Review Article

Recent developments and trends of automatic nucleic acid detection systems

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ABSTRACT

Nucleic acid detection, widely used in clinical diagnosis, biological analysis, and environmental monitoring, is of great significance for disease diagnosis and basic research. With the outbreak of COVID-19, the demand for fast and high-throughput nucleic acid detection from large numbers of samples has increased sharply. Automated nucleic acid detection systems can meet these needs, and also play important roles in disease screening and infectious disease prevention and control. In this review, we introduce and compare the current mainstream nucleic acid automatic detection instruments and equipment, then discuss the future demands of nucleic acid detection.

Contents

1. Introductions

As one of the most basic molecules of life, nucleic acids widely exist in all animal cells, plant cells, and microorganisms, and play crucial roles in their growth, genetics, and variation.[1] After Watson and Crick proposed the double-helix structure of DNA in 1953, modern molecular biology has significantly developed and advanced to a higher level with nucleic acid analysis.[2]

Nucleic acid detection technology focuses on a specific nucleic acid as its target. It includes many steps, such as sample processing, nucleic acid extraction, amplification, and product detection.[3] In clinical practice and other bioanalytical fields, the nucleic acid extracted from a sample is used as a template for amplification, and these results will be an important basis for disease diagnosis and other biological analysis. Compared with traditional cell detection and immune detection, nucleic acid detection has higher

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sensitivity and specificity, a short window period, and is widely used in the diagnosis and screening of infectious and hereditary diseases, tumor molecular diagnosis, and biological and medical research.[4]

Nucleic acid detection mainly includes three steps: 1) nucleic acid extraction, 2) amplification system preparation, and 3) polymerase chain reaction (PCR) amplification. It is mainly completed in three or four independent closed areas of a molecular laboratory. To avoid contamination, scientists walk through a special corridor and materials are transferred through designated transfer windows between the closed areas.[5]

However, with the continuous progress of nucleic acid detection, a new nucleic acid detection system with simpler operation procedures, less time consumption, and lower cost is expected. Such a system can be applied to point-of-care testing (POCT), without professional operators and precision auxiliary equipment, and can even be used in community clinics outdoors. Therefore, research on POCT nucleic acid analysis systems integrating nucleic acid extraction, amplification, and detection has been conducted worldwide.[6–7] An increasing number of researchers from various fields have devoted themselves to this research and are committed to solving numerous problems in the application of nucleic acid detection.[8–9]

Following the outbreak of COVID-19 across the world, the explosive growth in the demand for nucleic acid detection has placed great pressure on various countries and institutions. Laboratory inspectors must complete thousands to tens of thousands of nucleic acid tests each day. This high work intensity and significant infection risk will cause new problems and hidden dangers. Thus, various companies and scientific research institutions have developed a whole process automation system for nucleic acid detection.[10–11] Simultaneously, to meet the needs of on-site rapid detection, integrated rapid detection instruments have also been developed. Here, we describe the development status and trends of nucleic acid detection in China and other countries in the world.

2. Whole process automation system and integrated detection machine

2.1. Whole process automation nucleic acid detection system (WAS)

The WAS includes nucleic acid extraction, amplification system preparation, and nucleic acid amplification analysis modules. Each module is relatively independent and through mechanical automation and integration, all the modules are controlled by an automatic center to utilize automation or even unmanned systems. The WAS has the following advantages: (1) high automaticity;

(2) low biosafety risk; (3) high throughput system. Being high throughput is the most significant feature of this system. Examples include *Cobas® 6800/8800 (Roche)* and *Panther Fusion® Assays (Hologic)*.

2.2. Integrated nucleic acid detection machine (IDM)

The WAS generally covers a wide area, which affects its application in POCT. The IDM is small in size and also has a high degree of automation with low biosafety risk. This integrated machine can also complete all the procedures for nucleic acid detection. However, compared with the WAS, the IDM is a separate device, and each module in IDM is not independent but integrated into the system. One example is the *GeneXpert® system.*

These two detection systems have similar functions, but they are applicable to different scenarios or conditions. The WAS consists of three or more modules, and this system is often used for rapid detection and screening of a large number of samples in large hospitals and institutions. This equipment is frequently installed in independent rooms or spaces because of its large space occupation. The IDM, a separate device, is mainly used for community detection institutions, on-site detection, and POCT.

3. Detection principle and development status of automatic nucleic acid detection systems

The composition and principle of a nucleic acid detection automation system are shown in Fig. 1.

In accordance with the workflow, the system can be divided into four modules that can work independently: automatic dispensing (cup dividing), automatic nucleic acid extraction, automatic amplification system preparation, and automatic amplification analysis modules. The materials between each module are transferred through a handling device (manipulator or special conveyor), and each module is coordinated by the master control terminal through the communication bus. To prevent pollution and biosafety risk, each module or system adopts a high-efficiency particulate air filter (HEPA) independently to keep negative pressure.

