processes

MDPI

Review

Methods and Advances in the Design, Testing and Development of In Vitro Diagnostic Instruments

Lei Wang [1,2], Wenchang Xu [1,*], Biao Wang [1,3], Xiaonan Si [1,3] and Shengyu Li [1,3]

[1] CAS Key Lab of Bio-Medical Diagnostics, Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences, Suzhou 215163, China
[2] Jinan Guoke Medical Technology Development Co., Ltd., Jinan 250001, China
[3] School of Biomedical Engineering, Division of Life Science and Medicine, University of Science and Technology of China, Hefei 230026, China
* Correspondence: xuwc@sibet.ac.cn

Abstract: With the continuous improvement of medical testing and instrumentation engineering technologies, the design, testing and development methods of in vitro diagnostic instruments are developing rapidly. In vitro diagnostic instruments are also gradually developing into a class of typical high-end medical equipment. The design of in vitro diagnostic instruments involves a variety of medical diagnostic methods and biochemical, physical and other related technologies, and its development process involves complex system engineering. This paper systematically organizes and summarizes the design, testing and development methods of in vitro diagnostic instruments and their development in recent years, focusing on summarizing the related technologies and core aspects of the R&D process, and analyzes the development trend of the in vitro diagnostic instrument market.

Keywords: in vitro diagnostic instruments; design; testing and development methods; system engineering; market trends

Citation: Wang, L.; Xu, W.; Wang, B.; Si, X.; Li, S. Methods and Advances in the Design, Testing and Development of In Vitro Diagnostic Instruments. *Processes* **2023**, *11*, 403. https://doi.org/10.3390/pr11020403

Academic Editor: Yo-Ping Huang

Received: 30 November 2022
Revised: 15 January 2023
Accepted: 25 January 2023
Published: 29 January 2023

1. Introduction

In vitro diagnostic (IVD) instruments are the products that are used outside the human body to obtain clinical diagnostic messages by testing human samples (various body fluids, cells, tissue samples, etc.) and then determine disease or engine block function. In vitro diagnostic instruments are known as the "doctor's eyes" in the medical field and are an important part of modern laboratory medicine [1,2]. The clinical application of IVD instruments runs through the whole process of disease diagnosis and treatment, including disease prevention, preliminary diagnosis, treatment plan selection and efficacy evaluation, providing doctors with a large number of useful clinical diagnostic information and increasingly becoming an important component of human disease diagnosis and treatment [3,4].

In vitro diagnostic instruments, as a class of highly specialized instruments, need to be designed and developed on the basis of medical laboratory science and in vitro diagnostic methodology. With development in many fields, such as in vitro diagnostic methodology, test-related technology, and instrumentation engineering technology, the design of in vitro diagnostic equipment is becoming more fine-tuned and gradually entering advanced levels of high-end medical equipment research and development. In vitro diagnostics can be divided into biochemical diagnostics, immunodiagnostics, molecular diagnostics, microbiological diagnostics, blood diagnostics, point of care testing (POCT), etc. [5,6]. The related technologies involved include electrochemical technology, spectroscopic technology, chromatographic technology, mass spectrometry, electrophoresis, flow cytometry, labeled immunology, molecular biology, biosensing technology, etc. [7–9]. There are many kinds of in vitro diagnostic instruments, and the R&D process generally includes product project de-

sign, product prototype development, product finalizer development, product acceptance and other stages, each of which is based on certain rules.

This paper systematically organizes and summarizes the design, development, testing methods and recent developments of in vitro diagnostic devices, focusing on the relevant technologies and core aspects of the R&D process. Section 2 first introduces the development process of in vitro diagnostic methods and in vitro diagnostic devices, then summarizes and analyzes the related technologies and applications of in vitro diagnostic devices, classifies and gives examples of in vitro diagnostic devices from the application perspective, and finally focuses on the process of in vitro diagnostic device development and its key aspects. Section 3 analyzes the market development trend of in vitro diagnostic devices and introduces the key directions for future development. Section 4 provides a preliminary discussion from the direction of in vitro diagnostic device technology development. There are many kinds of in vitro diagnostic instruments, involving a huge range of fields and knowledge, and the design, testing and development of technologies are constantly evolving. It is impossible to provide a complete and detailed summary of the whole scope of in vitro diagnostic device R&D; this paper is only a preliminary compilation from one perspective.

2. Development of In Vitro Diagnostic Methods and Instruments

The design, testing and development of in vitro diagnostic instruments is based on medical testing and in vitro diagnostic methodology. In vitro diagnostic equipment, as systematic instruments that integrate testing and engineering, has a wide variety of types and models, involving electrochemistry, spectroscopy, chromatography, mass spectrometry, electrophoresis, flow cytometry, labeling immunology, molecular biology and other disciplines, and can support a variety of testing programs [10]. Meanwhile, similar to the R&D process of other types of comprehensive devices, the R&D of in vitro diagnostic devices needs to go through key stages, such as product project design, prototype development, product finalization and product acceptance, in addition to the clinical trials and legal and regulatory processes required by the medical industry in particular. This section will provide a systematic overview and summary of in vitro diagnostic methodological advances, related technologies and applications, in vitro diagnostic device classification and the R&D process.

2.1. Development of In Vitro Diagnostic Methodology

Along with the development of biochemistry, immunology, molecular biology and other fields, the development of the in vitro diagnostic industry can be divided into three stages.

Phase I: Before the 20th century, the invention of the microscope gave birth to the traditional means of microbiological microscopy-based inspection [11].

Phase II: In the early 20th century, the development of modern medicine and the discovery of enzyme-catalyzed reactions and antigen-antibody reactions laid the foundation for biochemical and immunological diagnosis, and in vitro diagnosis gradually emerged [12].

Phase III: After the 1950s, the use of technologies such as DNA double helix structure [13], monoclonal antibody technology [14] and giant molecule labeling technology [15] propelled the leapfrog development of molecular diagnostics as well as the whole in vitro diagnostic industry.

Phase III is the major development stage of core technologies in the in vitro diagnostics industry. From 1950 to 1970, the in vitro diagnostic industry entered the development phase. From 1970 to 1980, the industry entered a period of rapid development. Since 1980, new techniques for in vitro diagnosis have gradually emerged. The evolution diagram is shown in Figure 1.

