： The relative deviation of temperature duration and system temperature time is within ±5%.
3	Fluorescence intensity detection	Repeatability of fluorescence intensity detection: The coefficient of variation (CV, %) of each calibrated dye with high, medium and low concentrations was not more than 1.5% in the range of product determination.
		Fluorescence intensity detection precision: Within the product measurement range, 10 test holes were randomly selected and each calibration dye with high, medium and low concentrations was used for detection. The coefficient of variation (CV, %) of fluorescence value at the same concentration was not more than 5%.
		Fluorescence interference of different channels: the fluorescence detection intensity of other channels is not higher than the fluorescence threshold of the target channel.
		Fluorescence linearity: The linear regression coefficient R should not be less than 0.990 for the detection of a series of diluted fluorescent dye substances (5 gradients).
4	Sample test repeatability	When detecting high, medium and low concentration nucleic acid samples, the coefficient of variation of CT value of corresponding channel should not be more than 3%
5	Samples of the	Linear evaluation of samples (5 gradients) was carried out, and

	linear	the absolute value of linear regression coefficient R should not be less than 0.980
6	Accuracy and precision of dispensing probe:	Sampling probe: Accuracy:100ul±5ul, Repeatability:CV≤2%; Accuracy:600ul±12ul, Repeatability:CV≤2%. Reagent probe: Accuracy:10ul±0.5ul, repeatability CV≤4%; accuracy:50ul±2.5ul，repeatability CV≤3%.
7	UV-radiation intensity requirements	Ultraviolet radiation intensity: no less than 400mW/㎡.
8	Main functions of software	a) The product can indicate the status of reagents, consumables and wastes b)The product can display the remaining quantity of reagents and consumables; It can display the state of purified water, waste liquid container, solid waste bin, consumables, etc.
		The product has the function of self-inspection.
		The product has troubleshooting prompt such as tips consumable grasping failure, door being opened during operation, “Air Suction” or “Tip Blockage”, and the temperature state of the incubation reaction zone can be monitored in real time.
		After networking, the software can be upgraded remotely.
		a)The software has the function of systematic diagnosis, which is used to check the main functions of the instrument, including: scanning module inspection, cleaning PCR, simultaneous pump quantitative/airtight test, and incubation test routines;

		b)The software has the function of system setting, and users can set custom software functions through the system, mainly including: general setting, report setting, LIS communication setting, administrator password, backup plan, and about;
		c)The software has maintenance function to support users to carry out daily maintenance of the instrument, mainly including: maintenance, cleaning, debugging, system; Maintenance includes instant backup, recovery of the system, clearing historical data, cleaning includes daily cleaning, cleaning PCR, UV lamp disinfection, debugging includes monomer adjustment, sensor list, the system includes shutting down the system, closing the software, reinitialization, switching users.
		d)The software has test and analysis function, and users can conduct test management according to clinical application requirements. Mainly includes: consumables and calibration, quality control, test request, the test run, the result analysis, which contains consumables consumables, reagent management, calibration contain liquid calibration, the calibration set, quality includes quality control, quality control, test request contains patients request, quality inspection and selection test request, test runs including fluorescence curve, anomaly detection.
		e)The software has the shortcut operation button of the common function, is convenient for the user to operate. Mainly include: abnormal detection, reagents, consumables, quality control, logs, main interface, test request, fluorescence curve, test results.
9	Safety	The electrical safety of the instrument shall meet the

	requirements	requirements of IEC 61010-1:2017, IEC 61010-2-101:2018, IEC 61010-2-101: The basic safety features of the product are shown in Appendix B.
11	EMC Requirements	EMC should meet the requirements of BS EN 61326-1-2013 and BS EN 61326-2-6-2013.
12	Data interface	The test results in AutoBio-ACD format can be uploaded through the RJ45 interface using TCP/IP protocol. Test results in AUTOBIO-ACD format can be uploaded through serial interface using RS232 protocol The 1D/2D barcode information can be obtained through USB2.0, USB3.0 interface, using HID protocol, connected to external barcode reader; It can be used by removable storage devices through USB2.0 and USB3.0 interfaces. Data storage format: LDF and MDF files
13	User access mechanism	User access control mode: different user privileges use different passwords; The user access control rights are divided into three categories: operator, administrator and maintainer. Maintainers have full access to software functions. Administrators have all rights to software functions except for individual adjustments. The operator has all privileges in the software functions except for individual adjustment system setup system diagnosis instant backup and recovery system.

3. Quality Control



3.2.3 Experimental Design Scheme

3.2.3.1 Appearance

Visual inspection results should meet the requirements.