Sample dispensing can inactivate the bacteria or virus and transfer the sample solution to the nucleic acid extraction module safely and accurately. The nucleic acid extraction process aims to isolate and purify the nucleic acids from the sample, including DNA and RNA, to meet the requirements of amplification. PCR or other techniques are used to perform the specific nucleic acid amplification step, and the results are detected by measuring fluorescent signals or other information.
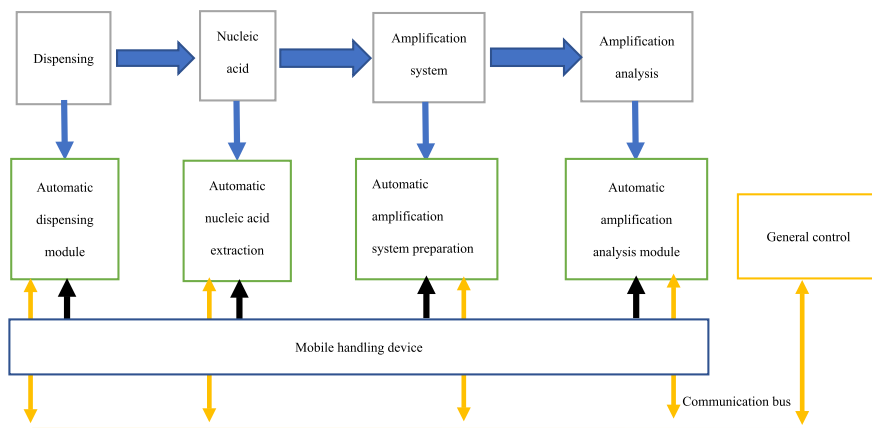

Fig. 1. Architecture diagram of a nucleic acid detection automation system.

With the development of molecular biology technology, detection methods are becoming increasingly abundant. However, the goal of most nucleic acid detection is to obtain a highly purified nucleic acid template from biological samples. The quality of the nucleic acid template has a significant impact on the detection sensitivity and specificity. Therefore, the nucleic acid extraction method is a key part of the nucleic acid detection process. Researchers worldwide have invested in nucleic acid extraction research and methods development, and have also been exploring the application of extraction reagents and materials for a long time.[12–13] A series of classical nucleic acid extraction methods, such as the phenol/chloroform extraction method, silica matrix method, magnetic bead method, and anion exchange method, has been invented.[14–17] With the progress in micromachining technology, the magnetic bead extraction method has been greatly recognized.[18] Magnetic bead extraction systems are applied in most nucleic acid detection instruments.

Nucleic acid amplification is the most applied method for nucleic acid detection, and PCR is the most widely used technique for DNA or RNA amplification. PCR is a method of enzymatic synthesis of specific DNA fragments in vitro. It consists of several sequential reactions, including high-temperature denaturation, low-temperature annealing (renaturation), and suitable temperature extension. The amplification product can be a new template, allowing the target DNA to be amplified rapidly. It is strongly specific, highly sensitive, simply operated, and timesaving. The amplification method in most automatic instruments is real-time PCR. In recent years, the use of numerous amplification steps and probes has enabled the simultaneous detection of multiple targets.[19] Additionally, isothermal amplification has attracted increased attention. Compared with other methods, it is cheaper and less time consuming, while requiring simpler equipment and more convenient operation.[20]

4. Mainstream products

Here, we describe some mainstream products throughout the world.

Most automatic instruments are composed of four modules: sample introduction, sample transfer, sample purification, and amplification detection. The whole process from sample transfer, sample preparation, amplification detection, result calculation, to data uploading can be automated. These systems include *Cobas® 6800/8800 (Roche)*[21] (Fig. 2a), *NeuMoDx™ 288 (Qiagen)*[22](Fig. 2b), *Panther Fusion® Assays (Hologic)*[24](Fig. 2d), *ANAS9850 (Amplly Biotech, China)*[25] (Fig. 2e), *AutoMolec 3000/1600 (Autobio Diagnostics, China)*[27]·*AutraMic mini (Liferiver, China)*[28] (Fig. 2g), *AutoSAT (Rendu, China)*[29] (Fig. 2h), and *High-protection-level nucleic acid automatic detection system (Cohere, China)*[30] (Fig. 2i). The only manually operated steps in most instruments are placing in reagent consumables and sample tubes for the automatic instruments. These systems are fully automated testing platforms that truly realize "sample in and result out", which can be applied to the needs of laboratories with different testing fluxes of low, medium, or high. The user-defined workflow and comprehensive detection menu can carry out the detection of multiple pathogens simultaneously.