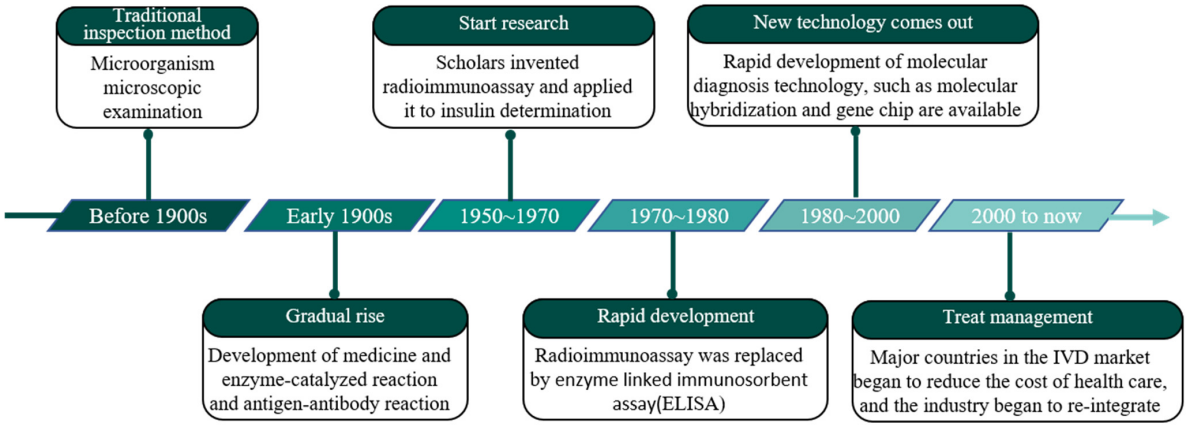

Figure 1. Historical development diagram of in vitro diagnostic industry.

According to different diagnostic principles and methods, in vitro diagnostic methodology can be divided into biochemical diagnosis, immunodiagnosis, molecular diagnosis, microbiological diagnosis, blood diagnosis, POCT, etc. Table 1 collates the main current in in vitro diagnostic methods, as well as the related principles, technologies and application areas.

Table 1. Classification of the main methodology of in vitro diagnostics.

Method	Principle	Technology	Applications
Biochemical diagnosis	Test the sample based on biochemical reaction	Dry Chemistry	Biochemical testing in the clinical emergency biochemical program testing [16]
		Others	Blood test, urine test, liver function, kidney function, etc. [17]
Immunization diagnosis	Immunology-based test using the specific reaction of antigen and antibody binding to each other	Immune colloidal gold technique	Hepatitis B, HIV, female pregnancy, drug testing, etc. [18]
		Latex turbidity	Specific body fluid protein testing [19]
		Chemiluminescence	Infectious disease, endocrine, tumor, drug, blood group testing, etc. [20]
		Radioimmunoassay	Hormone, trace protein, tumor marker and drug trace substance testing [21]
		Enzyme immunization	Infectious disease, endocrine, tumor, drug, blood group testing, etc. [22]
		Fluorescence immunization	Bacteria, virus, skin activity testing [23]
		Time-resolved fluorescence	Hormones, viral hepatitis, tumor testing, etc. [24]
Molecular diagnosis	Test the changes of the structure or expression levels of genetic material in patients using molecular biology methods	Polymerase Chain Reaction (PCR)	Bacteria, virus testing [25]
		Fluorescence original position crossbreeding	Genetic profiling, virus testing [26]
		Gene chips	Drug screening, new drug development, disease diagnosis, etc. [27]
		Gene sequencing	Genetic mapping, Down's syndrome screening, etc. [28]
Microbiological diagnosis	Test the types and numbers of microorganisms by microscopy	Drug sensitivity test	Laboratory diagnosis [29]
		Morphological observation	Bacteria, fungus testing [30]
		Automated microbiological analysis	Bacteria, fungus testing [31]
Blood diagnosis	Test the blood components such as red blood cells, white blood cells and hemoglobin	Smear and microscopy	Blood type testing [32]
		Blood cell image analysis	Red blood cell, white blood cell, platelet testing [33]
		Flow cytometry	Lymphocyte subpopulation testing, immunophenotyping, etc. [34]
POCT	Test the samples using portable analytical instruments and supporting reagents in the sampling site	Point of care testing	Cardiovascular disease markers, metabolic disease markers, coagulation characteristics testing, etc. [35]

2.2. Technologies Related to In Vitro Diagnostic Devices and Their Applications

Although there is a wide range of in vitro diagnostic instruments for clinical applications, the design and development of these instruments are always based on the generation and development of related technologies. In vitro diagnostic equipment is based on clinical and laboratory inspection methodology, combined with automation technology, optical technology, electronic information technology, biological sensing technology, computer technology, etc.; it gradually developed into systematic and engineered modern instruments and equipment to meet the needs of clinical testing and medical services. This section summarizes and analyzes the technologies and applications related to in vitro diagnostic equipment commonly used at present.

2.2.1. Electrochemical Analysis Technology

Electrochemical analysis is an analytical technique in which a solution of a substance to be measured is formed into a chemical cell, and the concentration of the substance to be measured is converted into electrical parameters by measuring variables such as potential, current, power and resistance of the cell, and thus detected. Electrochemical analysis techniques can be divided into potential analysis, electrical conductivity analysis, electrolysis analysis, electrical capacity analysis, Volt-Anne analysis and electrochemical biological sensing unit techniques [36].

Most of the instruments used for electrolyte analysis, blood gas and acid-base analysis in clinical chemistry tests are based on electrochemical analysis techniques. Electrochemiluminescence immunoassay (ECLIA) is a new technique that combines a chemiluminescent process caused by an electrochemical reaction with an immunoreactive process [37]. ECLIA has both the high sensitivity of luminescence detection and the high specificity of immunoassay. Electrochemical automated immunoassay analyzers based on ECLIA technology have been widely used in clinical immunology tests involving the determination of tumor markers, hormones, enzymes, antigens or antibodies, vitamins, cytokines and various metabolites.

2.2.2. Spectrum Analysis Technology

The interaction between light and matter is a universal physical phenomenon in nature. The technique to characterize and quantitatively examine a substance with the help of the different wavelengths and intensities of the absorption, emission or scattering spectra produced when the interaction between these two occurs is called spectrum analysis technology.