3.2.3.2 Temperature

1) Low temperature of reagent area

Trial methods

a. Open the product and preheat for 30 mins.

b. Take the 6 probes on the temperature tester and place them in the liquid at 6 positions inside the refrigerated reagent area

c. Close the cover of the reagent area.

d. After the temperature is balanced, test the temperature every 30 seconds for 10 minutes and record the data

e. The data obtained from the test shall meet the requirements in the table.

2) Low temperature of reaction area

Trial methods

f. Open the product and preheat for 30 mins.

g. Fix the probe with adhesive tape in the middle of the outer wall of the extractor reaction chamber.

h. Put the extract strip into any incubator of the incubator module.

i. After the temperature is balanced, the temperature was measured every 5 seconds and a total of 20 data were recorded.

j. The data obtained from the test shall meet the requirements in the table.

3) High temperature incubation

Trial method:

a. Open the product and preheat for 30 mins.

b. Add 260 μl purified water to the extraction strip reaction chamber.

c. Put the extract strip into any incubator of the incubator module,the temperature probe is fixed in the liquid.

d. After the temperature was balanced, the temperature was measured every 5 seconds and total of 20 data were recorded.

e. The data obtained from the test shall meet the requirements

Temperature index of thermal cycle module

4) Temperature indicator of the thermal unit

4.1) Heating rate

According to the representative of a specific temperature, run a 45 (constant temperature 30 s) and 95 (constant temperature 30 s) between circulating temperature control program On module randomly selected from 6 amplification module, select each module in the hole near the internal sensor, the temperature measuring equipment of thermal probe, respectively in the module test hole, the other end connection temperature tester Turn on the temperature tester, confirm the product works normally, run the routine, and use the temperature tester to record the temperature change during the period from the time when the product display temperature reaches the set temperature and the constant temperature is 10s to the end of the constant temperature.

Steps	Temperature	Times
1	45℃	30 s
2	95 ℃	30 s
3	45 ℃	30 s

a. Average heating rate:

Timing starts from room temperature. After preheating 74s, take a temperature point within the range of 50±0.5 and denote the temperature as T_a , take a temperature point within the range of 90±0.5 and denote the temperature as T_b , and denote t from the time T_a to arrive at the time T_b . Calculate the average heating rate according to Formula (1), and the result shall meet the specified requirements

$$V_{ascendM} = \frac{T_b - T_a}{t} \quad \text{.....（1）}$$

In the Formula(1):

$V_{ascendM}$ ——Average heating rate;

T_b ——Any temperature point within 90℃±0.5℃;

T_a ——Any temperature point within 50℃±0.5℃;

t—— From T_a to T_b

The calculated results can meet the requirements.

b. The maximum heating rate:

Set the time interval of temperature acquisition as△t, △t≤1s and as small as possible. Start the timing from room temperature. After preheating 74s, the maximum instantaneous temperature change (△T max) in the process of scanning temperature rising from 50±0.5 to 90±0.5 was calculated according to formula (2)

$$V_{ascend\ max} = \frac{\triangle T_{max}}{\triangle t} \quad \text{.....（2）}$$

In the formula(2)

$V_{ascendmax}$ ——The maximum heating rate

△Tmax——Maximum instantaneous temperature change from 50±0.5 to 90±0.5

△t——Temperature acquisition time interval (no more than 1s)

The calculated results can meet the requirements.

4.2) Cooling rate

According to the representative of a specific temperature, run a 45 (constant temperature 30 s) and 95 (constant temperature 30 s) between circulating temperature control program On module randomly selected from 6 amplification module, select each module in the hole near the internal sensor, the temperature measuring equipment of thermal probe, respectively in the module test hole, the other end connection temperature tester Turn on the temperature tester, confirm the product works normally, run the routine, and use the temperature tester to record the temperature change during the period from the time when the product display temperature reaches the set temperature and the constant temperature is 10s to the end of the constant temperature

Steps	Temperature	Times
1	45℃	30 s
2	95 ℃	30 s
3	45 ℃	30 s

a. Average cooling rate

Timing starts from room temperature. After preheating 74s, take a temperature point within the range of 50±0.5 and denote the temperature as T_a , take a temperature point

within the range of 90±0.5 and denote the temperature as T_b, and denote t from the time T_a to arrive at the time T_b. Calculate the average cooling rate according to Formula (3), and the result shall meet the specified requirements

$$V_{descendM}=\frac{T_B-T_A}{t} \quad \text{.....（3）}$$

In the Formula(3)