The operation steps, nucleic acid extraction, amplification system preparation, and PCR amplification are almost the same in these instruments. Magnetic glass beads that can bind negatively charged nucleic acids are suitable for nucleic acid extraction in the automated instruments. The purpose of the automation steps is to move reagent tubes and transfer liquids, and different instruments have slight differences in operation sequence. Although several isothermal amplification methods have been applied in some systems, PCR amplification is still the first choice for the detection.

Cobas®8800 has the maximum sample loading capacity among these instruments. The large sample loading capacity allows the personnel to leave the machine for 8 h or more, which gives this system clear advantages for overload sample screening. *Panther Fusion® Assays* and *ANAS9850* can complete the detection of 500–600 samples in 8 h.

GeneXpert® Infinity-80 system (Fig. 2c) is the most famous POCT product. The feature of POCT products is "sample in, result out". This instrument is composed of a special microfluidic kit and a building block accumulation reaction module. The sample preparation, nucleic acid extraction and purification, amplification reagent preparation, and amplification detection are completed in a special kit. The independent reaction unit can allow for timely detection without being limited by batch.
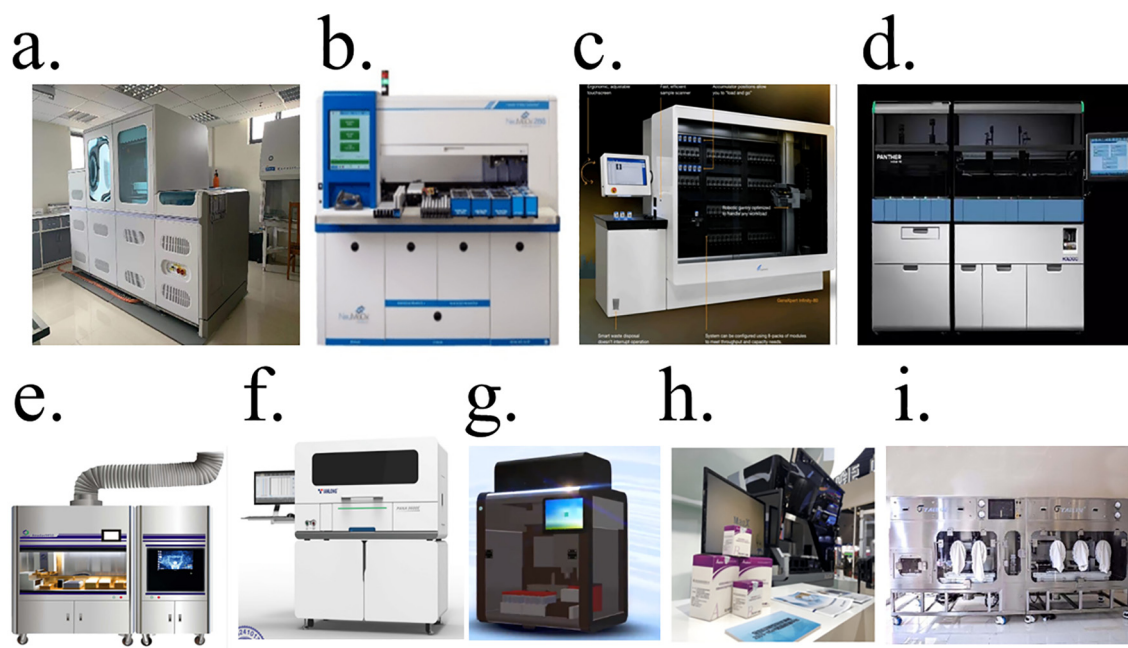

Fig. 2. Mainstream products in the world a. Cobas® 6800; b. NeuMoDxTM 288; c. GeneXpert® Infinity-80; d. Panther Fusion® Assays; e. ANAS9850; f. PANA9600E; g. AutraMic mini; h. AutoSAT; i. High-protection-level nucleic acid automatic detection systems. All pictures were downloaded from each company's website homepage.[21–30]

Although the detection capacity is less than other systems, it still has a wide application prospect under specific conditions, such as a primary clinic and other hospitals without a molecular biology lab.

The Panther Fusion system increases the power to run real-time PCR, transcription-mediated amplification (TMA), and RT-TMA assays on a single, fully automated platform. In addition, patient samples can be loaded into the Panther system for testing immediately after they arrive in the laboratory, which can improve efficiency and workflow.

ANAS985 was approved by CFDA in 2015. It is the earliest integrated nucleic acid detection machine in China. The whole process from sample input to PCR quantitative report is automated, and 96 samples can be detected within 3 h.