According to the way the spectra are generated, spectral analysis techniques can be divided into three main categories: absorption spectrum analysis, emission spectrum analysis and scattering spectrum analysis. In vitro diagnostic instruments using spectrum analysis technology are relatively simple in structure, easy to operate and relatively sensitive in method, and are widely used in clinical testing.

The molecular or atomic spectra obtained based on the molecular or atomic pair selective absorption of a substance radiation energy are the absorption spectra. Absorption spectrum analysis includes the ultraviolet-visible light spectrophotometric method, infrared spectroscopy and atomic absorption spectrophotometric method. The ultraviolet-visible light spectrophotometric method is mainly used for substances' quantitative examination, such as serum inorganic phosphorus. The atomic absorption spectrophotometric method is mainly used for the analytical determination of metal elements, such as the determination of zinc, magnesium and copper content in blood [38].

The emission spectrum is the spectrum that occurs when a molecule, atom or ion of a substance receives external energy, jumps from a ground state to a higher energy state, and then returns from the higher energy state to the ground state. Emission spectrum analysis technology involves the use of emission spectra for the characterization of substances using quantitative examination. Fluorescence spectrophotometry is used more often in

clinical tests; for example, the determination of vitamin content can be conducted using fluorescence spectrophotometry [39].

When photons act on non-uniform dielectrics, the phenomenon of light scattering is produced; the method to obtain the content of the substance to be measured by measuring the intensity of diffused light is the scattering spectrum analysis method. When the light from the light source passes through the sample to be measured, the antigen in the sample and its specific antibody form an antigen-antibody complex, which increases the solute particles and enhances light scattering, and the intensity of diffused light is proportional to the content of the complex. Thus, the antigen content can be measured from the change in diffused light intensity. Automated scattering turbidity analyzers based on scattering turbidimetry are widely used in clinical applications, mainly for measuring protein substances in the body, such as serum immunoglobulins, C-reactive proteins and trace proteins in urine [40].

2.2.3. Chromatography Analysis Technology

Chromatography is a general term for a class of separation and analysis techniques that are mainly used for the separation and analysis of complex multi-component mixtures. Chromatography uses the difference partition factor between two phases of a mixture in which the components are immiscible, while the components of the substance are separated and then characterized or quantitatively measured.

There are many types of chromatographic methods; they can be divided into liquid phase chromatography and gas chromatographic methods according to the nature of the moving phase; adsorption chromatography, distribution chromatography, ion exchange chromatography, etc. according to the separation principle; column chromatography and planar chromatography according to the fixed phase state. The chromatographic techniques commonly used in clinical tests have also become column chromatography techniques. High performance liquid chromatography (HPLC) is based on the classical chromatographic method by changing the moving phase to be delivered with high pressure and filling the column with small particle-size packing, so that the column effect is greatly improved, and the efficiency of the continuous detection of the effluent is increased by connecting a high-sensitivity detecting device. HPLC is suitable for the separation and purification of physiologically active giant molecule substances, such as proteins, enzymes, hormones, monoamino acid, nucleic acids, etc. [41].

2.2.4. Mass Spectrum Analysis Technology

Mass spectrum (MS) are plots of charged atoms, molecules or molecular fragments according to the magnitude series arrangement of the mass-to-charge ratio. The mass spectrometer is a class of instruments capable of dissociating particles of matter into ions and separating them according to their spatial location, temporal sequence or orbital stability by an appropriate electric or magnetic field to achieve a mass-to-charge ratio, and analyzing the substance after checking the intensity [42].

While mass spectrum is capable of qualitative analysis, it cannot analyze mixtures, whereas chromatography is effective in separating mixtures; the combination of the two allows for simultaneous separation and identification, thus giving rise to the mass spectrum coupling technique. The current mass spectrometer is mainly available in the following coupling methods: gas phase chromatography-mass spectrometry coupling technique [43], liquid chromatography-mass spectrometry (LC-MS) coupling technique [44], tandem mass spectrometry techniques [45], etc. Mass spectrometry is widely used in clinical medicine because of its high analytical sensitivity, low sample consumption, fast analysis speed and the ability to integrate separation and identification simultaneously.

When abnormal bone metabolism, diabetes, cardiovascular disease, tumors and other diseases occur, vitamin D is reduced or deficient in different groups. The LC-MS technique is highly specific and capable of measuring 25-(OH)D_2 and 25-(OH)D_3 simultaneously; it is considered as the gold standard for the detection of 25-(OH)D and is becoming increasingly

common in clinical applications [46]. In addition, mass spectrum analysis technology is widely used in some drug and doping tests.

2.2.5. Electrophoresis Analysis Technology

Electrophoresis is a phenomenon in which charged particles dispersed in a medium move toward the positive pole of an electric field under the action of an electric field, and positively charged particles move toward the negative pole of an electric field. The technique of separating substances using the phenomenon of electrophoresis is called electrophoresis analysis technology. Electrophoresis analysis techniques are mainly used for the separation, identification and quantitative examination of proteins and nucleic acids [47]. Electrophoresis techniques can be divided into mobile interface electrophoresis, zonal electrophoresis and steady-state electrophoresis, which can be further divided into two categories according to the solid phase support medium: one is filter paper, acetyl cellulose film electrophoresis, and the other is agarose gel, polyacrylamide gel electrophoresis.

The development and application of an isoelectric focusing cataphoresis meter, bi-directional electrophoresis system, vertical electrophoresis system and fully automatic electrophoresis analyzer have made the separation of electrophoretic zones increasingly clear and easy to operate [48,49]. The commonly used horizontal electrophoresis analyzers are mainly used for the analysis of serum proteins, hemoglobin, isoenzymes, etc.

2.2.6. Flow Cytometry Analysis Technology

Flow cytometry (FCM) combines lasers, fluid mechanics, computers and electronic measurement techniques to achieve rapid and accurate cell measurements at the moving phase. FCM can measure cell size, shape, nucleoplasm ratio, pigment content, cytoplasmic granules, etc. [50,51]. After fluorescent dye cells, it can also measure nucleic acid content in the nucleus, chromatin structure, cell surface antigen or sugar molecules, cytoskeleton, etc. With the advantages of high sensitivity, hi resolution, high sorting purity, high reproducibility and integrated analysis of multiparametric information, FCM provides rapid and accurate quantitative examination and sorting of cells and their associated components.

In clinical immunoassays, FCM is mainly used to analyze and measure cell surface antigens by examining differentiated antigens on the cell surface for lymphocyte subpopulations, hematopoietic stem cells, antigen-presenting cells, etc. [52].