$V_{descendM}$——Average heating rate；

T_B ——Any temperature point within 50℃±0.5℃；

T_A ——Any temperature point within90℃±0.5℃；

t—— from T_A to T_B

The calculated results can meet the requirements.

b. The maximum cooling rate:

Set the time interval of temperature acquisition as△t, △t≤1s and as small as possible. Start the timing from room temperature. After preheating 74s, the maximum instantaneous temperature change (△T max) in the process of scanning temperature descends from 90±0.5 to 50±0.5 was calculated according to formula (4)

$$V_{\text{descend max}}=\frac{\triangle T\,\text{max}}{\triangle t} \quad \text{.....（4）}$$

In the formula,

$V_{\text{descend max}}$ ——The maximum cooling rate:

△Tmax——the maximum instantaneous temperature change (△T max) descending from 90℃±0.5℃ to 50℃±0.5℃.

△t——Temperature acquisition time interval（no more than 1s）.

The calculated results can meet the requirements.

5) Temperature control precision of module:

Run a temperature cycle file program, take one temperature point in the range of 60℃±5℃ and 95℃±5℃ respectively, set the constant temperature for 3min, and cycle for 5 times. Six amplification modules were randomly selected, and the hole position close to the internal sensor in each module was selected. The temperature sensing probes of the temperature measuring equipment were put into the test holes of the modules respectively, and the other

end was connected with the temperature tester. Open the temperature tester, confirm that the product works normally, run the routine, display the temperature reached the set temperature, constant temperature 10s, timing 30s, record the highest temperature and the lowest temperature, the difference between the two is half △Ti. Five consecutive cycles were recorded, and the maximum △Ti (I =1,2,3,4,5) should meet the requirements.

Step	Temperature	Time
1	60℃	3min
2	95℃	3min

6) Temperature accuracy

A temperature cycle file program was performed. One temperature point was selected in the range of 60℃±5℃ and 95℃±5℃ respectively, and the constant temperature was set for 3min. The number of cycles was once. Six amplification modules were randomly selected, and the hole position close to the internal sensor in each module was selected. The temperature sensing probes of the temperature measuring equipment were put into the test holes of the modules respectively, and the other end was connected with the temperature tester. Turn on the temperature tester to confirm that the product works normally, run the routine, and show that the temperature reaches the set temperature. After the constant temperature is 10s, time 60s, and record the temperature Ti (I =1,2,3,4,5,6,) every 10s. The average value is Tm and the absolute value of the difference between the set temperature should meet the requirements.

Step	Temperature	Time
1	60℃	3min
2	95℃	3min
3	60℃	3min

7) Module temperature uniformity:

Run a temperature file program, take one temperature point in the range of 60℃±5℃ and 95℃±5℃ respectively, set the constant temperature for 3min, and cycle for 5 times. Six amplification modules were randomly selected on the module, and the hole position close to the internal sensor in each module was selected. The temperature sensing probes of the temperature measuring equipment were put into the test holes of the module respectively, and the other end was connected with the temperature tester. Turn on the temperature tester

to confirm that the product is working normally, run the routine, and display the temperature in any hole to reach the set temperature. After the constant temperature is 10s, time 60S. The recorded temperature was Ti (I =1,2... 6), take the maximum and minimum value of Ti, calculate the temperature difference △T of each detection hole position, and the temperature difference should meet the requirements.

Step	Temperature	Time
1	60℃	3min
2	95℃	3min

8) Temperature duration accuracy

Run a file that cycles between 45℃ (constant temperature time t, t=180s) and 95℃ (constant temperature time t, t=180s). One amplification module was randomly selected on the module, and the hole position close to the internal sensor was selected in the module. The temperature sensing probe of the temperature measuring tool was put into the test hole of the module, and the other end was connected with a temperature tester. Turn on the temperature tester, confirm the product works normally, run the routine, take 95℃±0.5℃ as the timing reference point, start the timing from the temperature displayed (temperature tester) reaches the timing reference point for the first time, and finish the recording time as TI (I =1,2,3,4,5), and continuously record 5 cycles. The relative deviation calculated according to Equation (5) shall meet the requirements.

$$\text{Relative deviation} = \frac{(t_m - t)}{t} \times 100\% \qquad \text{.....（5）}$$

In the Formula(3)

tm——The average value of the 5 cycles is recorded

t—— Compilation of constant temperature time

Steps	Temperature	Times
1	45℃	3min
2	95℃	3min

3.2.3.3 Fluorescence intensity detection

1) Repeatability of fluorescence intensity

Within the measurement range of the product, one detection channel is randomly selected and the calibration fluorescent dye solution is configured for detection. One detection hole is randomly selected for each calibrated dye with high, medium and low concentrations, and the detection is repeated for 10 times. The photoelectric module collects

the data of the target channel.