AutoSAT by Rendu meets the original tube loading to the instrument. The amplification method in this system is isothermal amplification, which is different from other systems. This machine can complete 500–700 tests in 24 h.

High-protection-level nucleic acid automatic detection system was developed by Shanghai Cohere Electronic Technology Company and the University of Shanghai for Science and Technology. Combined with a mechanical automatic handling system, a set of fully automatic nucleic acid detection platform systems with P2 + biological protection grade was constructed. This detection system was especially developed for COVID-19, and this platform can also be used for high-throughput unmanned detection of other pathogens, which effectively reduces the risk of infection among operators. This system can meet the needs of different application scenarios and detection quantities.

The performance parameters of each product are compared as follows (Table 1). In response to COVID-19, all companies have developed the detection agents for the coronavirus.

Table 1
Product performance comparison.

Brand	Product	Maximum throughput	Analysis method	Target in assay menu	Advantages	Disadvantages
Roche (Swiss) [a]	Cobas® 6800 Cobas® 8800	384/8h 1056/8h	qPCR qPCR	>30	Perform 3 tests simultaneously on up to 4 thermocyclers; Unmatched flexibility in molecular testing; Monthly maintenance; Multiple detection targets;	Large floor area; Expensive;
Thermo (US) [b]	NeuMoDx™ 288	144/8h	qPCR	>10	Fluorescence detection at five wavelengths enabling multiplexed amplification reactions; Flexible specimen tube compatibility;	Long detection time for large amount samples;
Cepheid (US) [c]	GeneXpert® Infinity	80/2.5 h	qPCR	>30	Room temperature reagents storage; Sample tested independently; Multiple detection targets	Manual sampling into chip kit; Less sample capacity
Hologic (US) [d]	Panther Fusion® Assays	500/8h	qPCR & TMA	>12	Run up to 5 PCR reactions from a single patient extraction; Minimal hardware/software changes allow additional testing without extensive retraining or SOP updates;2 analysis methods	Large floor area; Expensive;
Amplly (CN) [e]	ANAS9850	600/8h	qPCR	18	Less contaminated; Less floor area;	Less detection targets for Respiratory pathogens
Tianlong (CN) [f]	PANA9600E	96/1h	N/A			Without amplification
Autobio (CN) [g]	Automolec 3000/1600	96/2h	qPCR	Covid-2019	Detection without waiting	Long detection time for large amount samples; Just for Covid-2019 test;
Liferiver (CN) [h]	AutraMic mini	96/2h	qPCR	>20	Less floor area; Multiple detection targets;	Amplification in another instrument;
Rendu (CN) [i]	AutoSAT	600/24 h	SAT	>12	Isothermal amplification; Less floor area; Less time consuming for test only	Mineral oil needed; Reagents not compatible
Cohere (CN) [j]	High protection level nucleic acid automatic detection system	600/8h	qPCR & LAMP	>30	2 analysis methods including isothermal amplification;High level protection; Acceptable for highly communicable infections pathogen detection	Large floor area;

a https://diagnostics.roche.com.
b https://www.neumodx.com.
c https://www.cepheid.com.
d https://www.hologic.com.
e http://www.amplly.com.
f https://www.instrument.com.cn/netshow/SH101136/C18808.htm.
g https://www.autobio.com.cn.
h https://www.liferiver.com.cn.
i http://www.rdbio.com.
j http://www.cohere.com.cn.

5. Prospect of automatic nucleic acid detection systems

Automatic detection systems are developing toward better specificity, higher sensitivity, faster detection speed, and lower price. Meeting the needs of different application scenarios will be the focus of product development in the future.

Currently, the sample nucleic acid extraction step takes the most time in an automatic detection system. Hence, the nucleic acid extraction could be less time consuming through the optimization of extraction methods and automatic devices. At the same time, a more appropriate biosafety protection strategy can be used in the system to prevent cross contamination among samples and reduce biosafety risks.

By improving and optimizing nucleic acid amplification technology, we can improve the specificity and sensitivity of the amplification reaction. The development of emerging pathogen detection and new subtype detection is also important to meet the needs of different institutions. Combining CRISPR technology, third-generation sequencing, biosensor, nanotechnology, microfluid technology, and other analysis technologies will achieve more precise detection for different detection targets.

The further improvement of the automation efficiency of instruments is also needed. Through combining new information processing, transmission technology, and artificial intelligence technology, the instruments will be more convenient to use.

With the global spread of the current pandemic, the demand for real-time diagnosis technology is increasing. Accurate, fast, easy-to-use, and easy-to-popularize real-time disease diagnosis technology will become the focus of researchers. Many countries have invested heavily in the research and development of new in vitro diagnostic products and established a sustainable platform for the prevention and control of emerging infectious diseases to meet more severe challenges in the future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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