2.2.7. Labeled Immunization Analysis Technology

The labeled immunoassay technique is the basic technique of various clinical immunological assays, which refers to the labeling of antigens or antibodies with different substances to give them a tracer function, and the specific reaction of antigen-antibody to achieve the purpose of detecting the substances to be tested in clinical specimens. At present, the main labeled immunological techniques for clinical immunological testing include fluorescent immunological techniques [53], radioimmunological techniques [54], enzyme immunological techniques [55], chemiluminescent immunological techniques [56] and electrochemiluminescent immunological techniques [57], etc. The main labeling substances used as tracers are fluorescein, radionuclides, enzyme proteins, chemiluminescent agents and electrochemiluminescent agents.

2.2.8. Molecular Biology Analysis Technology

Molecular biology analysis techniques have become one of the important techniques in clinical testing for the detection of nucleic acids of various pathogenic microorganisms, the detection of genes responsible for hereditary diseases, and the detection of some disease-related genes and metabolite genes.

The most common molecular biology technique used in clinical testing is polymerase chain reaction (PCR), a technique for amplifying genes in vitro [58]. Currently, the most used in clinical test is the fluorescent quantitative PCR technique, which is able to perform quantitative examination of target molecules [59], such as the quantitative determination of

viral nucleic acids for hepatitis B and C, which is an important basis for disease diagnosis and drug efficacy observation.

2.3. Classification of In Vitro Diagnostic Instruments

In vitro diagnostic products are mainly composed of diagnostic equipment and diagnostic reagents. Among them, in vitro diagnostic instruments belong to the category of clinical testing and analysis instruments. There is a wide range of in vitro diagnostic devices, covering almost all items of medical tests. Table 2 summarizes the current classification of common in vitro diagnostic devices, as well as representative products.

Table 2. Classification of in vitro diagnostic instruments.

Classification	Representative Instruments
Hematology analysis instruments	Blood group analyzer, blood cell analyzer, blood cell morphology analyzer, coagulation analyzer, platelet analyzer, blood flow analyzer, red blood cell sedimentation analyzer, flow cytometry analyzer, etc. [60].
Biochemical analysis instruments Electrolyte and blood gas analysis instruments	Biochemistry analyzer, blood glucose analyzer, etc. [61]. Electrolyte analyzer, blood gas analyzer, electrolyte blood gas analyzer, electrolyte blood gas test electrode, etc. [62].
Immunoassay instruments	Enzyme-linked immunoassay analyzer, chemiluminescence immunoassay analyzer, fluorescence immunoassay analyzer, immunochromatographic analyzer, immunoblotter, immunoscattering turbidity analyzer, biochemical immunoassay analyzer, etc. [63].
Molecular biology analysis instruments	Gene sequencer, sanger sequencer, nucleic acid amplification analyzer, nucleic acid amplification instrument, nucleic acid molecular hybridization instrument, etc. [64].
Microbiological analysis instruments	Microbial turbidimeter, microbial culture monitor, microbial drug sensitivity culture monitor, microbial identification instrument (non-mass spectrometry), microbial mass spectrometry identification instrument, microbial identification drug sensitivity analyzer, bacterial endotoxin/fungal dextran detector, H. pylori analyzer, etc. [65].
Scanning image analysis instruments	Medical microscopes, image scanners, image analyzers, etc. [66].
Radionuclide specimen determination instruments	Radioimmune γ counting device, liquid scintillation counting device, radioactive level scanning instrument, etc. [67].
Urine and other body fluid analysis instruments	Dry chemical urine analyzer, urine organic fraction analyzer, urine analyzer, stool analyzer, sperm analyzer, reproductive tract secretion analyzer, other body fluid analyzer, other body fluid morphology analyzer, etc. [68].
Other medical analysis instruments	Flow dot meter, minor element analyzer, mass spectrometry monitoring system, liquid chromatography analyzer, chromatography column, osmotic pressure assayer, circulating tumor cell analyzer, biochip analyzer, cataphoresis meter, etc. [69].
Sampling instruments	Automatic sampler, sample processing and spiking system [70].
Sample pre-processing instruments for morphological analysis	Blood cell analysis sample pre-processor, pathology analysis sample pre-processor, flow cytometry sample lyser, etc. [71].
Sample separation instruments	Medical centrifugal machine, nucleic acid extraction and purification instrument, etc. [72].
Culture and incubation instruments	Medical culture/constant temperature oven, anaerobic incubator, incubator, platelet shaker, etc. [73].
Inspection and other utility instruments	Automatic sample adding instrument, sample processing system, medical cryogenic equipment, medical refrigeration equipment, medical freezing equipment, etc. [74].
Medical bioprotective instruments	Biological safety cabinets, clean working bench, etc. [75].

2.4. Systematic Design, Testing and Development Process of In Vitro Diagnostic Instruments

Similar to the R&D process of other kinds of comprehensive instrumentation, the design and development of in vitro diagnostic devices is also a systematic project. There is no certain rule for product development, which needs to be planned according to the specific in vitro diagnostic device and the characteristics of the R&D and production team.

However, from the engineering perspective, the design, testing and development process of in vitro diagnostic devices generally go through important stages, such as product project design, product prototype development, product finalization and product acceptance. In addition, due to the special characteristics of the medical industry, in vitro diagnostic devices often have to go through clinical trials and evaluations, as well as relevant law and regulation validation stages before they are put into production. This section will give an overview of the R&D process of in vitro diagnostic devices.

2.4.1. Product Design

Overall Solution Design and Evaluation

According to policies and regulations, industry standards and regulatory documents, the project manager should complete the overall scheme design of the product. This generally includes product design requirements, test methods, test principles, product structural components, product functional indicators, product performance index, product main workflow, modular structure design, product cost budget, etc. If the product has to enter other countries or regional markets, it needs to meet the requirements of local regulations. Then, the relevant personnel review the overall plan, make comments or suggestions for modification, and make continuous improvements.

Form Design and Evaluation

Based on the structural composition and size of each module, the structure, specifications and design style of the product shape are broadly determined. Good form design can make the product dominant in the market, enhance the product's comprehensive competitive power, reduce production costs, and increase favor with customers. Therefore, form evaluation is an indispensable part of the R&D process [76].