The mean M and standard deviation SD of the measurement results of the calibrated dyes at each concentration were calculated respectively, and the coefficient of variation CV was obtained according to Equation (6). The results should meet the requirements of 2.3.1.

$CV = SD / M \times 100\% .....$（6）

In the Formula(3)

CV ——Coefficient of variation；

SD ——Standard deviation；

M ——Mean of the measured result；

Fluorescence intensity detection precision

Within the product measurement range, 1 detection channel and 10 detection holes were randomly selected. The calibration fluorescent dye solution was prepared for detection, and each calibration dye with high, medium and low concentrations was detected once. The photoelectric module collected the data of the target channel.

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The mean M and standard deviation SD of the measurement results of the calibrated dyes at each concentration were calculated respectively. The coefficient of variation CV was obtained according to Equation (6), and the results should meet the requirements.

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2) Fluorescence interference of different channels.

One detection hole was randomly selected, and two channels were randomly selected for detection. Fluorescence solution of non-target channel was configured respectively. The photoelectric module collected data of all channels, and the results should meet the requirements after fluorescence correction.

One detection hole was randomly selected, and two channels were randomly selected for detection. Fluorescence solution of non-target channel was configured respectively. The photoelectric module collected data of all channels, and the results should meet the

requirements after fluorescence correction.

3) Fluorescent linear

After gradient dilution of standard fluorescent dye with known concentration (dilution with 5 gradients), test 3 Wells in parallel for each concentration gradient. Calculate linear correlation coefficient R by taking dilution ratio and mean value of fluorescence determination according to Equation (7), and the result should meet the requirements of 2.5.2.

$$r = \frac{\sum\left[\left(X_i - \overline{X}\right)\left(Y_i - \overline{Y}\right)\right]}{\sqrt{\sum\left(X_i - \overline{X}\right)^2 \sum\left(Y_i - \overline{Y}\right)^2}} \quad \text{.....（7）}$$

In the Formula(3)

X_i： Dilution ratio

$\overline{X}$： Mean dilution ratio

Y_i： Fluorescence mean value of each concentration

$\overline{Y}$： Fluorescence mean values at different concentrations

3.2.3.4 Repeatability of sample test

Influenza A virus/influenza B virus/respiratory syncytial virus nucleic acid detection kit (PCR-fluorescent probe) and human CYP2C19 gene detection kit (PCR-fluorescent probe) were selected: The corresponding samples with high, medium and low concentration levels were detected respectively for 10 times, and the mean value M and standard deviation SD of the corresponding channel CT values were calculated respectively for 10 times. The coefficient of variation Cv was obtained according to the formula Cv =SD/M×100%, and the results should meet the requirements of 2.4.

3.2.3.5 Sample liner

Hepatitis B Virus Nucleic Acid Detection Kit (PCR-Fluorescent Probe) : The high value sample close to 2.5×108IU/mL was diluted by 10 times gradient (5 gradients), and the corresponding reagents were selected according to the test items. Each concentration gradient was tested in parallel for 3 Wells, and the linear correlation coefficient R was calculated by taking the mean value of CT and the numerical mean value of concentration against each other, and the results should meet the requirements of 2.5.1.

Hepatitis C Virus Nucleic Acid Detection Kit (PCR-Fluorescent Probe) : The high value sample close to 2.5×108IU/mL was diluted by 10 times gradient (5 gradients), and the

corresponding reagents were selected according to the test items. Each concentration gradient was tested in parallel for 3 Wells, and the linear correlation coefficient R was calculated by taking the mean value of CT and the numerical mean value of concentration against each other, and the results should meet the requirements of 2.5.1.

3.2.3.6 Dispensing probe accuracy and repeatability

Sampling probe test method

a) Open the product and preheat for 30 minutes.

b) Pour purified water into the specified container

c) The eight empty cups of the containers were numbered 1-- 8, and their weight was recorded as M1 -- M8 by an electronic balance.

d) Place the container into the hole in the sample position of the left unit of the product in sequence, and run the software to add the sample needle 100ul.

e) After adding the samples, the samples were respectively weighed and marked as MJ1 -- MJ8.

f) According to the weight after adding samples and the weight of the empty cup, the injection mass m was calculated.

g) Calculate the added sample volume according to Equation (8).

h) Repeat the above test for 10 times;

i) Calculate the average value of each probe test and the results should meet the needle accuracy requirements of the sample in Table 2.