Software System Design and Evaluation

In order to give software developers, users, and testers a clear understanding of the functions of the software, the software description should be as detailed as possible, including the interface style, user requirements, description of the requirements of related products, and the relationship between the software and total system interface, operating environment, system functions, security requirements, etc. Among them, the software architecture design mainly describes the type of software components and important functional modules of system operation; software description documents need to be prepared under the software and network security–related regulations, standards and technology governing principles.

Hardware System Design and Evaluation

First, patent analysis is conducted to avoid patent disputes on design results based on each module on issues such as the possibility of infringement, derivative patents, and patentability of technological achievement. Next, the development of the hardware system technical specification instruction manual is carried out, including a detailed description the composition of the hardware system, the requirements of the system development, the main technical indicators, the demand analysis of the hardware, the layout structure of each module, the design of the interface with the computer, etc. Then, the relevant personnel review the technical specification instruction manual, make comments or suggestions for modification, and then continuously improve it, entering the next process after the review is passed.

Core Module Design and Evaluation

The following are developed: the core module technical specification instruction manual, a detailed description the structure of the module design, the software support required, the functions to be implemented, the performance index and the operating environment required to form the core module design plan. Then, the relevant personnel

review the module project design, make comments or suggestions for modification, and then continuously improve it, proceeding to the next process after the review is passed [77].

2.4.2. Product Prototype Development

Hardware System Detailed Design and Evaluation

First, the basic modular structure of the product and the approximate size of each module are determined in order to realize the function and performance of the product. Next, the software that the system depends on to run is determined, including the executive system, interface software and the approximate size of each module. Then, a detailed design and confirmation of mechanical drawings, circuit boards, and tools such as the software used and their version numbers are established.

The detailed design of the hardware system needs to be completed as follows. (1) Electrical structure design: determine the number of selected control components and their parameters, and how the modules fit together. (2) Physical construction design: determine how to match mechanical parts and installation according to the size of electrical components. (3) Functional structure design: determine the parameter selection. (4) Device selection: according to the parameters in the component manual, determine the device model; or according to the design of mainstream models, under the premise of achieving the same function, select the largest sales volume and sufficient supply of components. (5) Draw the printed circuit board: according to the circuit function needs, design the schematic drawing and then design the printed circuit board diagram. (6) Hardware system detailed design review: the relevant personnel should review the hardware system detailed design, make changes or suggestions, and then continue to improve, with the review passed into the next process.

Software System Detailed Design and Evaluation

The detailed design of the software system needs to specify the main business requirements, inputs, outputs, main functions, performance index and operating environment of the software system; the language, tools, and methods used for software development and the names, full versions, and suppliers of the said supporting and application software need to be determined. At the same time, the number of R&D staff, development time, workload and total number of lines of code need to be clarified, the level of software security needs to be specified, and the reasons for the determination need to be detailed.

The software life cycle is divided into five phases, namely requirements analysis, design, coding, testing and maintenance. The following core points need to be completed for the detailed design of the device system. (1) System hardware topology: provide a physical topology diagram based on software design specification and describe the physical connection relationship between software or component modules, multi-purpose computer, and medical device hardware. (2) System structural drawing: Represent the relationship between the component modules and between the component modules and peripheral interface using the structural drawing and describe the functions, module relationships and peripheral interface of the component modules based on the system structural drawing. (3) User interface relationship diagram: describe the relationship between the user interface, based on the user interface relationship diagram, to describe the functions and module relationships of the software system, in the form of a diagram listing the relationship between each module within the system. (4) Core algorithm: describe the calculation formula and specific calculation steps used in the core algorithm. (5) The process logic, in the form of the process flow diagram, shows the logical flow of the realization program. (6) Operating environment: specify the hardware configuration, software environment and network conditions required for software operation, including hardware configuration (including processor, memory and peripheral devices), software environment (including system software, support software and security software), and network conditions (including network architecture, network type (WAN, LAN, personal domain network) and bandwidth). (7) Interface: describe the interface relationship between the upper-level

modules and lower-level modules related to this program. (8) Contraindications: use stand-alone software to describe the software contraindications or service restriction, software components to describe the contraindications of medical device products or service restrictions, and imported medical device software to describe the country of origin.

Design Engineering

Design engineering is the activity process of expressing a design plan, including planning and vision, in physical form [78]. (1) Electrical and optical module design. The printed wiring board is made from the electrical schematic drawing. The electrical schematic drawing is mainly based on the electrical performance of each component for reasonable construction, through which the diagram can accurately reflect the important functions of the printed wiring board, as well as the relationship between the various components. After the electrical schematic drawing design is completed, the individual components selected are then packaged by the drawing software to generate and realize a grid with the same appearance and size of the components. The individual components are then placed according to the size of the printed wiring board; when placing the components, it is necessary to ensure that the leads of the individual components do not cross. While carrying out the design of the wiring diagram, first draw the wiring floor plan according to the electrical schematic drawing; then, determine the placement of the electrical components according to floor plan and assemble the circuit board and other components into modules according to design drawing. (2) Module validation. According to the functions and performance index that each module can achieve at this stage, the design engineer prepares the module validation plan; the test engineer carries out the validation according to the validation plan, and issues the validation report after the validation. (3) Electrical and optical module design review. The relevant personnel review the electrical and optical module design, put forward modification comments or suggestions, and then continuously improve it, entering the next process after the review is passed. (4) Mechanical and hydraulic circuit module design. First, determine the components according to the design, then process or purchase the components according to the drawing and assemble them into modules according to design drawing. (5) Module validation. According to the functions and performance index that each module can achieve at this stage, the design engineer prepares the module validation scheme; the test engineer carries out the validation according to the validation scheme and issues the validation report after the validation. (6) Mechanical and liquid circuit module design review. Relevant personnel review the mechanical and liquid circuit module design, put forward modification opinions or suggestions, and then continuously improve it, entering the next process after the review is passed.

Whole Instrument Design and Verification

Carry out assembly coupling and functional verification of the whole machine. (1) Whole instrument assembly. According to the assembled electrical module, optical module, mechanical module and hydraulic module, install into the whole machine according to the drawing requirements. (2) The initial adjustment is made before powering on. Before powering on the whole machine, check the relative position of each module again according to the requirements of the drawings to avoid collision and interference after powering on. For example, check whether the internal assembly wiring is correct and wiring is reasonable, whether the fasteners are firmly installed, whether the moving parts are flexible in rotation, etc. (3) Whole machine function and performance verification. According to the functions and performance index that can be achieved by the product at this stage, the design engineer prepares the preliminary verification plan for the whole machine, and the test engineer carries out verification according to the preliminary verification plan, and issues a verification report after verification. (4) Electrical safety verification. According to the requirements of regulations and national standards, the electrical safety of the whole machine is verified. (5) Environmental adaptability verification. Verify that the product meets the environmental requirements of the design input, including climate environmental

baseline and mechanical environmental baseline. (6) Electromagnetic compatibility (EMC) verification. According to the requirements of regulations and national standards, the EMC of the whole machine is verified. (7) Whole machine review. Relevant personnel review the safety and effectiveness that should be achieved at this stage of the whole machine, put forward modification opinions or suggestions, and then continuously improve it, entering the next process after the review is passed [79,80].

2.4.3. Product Finalization Development

Module Improvement and Mold Development

Engineer the mold to improve it according to the finalized design structure. Carry out packaging and label design as well as packing container and accessory case design, and refine module process documentation. The test engineer prepares test cases based on the functions and performance of each module of the instrument, which translates the behavior of software testing into manageable patterns and also quantifies the testing work. A list of key components is prepared, and the test engineer implements the test according to the raw material.

Module Validation

According to the generated BOM list and progress plan, the procurement department purchases small quantities of raw material. According to the functions realized by each module and performance index, the design engineer improves the module validation scheme. According to the developed validation scheme, test engineers implement the validation and make relevant records. According to the comments or suggestions made by experts during the module review, the modules are improved, and the technical documents are updated.

Whole Instrument Improvement

Develop the whole instrument initial inspection, commissioning and aging standards. According to the experience accumulated during the operation of the whole machine preliminary inspection and whole machine aging and the requirements of design, prepare the whole machine preliminary inspection and whole machine aging standards to guide production. According to the product assembly, design engineers should inspect the key main base, design each key main base required for tooling and inspection, and follow inspection acceptance criteria and instructions for use; if software is involved, the code should be unified for archiving.

Whole Instrument Validation

Validation of the overall functionality and performance of the developed medical device products. (1) Develop the whole machine verification program: According to the whole machine function and performance index, design engineers should improve the whole machine verification program, which includes the whole machine function verification program, the whole machine performance verification program, the whole machine reliability verification program and the whole machine type verification program. (2) Implementation of verification: According to the developed verification program, test engineers should implement verification and make relevant records. (3) Initial inspection of the whole machine: check the tightness of the installation of the whole machine, as well as the relative position of each independent component and module. (4) Whole machine aging: After the completion of the product assembly and commissioning, the whole machine will be continuously energized for a period of time (depending on the product and time) according to the requirements specified in the process documents, with the purpose of discovering and eliminating the early failure of electronic components through aging, as well as improving the working reliability of electronic equipment and service limits, while stabilizing the whole machine parameters and ensuring the quality of commissioning. In general, the whole machine is considered according to the following

aspects of power-up aging: temperature, cycle period, accumulation time, test times, test blanking time, etc. (5) Whole machine debugging: after the debugging of qualified modules assembled into the whole machine, the cooperation between the modules cannot all be in the best state and meet the technical indicators of the whole machine; thus, it is necessary to adjust the relative position of the modules and the tolerance clearance to the size required by the drawings of the whole machine, so that the work associated with each component is in the best state of cooperation. (6) Machine performance inspection: In order to verify whether the instrument can reach industry standards or product technical requirements of the performance index, and to determine whether there are performance bottlenecks in the software system and then optimize the software, the machine performance inspection is one of the most critical links. Item inspection should be in accordance with industry standards or product technical requirements of the performance index item. (7) Software testing: different testing software tools can be written as needed and then used to analyze and evaluate the possible problems of the test program [81,82].

2.4.4. Product Receiving Inspection

After completing the design and development of medical device products, we conduct the final acceptance of the products and carry out clinical evaluation and other work as needed. (1) Develop the whole machine verification program: Supplement the whole machine verification program for the changed design structure. (2) Implementation of verification: According to the developed verification scheme, test engineers implement verification and make relevant records. (3) Clinical applicability evaluation: Medical device products are intended for clinical application, so it is necessary to analyze and evaluate whether the design meets clinical requirements before the product is marketed. Clinical performance and safety data (including favorable and unfavorable data) collected during the clinical evaluation should be included in the analysis. The depth and breadth of the clinical evaluation, the type of data needed and data volume should be appropriate to the design characteristics, gordian technique, scope of application and degree of risk of the product, and also to the level and extent of nonclinical studies. The clinical evaluation should confirm the scope of application of the product (such as the applicable population, application site, contact mode with human body, indications, degree and stage of disease, service requirement, service environment, etc.), method of application, contraindications, precautions, warnings and other clinical use information.

3. Market Trends of In Vitro Diagnostic Instruments

In recent years, the in vitro diagnostic market has maintained rapid growth. With the continuous improvement of living standards, people's demand for health is also increasing day by day, and in vitro diagnostics as an important means of disease diagnosis have also gained rapid development. In recent years, many countries have vigorously pushed forward the reform of the medical system, abolishing the markup of drugs and consumables, and a series of hospital reform policies, such as medical insurance cost control as well as controlling the consumption ratio and drug ratio, have been introduced [83]. Hospitals, as an economy, have been devoting more attention to medical services and diagnostic departments to compensate for the loss of their revenue from drugs and consumables, and medical services and in vitro diagnostics will develop rapidly in this and other aspects.

The three key directions for the future of the in vitro diagnostics industry are as follows.

3.1. Chemiluminescence

Immunodiagnostics is the largest segment in the in vitro diagnostic market, accounting for about 40% market share. With the continuous development and progress of technology, the low-end immunodiagnostic field has achieved better results, but the high-end immunodiagnostic field is still monopolized by several giant companies, the most representative of which is the chemiluminescence field. At present, chemiluminescence has replaced enzyme-linked immunoassay as the mainstream immunodiagnostic method; the market

size has reached more than 70% of the total immunodiagnostic market and is basically monopolized by the leading companies. In the future, with the breakthrough of key core technologies and the introduction of advanced technologies, coupled with the support of policies to encourage innovation, important breakthroughs will be made in the field of chemiluminescence, which is expected to gradually realize leapfrog development in the field of high-end immunodiagnostics [84–87].