j) The probe repeatability of the sample was calculated according to Equation (9)

k) Perform a 600ul sample add test for the left unit sample needle according to the above test

l) Complete the sample adding test of 100ul and 600ul for the sample needle of the right unit according to the above steps; The results shall meet the requirements of 2.6

Reagent probe test method

m) Open the product and preheat for 30 minutes.

n) Pour purified water into the specified container;

o) The empty cups of 20 containers were numbered 1-- 20 respectively, and their weight was recorded as M1 -- M20 with an electronic balance

p) Place the container into the hole in the reagent position of the right unit of the product in sequence, and run the software to add the reagent probe 10ul.

q) After adding samples, the samples are respectively weighed and denoted asmJ1---mJ20;

r) According to the weight after adding samples and the weight of the empty cup, the injection mass m was calculated.

s) Calculate the sampling volume according to formula (8).

t) The average value of the calculated value should meet the requirements of probe accuracy in Table 2

u) Calculate the reagent probe repeatability according to formula (9).

v) Repeat the above experiment, then sample adding test to the probe of the left unit of 50μL;

w) Follow the above steps to complete the sample adding test of the reagent probe of the right unit 10ul and 50ul; The results shall meet the requirements of section 2.6

$$\nu = \frac{\mathrm{m}}{\rho} \text{.....（8）}$$

In the formula:

m ——Dispensing quality.

ρ ——Water density(See the annex C)

$$CV = \frac{SD}{\overline{X}} \times 100\% \text{.....（9）}$$

In the formula:

SD ——Standard deviation of injection quality test value

$\overline{X}$ ——The average value of the injection quality test

3.2.3.7 Ultraviolet radiation intensity

Test method:

Select a point in the purification area, PCR consumable area, thermal circulation area and solid waste bin, align the receiver with the UV light source when placed, close the protective door, turn on the UV lamp, read the UV radiation illuminance meter reading after stabilization, the value should meet the requirements.

Main function:

The product can indicate the status of reagents, consumables and wastes

Open the software, operate the instrument, add TIP consumables and PCR consumables; Click the "Reagent" and "Consumable" buttons respectively in the software to check the status information, which should meet the requirements

The product has the function of self-inspection

In the software ready state, unplug the network cable, click “Reinitialize”, the software display “Control machine connection error”, the software into the offline state; After the network cable is inserted, reinitialization is performed, the product should be able to connect to the control machine, and the software shows “Ready”.

The product has a fault prompt function

Perform the following operations respectively, and the results shall meet the requirements of 2.8.3

a）Manually remove part of the TIP consumable and the module starts testing the request

b） During the operation of the product, open the left or right door of the product to observe the status of the product.

c）Manually remove the reagent solution, the module starts the test request, and view the "Log" of the software interface.

d） Manually remove the reagent solution, the module starts the test request, and check the "Log" on the software interface, prompting "Hollow suction in the process of reagent extraction"; After the 2ml Tip consumables are blocked, the module starts the test request, and "Log" on the software interface is displayed, indicating "Probe blocked during reagent extraction".

e）Software ready status: view the temperature information area on the interface.

Remote service function

After connecting with the network, if a new software version is detected, users can upgrade the software to the new version. The result must meet the requirements.

3.2.3.8 Software function

（1）When running the software, view the software interface under the permission of the maintenance personnel. The requirements must be met

（2）When running the software and viewing the software interface under the permission of the administrator, the requirements must be met.

3.2.3.9 Safety requirements

Electronic safety test method should be executed according to IEC 61010-1:2017, IEC 61010-2-101:2018, IEC61010-2-010:2019.

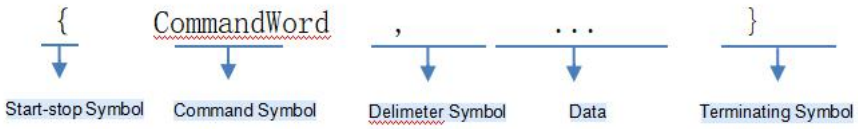
3.2.3.10 EMC Test Method

EMC test method should be executed according to BS EN 61326-1-2013 and BS EN 61326-2-6-2013.

3.2.3.11 Data Interface

a） Set LIS communication port to network port, IP to 192.168.37.18, and port to 6666; Open LIS/HIS debugging software, set IP to 192.168.37.18 and port to 6666; The Wire shark is used to obtain communication data over THE TCP/IP protocol. The data format is checked in UTF8 format. The data format must meet the Autobio-ACD format requirements.

Autobio-ACD format：



b）Set LIS communication port as serial port, port as COM1, baud rate as 9600, data bit as 8, parity check as none, flow control as none, and stop bit as none. Open LIS/HIS debugging software, set the port to COM2, baud rate to 9600, data bit to 8, parity check to none, flow control to none, and stop bit to none; The CommMonitor packet capture tool is used to obtain communication data through RS232 protocol, and the data format is verified in UTF8 format. The data format must meet the requirements of Autobio-ACD format

c）Use USB2.0/USB3.0, connect USB external bar code reader that meets HID protocol, edit a TXT document, use external bar code reader to scan 1D bar code and 2D bar code respectively, 1D/2D bar code information should be obtained;

d）If USB2.0 or USB3.0 is used to connect to removable storage devices, files can be copied in and out

e）It should meet the requirements to find the saved file related data in the computer.

3.1.13 User Access Mechanism

Run the software, select the administrator and maintenance administrator rights, and log in with the corresponding password, and select the operator login without password, both can log in successfully;

Run the software and select the rights of operator, administrator, and maintenance personnel respectively. The actual check rights should meet the requirements.

3.2.4 Time Schedule

No.	Test Item	Start Date
1	Appearance	2020-09-29
2	Temperature	2020-09-21
3	Fluorescence intensity detection	2020-09-24
4	Sample test	2020-10-09

	repeatability	
5	Samples of the linear	2020-10-09
6	Accuracy and precision of dispensing probe:	2020-09-16
7	UV-radiation intensity requirements	2020-09-23
8	Main functions of software	2020-12-28
9	Safety requirements	2020-09-15
10	EMC Requirements	2020-09-21
11	Fluorescence intensity detection	2020-09-24
12	Data interface	2020-09-18
13	User access mechanism	2020-09-25

1. Test Result Analysis

No.	Test Items	Standard requirements	Result
1	Appearance	The graphic symbols and words on the panel shall be accurate, clear, uniform, and shall not have obvious scratches;	Pass

		Fastener connection should be firm and reliable, not loose;	
		Moving parts should be stable, not be stuck or jump.	
2	Temperature	Low temperature incubation: 37℃±2℃;	Pass
		High temperature incubation:80℃±3℃。	
		Heating rate: a)The average heating rate shall be no less than 3.0℃/s from 50℃~90℃; b) The maximum heating rate shall be no less than 6.0℃/s from 50℃~90℃;	
		Cooling rate:a)The average cooling rate shall be no less than 2.5℃/s from 90℃~50℃; b) The maximum cooling rate shall be no less than 4.0℃/s from 90℃~50℃;	
		Temperature control precision of module:module control precision should be no large than 0.2℃.	
		Temperature accuracy: The absolute value of the difference between the measured value and the set temperature should not be greater than 0.1℃ at 60℃; b） The absolute value of the difference between the measured value and the set temperature should not be greater than	

		0.3℃ at 95℃.	
		Module temperature uniformity: a)Temperature uniformity at 60℃ should be within the range of ±0.15℃; b)Temperature uniformity at 95℃ should be within the range of ±0.5℃.	
		Repeatability of fluorescence intensity detection: The coefficient of variation (CV, %) of each calibrated dye with high, medium and low concentrations was not more than 1.5% in the range of product determination.	
3	Fluorescence intensity detection	Fluorescence intensity detection precision: Within the product measurement range, 10 test holes were randomly selected and each calibration dye with high, medium and low concentrations was used for detection. The coefficient of variation (CV, %) of fluorescence value at the same concentration was not more than 5%.	Pass
		Fluorescence interference of different channels: the fluorescence detection intensity of other channels is not higher than the fluorescence threshold of the target channel.	
		Fluorescence linearity: The linear regression coefficient R should not be	

		less than 0.990 for the detection of a series of diluted fluorescent dye substances (5 gradients).	
		When detecting high, medium and low concentration nucleic acid samples, the coefficient of variation of CT value of corresponding channel should not be more than 3%	
4	Sample test repeatability	Linear evaluation of samples (5 gradients) was carried out, and the absolute value of linear regression coefficient R should not be less than 0.980	Pass
5	Samples of the linear	Sampling probe: Accuracy:100ul±5ul, Repeatability:CV≤2%; Accuracy:600ul±12ul, Repeatability:CV≤2%. Reagent probe: Accuracy:10ul±0.5ul, repeatability CV≤4%; accuracy:50ul±2.5ul, repeatability CV≤3%.	Pass
6	Accuracy and precision of dispensing probe:	Ultraviolet radiation intensity: no less than 400mW/㎡.	Pass
7	UV-radiation intensity requirements	a) The product can indicate the status of reagents, consumables and wastes b)The product can display the	Pass