3.2. Gene Sequencing

Molecular diagnostics are in their infancy, with a low market concentration and small scale; however, the growth rate is high, leading the in vitro diagnostics industry with a growth rate of over 25%. As the most cutting-edge technology in life sciences, molecular diagnostics have been widely used in prenatal screening, infectious diseases, tumors and other fields. The development time of molecular diagnostic technology is relatively short, and the gap between the technical levels of related enterprises is relatively small, especially in the field of gene sequencing, where many excellent and innovative enterprises have emerged. Benefiting from the development trend of precision medicine and the continuous upgrading of molecular diagnostics technology, molecular diagnostics will continue to grow rapidly in the future, and gene sequencing, which is at the forefront of molecular diagnostics, will also become the main battlefield for high-quality enterprises [26–28].

3.3. POCT

POCT has many advantages, such as small space, ease of use, high efficiency and high accuracy, generally low price and so on. POCT is of great significance for disease prevention, by determining cause and prognosis, improving treatment effectiveness and reducing medical costs, and can meet the clinical testing needs of various medical institutions at all levels [88,89]. Especially in the time of vigorously promoting graded treatment and third party testing, low-cost, high-efficiency POCT products are more attractive for primary health care institutions and third party testing institutions. At present, the overall POCT market size is not large, but the development is fast; with the implementation of the graded treatment policy, the POCT market will develop rapidly, and the first layout of the enterprise with the policy and cost advantages will also usher in further development [90].

Enterprises can effectively enhance competitive power by occupying a reasonable position in the industry chain. The in vitro diagnostic industry mainly consists of upstream (electronic components, diagnostic enzymes, antigens, antibodies and other raw materials), midstream (diagnostic equipment, diagnostic reagents) and downstream (hospital testing departments, medical examination centers, independent laboratories, epidemic prevention stations, etc.). The upstream raw material market, midstream high-end diagnostic market and downstream large hospital market are dominated by leading enterprises; upstream raw material is especially monopolized by large enterprises, resulting in difficulties for other enterprises, which can only participate in the fierce competition in the middle and low-end market; even if progress is made in a certain segment, it cannot be well translated into overall advantages [91–93].

In recent years, this situation gradually began to improve; as innovation and import substitution policies continue to be introduced, innovative enterprises have strengthened the layout of the industry chain and R & D investment and made good progress in the upper, middle and lower reaches. Therefore, specialization and scale becomes the best choice for enterprises for breakthrough development.

4. Discussion

The most prominent sign of the development of medical diagnostic science is the automation of medical tests in place of traditional manual operation; manual analysis and determination has low precision, is slow, and is difficult to standardize, among other shortcomings. Among them, especially the design and development of in vitro diagnostic instruments and clinical applications are the most important, and are of great significance

to the development of clinical testing technology and modern medicine. The technical principles of most of the common clinical automated in vitro diagnostic instruments are based on traditional experimental techniques, such as electrochemical techniques, spectroscopic techniques, chromatographic techniques, mass spectrometry, electrophoresis, flow cytometry, labeled immunology, molecular biology, biosensing, etc. [94,95].

The development process of in vitro diagnostic equipment reflects the characteristics of semi-automation, automation, integration, miniaturization, high throughput and intelligence, which decreases the cost of testing and improves the precision and accuracy of analysis and measurement comprehensively, meeting the needs of different clinical and patient situations. In vitro diagnostic instruments can be used for common hematological analysis, urine analysis, biochemical analysis, immunological analysis, cellular immune function analysis, blood group identification, microbial culture and identification, molecular biology testing, etc., covering almost all the specialties and most of the testing items of clinical testing laboratories and thus becoming an important tool of modern medical testing [96].

There is a wide variety of in vitro diagnostic devices, and the R&D process cannot be defined by a fixed set of specifications. However, there is a set of referable processes for the design, testing and development of in vitro diagnostic devices for reference by integrating theoretical and technical concepts such as medical test methodology and engineering. In general, the design and development of in vitro diagnostic devices have to go through the stages of product project design, product prototype development, product finalization and product acceptance, and some instruments have to go through more complicated testing processes such as clinical application evaluation, which involve disciplines such as mechanics, electronics, optics, computer science, biochemistry, etc. [97–99]. In the process of testing and verification, experimental science, engineering, testing science, laws and regulations are involved. The process of marketing is influenced by many factors. It can be said that the success of an in vitro diagnostic device is much more than the success of design and development; it also needs to withstand clinical application and market testing [100,101].

5. Conclusions

This paper provided a systematic overview and summary of the methods and related advances in the design, testing and development of in vitro diagnostic instruments. The development of in vitro diagnostic instruments is based on the development of medical test methodology. At the same time, the design, testing and development of in vitro diagnostic instruments is a multidisciplinary intersection of complex systems engineering, which requires the integration of engineering and technology methods from many fields, such as mechanics, electronics, optics, inspection, computer science and controls, and has high requirements for materials, processes and protocols in processing and production. The R&D process generally includes product project design, product prototype development, product finalization and product acceptance stages. This paper summarizes and outlines the tasks and considerations to be accomplished in these important stages. In addition, this paper analyzes the development trend of the in vitro diagnostic device market and proposes potential hotspots in product development and marketing in the future.

Author Contributions: Conceptualization, L.W.; Funding acquisition, L.W.; Investigation, W.X.; Methodology, L.W. and W.X.; Supervision, L.W. and W.X.; Validation, B.W.; Visualization, X.S.; Writing—original draft, S.L.; Writing—review and editing, L.W. and W.X. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Key Research and Development Plan Project of China (2022YFC2407006, 2022YFC2503305), the Key Research and Development Plan of Shandong Province (2021SFGC0104, 2021CXGC011103), the Youth Foundation of Shandong Natural Science Foundation of China (2020QF018), and the Suzhou Municipal Science and Technology Program Grant (GWZX201903).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Abbreviations

ECLIA	Electro-chemiluminescence immunoassay
EMC	Electromagnetic compatibility
FCM	Flow cytometry
HPLC	High performance liquid chromatography
IVD	In vitro diagnostic
LAN	Local area network
LC-MS	Liquid chromatography-mass spectrometry
MS	Mass spectrum
PCR	Polymerase chain reaction
POCT	Point of care testing
WAN	Wide area network