		remaining quantity of reagents and consumables; It can display the state of purified water, waste liquid container, solid waste bin, consumables, etc.	
8	Main functions of software	The product has the function of self-inspection.	Pass
		The product has troubleshooting prompt such as tips consumable grasping failure, door being opened during operation, “Air Suction” or “Tip Blockage”, and the temperature state of the incubation reaction zone can be monitored in real time.	
		After networking, the software can be upgraded remotely.	
		a)The software has the function of systematic diagnosis, which is used to check the main functions of the instrument, including: scanning module inspection, cleaning PCR, simultaneous pump quantitative/airtight test, and incubation test routines;	
		b)The software has the function of system setting, and users can set custom software functions through the system, mainly including: general setting, report setting, LIS communication setting, administrator	

		password, backup plan, and about;	
		c)The software has maintenance function to support users to carry out daily maintenance of the instrument, mainly including: maintenance, cleaning, debugging, system; Maintenance includes instant backup, recovery of the system, clearing historical data, cleaning includes daily cleaning, cleaning PCR, UV lamp disinfection, debugging includes monomer adjustment, sensor list, the system includes shutting down the system, closing the software, reinitialization, switching users.	
		d)The software has test and analysis function, and users can conduct test management according to clinical application requirements. Mainly includes: consumables and calibration, quality control, test request, the test run, the result analysis, which contains consumables consumables, reagent management, calibration contain liquid calibration, the calibration set, quality includes quality control, quality control, test request contains patients request, quality inspection and selection test request, test runs	

		including fluorescence curve, anomaly detection.	
		e)The software has the shortcut operation button of the common function, is convenient for the user to operate. Mainly include: abnormal detection, reagents, consumables, quality control, logs, main interface, test request, fluorescence curve, test results.	
		The electrical safety of the instrument shall meet the requirements of IEC 61010-1:2017, IEC 61010-2-101:2018, IEC 61010-2-101: The basic safety features of the product are shown in Appendix B.	
9	Safety requirements	EMC should meet the requirements of BS EN 61326-1-2013 and BS EN 61326-2-6-2013.	Pass
10	EMC Requirements	The test results in AutoBio-ACD format can be uploaded through the RJ45 interface using TCP/IP protocol. Test results in AUTOBIO-ACD format can be uploaded through serial interface using RS232 protocol The 1D/2D barcode information can be obtained through USB2.0, USB3.0	Pass

		interface, using HID protocol, connected to external barcode reader; It can be used by removable storage devices through USB2.0 and USB3.0 interfaces. Data storage format: LDF and MDF files	
11	Fluorescence intensity detection	User access control mode: different user privileges use different passwords; The user access control rights are divided into three categories: operator, administrator and maintainer. Maintainers have full access to software functions. Administrators have all rights to software functions except for individual adjustments. The operator has all privileges in the software functions except for individual adjustment system setup system diagnosis instant backup and recovery system.	Pass
12	Data interface	The graphic symbols and words on the panel shall be accurate, clear, uniform, and shall not have obvious scratches;	Pass

13	User access mechanism	Fastener connection should be firm and reliable, not loose;	Pass

3.2.5 Conclusion

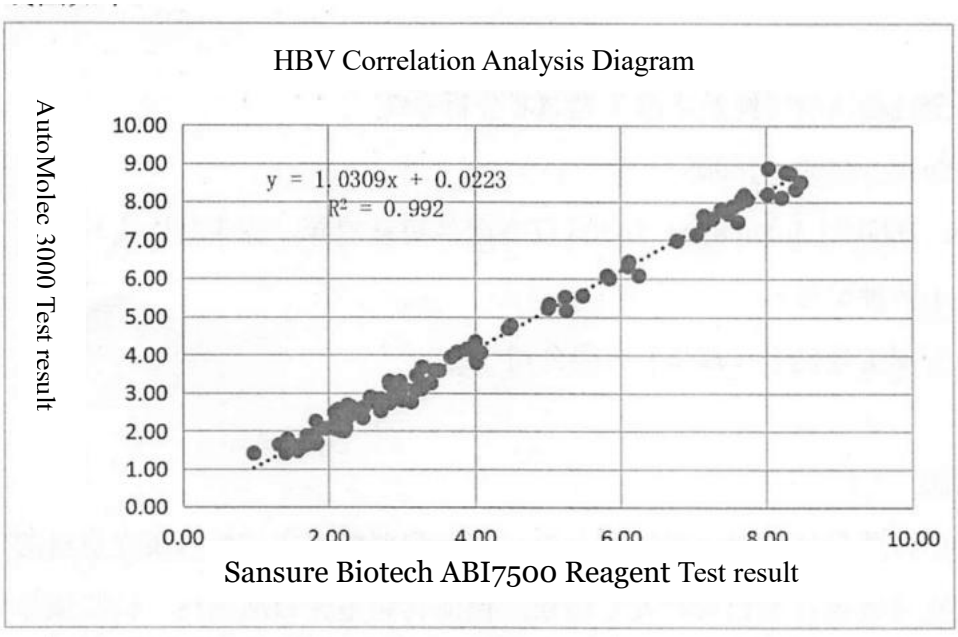
The performance of AutoMolec 3000 can meet the requirements of design.

3.3 Clinical performance study Report

3.3.1 Correlation analysis of test results:

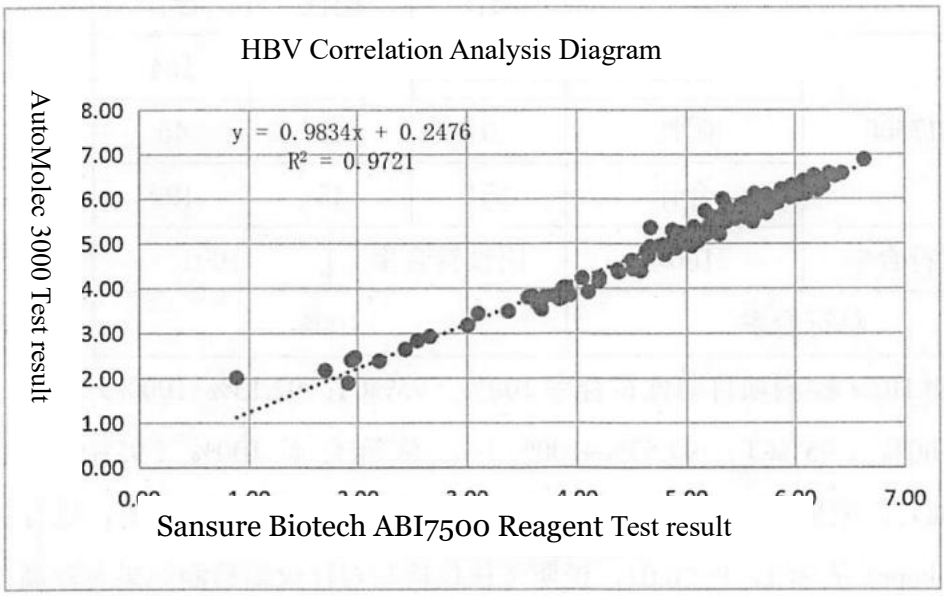
1) HBV DNA correlation analysis

Correlation analysis was performed on the concentration log value of clinical samples of HBV from the two testing systems, and the correlation curve is shown as follows:



The correlation curve equation of HBV detection results is γ =0309x+0.0223, and the correlation coefficient r=0.9960, indicating that the two groups of data are highly correlated.

2) HCV RNA correlation analysis



The correlation curve equation of HCV detection results is Y = 0.9834x + 0.2476, and the correlation coefficient r=0.9859, indicating that the two groups of data are highly correlated. The results showed that the negative coincidence rate of HBV test items was 100% C (95%CI: 93.69%-100%), the positive coincidence rate was 100% (95%CI: 96.79%-100%), and the total coincidence rate was 100% (95%CI:97.83% ~ 100%). SPSS26 statistical analysis software was used for data processing and statistical analysis, and Kappa test was carried out. The Kappa value was 1, P<0.01, indicating that there was a high consistency between the test instrument and the comparison instrument.

3.3.2 Statistic of detection result conformance rate

1) Statistics on the coincidence rate of HBV DNA test results

HBV DNA		AutoMolec 3000		
		Negative	Negative	Total
ABI7500	Positive	116		116
	Negative		57	57
	Total	116	57	173
Positive coincidence rate	100%	Negative coincidence rate		100%
Total coincidence rate		100%		

2) Statistics on the coincidence rate of HBV RNA test results

HCV RNA		AutoMolec 3000		
		Negative	Negative	Total
ABI7500	Positive	154		154
	Negative		45	45
	Total	154	45	199
Positive	100%	Negative coincidence rate		100%
Total coincidence rate		100%		

The results showed that HCV negative coincidence rate was 100% (95%CI: 92.13%-100%), positive coincidence rate was 100% (95%CI: 97.57%-100%), total coincidence rate 100% (95%CI:98.11% ~ 100%). SPSS26 statistical analysis software was used for data processing and statistical analysis, and Kappa test was carried out. The Kappa value was 1, $P<0.01$, indicating that there was a high consistency between the test instrument and the comparison instrument.