References

1. Peng, P.; Liu, C.; Li, Z.D.; Xue, Z.R.; Mao, P.; Hu, J.; Xu, F.; Yao, C.Y.; You, M.L. Emerging ELISA derived technologies for in vitro diagnostics. *TrAC Trends Anal. Chem.* **2002**, *152*, 116605. [CrossRef]
2. Müller, H.; Holzinger, A.; Plass, M.; Brcic, L.; Stumptner, C.; Zatloukal, K. Explainability and causability for artificial intelligence-supported medical image analysis in the context of the European In Vitro Diagnostic Regulation. *New Biotechnol.* **2022**, *70*, 67–72. [CrossRef] [PubMed]
3. Ma, F.; He, L.; Lindner, E.; Wu, D.Y. Highly porous poly(l-lactic) acid nanofibers as a dual-signal paper-based bioassay platform for in vitro diagnostics. *Appl. Surf. Sci.* **2021**, *542*, 148732. [CrossRef]
4. Cao, S.; Xie, Z.; Xiao, G.; Sun, X.; Diao, H.; Zhou, X.; Yue, Z. Photoelectrochemical sensors based on heterogeneous nanostructures for in vitro diagnostics. *Biosens. Bioelectron. X* **2022**, *11*, 100200.
5. Iglesia, T.B.; Yañez-Vico, R.M.; Iglesias-Linares, A. Diagnostic performance of cone-beam computed tomography to diagnose in vivo/in vitro root resorption: A systematic review and meta-analysis. *J. Evid.-Based Dent. Pract.* **2022**, 101803. [CrossRef]
6. Guliy, O.I.; Evstigneeva, S.S.; Bunin, V.D. Electrical sensor system for in vitro bacteria biofilm diagnostics. *Biosens. Bioelectron. X* **2022**, *11*, 100174. [CrossRef]
7. Geraldi, A.; Giri-Rachman, E.A. Synthetic biology-based portable in vitro diagnostic platforms. *Alex. J. Med.* **2018**, *54*, 423–428. [CrossRef]
8. Chi, X.; Huang, D.; Zhao, Z.; Zhou, Z.; Yin, Z.; Gao, J. Nanoprobes for in vitro diagnostics of cancer and infectious diseases. *Biomaterials* **2012**, *33*, 189–206. [CrossRef]
9. Takazawa, T.; Sabato, V.; Ebo, D.G. In vitro diagnostic tests for perioperative hypersensitivity, a narrative review: Potential, limitations, and perspectives. *Br. J. Anaesth.* **2019**, *123*, e117–e125. [CrossRef]
10. Grifa, R.A.; Pozzoli, G. Performance evaluation of in vitro diagnostic medical devices: Methodology and differences compared to studies on other medical devices. *Microchem. J.* **2018**, *136*, 279–282. [CrossRef]
11. Konkol, N.R.; McNamara, C.J.; Hellman, E.; Mitchell, R. Early detection of fungal biomass on library materials. *J. Cult. Herit.* **2012**, *13*, 115–119. [CrossRef]
12. Zhao, F.; Shi, R.; Liu, R.; Tian, Y.; Yang, Z. Application of phage-display developed antibody and antigen substitutes in immunoassays for small molecule contaminants analysis: A mini-review. *Food Chem.* **2021**, *339*, 128084. [CrossRef]
13. Neidle, S. Beyond the double helix: DNA structural diversity and the PDB. *J. Biol. Chem.* **2021**, *296*, 100553. [CrossRef]
14. Siegel, D.L. Recombinant monoclonal antibody technology. *Transfus. Clin. Biol.* **2002**, *9*, 15–22. [CrossRef] [PubMed]
15. Watson, P.; Jones, A.T.; Stephens, D.J. Intracellular trafficking pathways and drug delivery: Fluorescence imaging of living and fixed cells. *Adv. Drug Deliv. Rev.* **2005**, *57*, 43–61. [CrossRef] [PubMed]
16. Balk, E.M.; Ioannidis, J.P.A.; Salem, D.; Chew, P.W.; Lau, J. Accuracy of biomarkers to diagnose acute cardiac ischemia in the emergency department: A meta-analysis. *Ann. Emerg. Med.* **2001**, *37*, 478–494. [CrossRef] [PubMed]
17. Fu, Q.; Qi, T.; Wu, Z.; He, Y.; Guan, S.; Luo, S.; Zhang, Q.; Luo, W.; Xiao, W.; Situ, B.; et al. A portable smartphone-based hemoglobin point-of-care testing platform for accurate anemia diagnostics. *Biosens. Bioelectron.* **2022**, *217*, 114711. [CrossRef]

18. Gurramkonda, C.; Zahid, M.; Nemani, S.K.; Adnan, A.; Gudi, S.K.; Khanna, N.; Ebensen, T.; Lünsdorf, H.; Guzmán, C.A.; Rinas, U. Purification of hepatitis B surface antigen virus-like particles from recombinant Pichia pastoris and in vivo analysis of their immunogenic properties. *J. Chromatogr. B* **2013**, *940*, 104–111. [CrossRef]
19. Gossen, P.D.; MacGregor, J.F. On-Line Particle Diameter for Poly(Vinyl Acetate) Latex Using Specific Turbidity Method. *J. Colloid Interface Sci.* **1993**, *160*, 24–38. [CrossRef]
20. Cao, Y.; Dai, Y.; Zhang, L.; Wang, D.; Yu, Q.; Hu, W.; Wang, X.; Yu, P.; Ping, Y.; Sun, T.; et al. Serum oncostatin M is a potential biomarker of disease activity and infliximab response in inflammatory bowel disease measured by chemiluminescence immunoassay. *Clin. Biochem.* **2022**, *100*, 35–41. [CrossRef]
21. Thumvijit, T.; Supawat, B.; Wattanapongpitak, S.; Kothan, S.; Tungjai, M. Effect of iodinated radiographic contrast media on radioimmunoassay for measuring thyroid hormones. *Appl. Radiat. Isot.* **2022**, *185*, 110261. [CrossRef] [PubMed]
22. Steininger, C.; Kundi, M.; Aberle, S.W.; Aberle, J.H.; Popow-Kraupp, T. Effectiveness of reverse transcription-pcr, virus isolation, and enzyme-linked immunosorbent assay for diagnosis of influenza a virus infection in different age groups. *J. Clin. Microbiol.* **2002**, *40*, 2051–2056. [CrossRef] [PubMed]
23. Song, L.W.; Wang, Y.B.; Fang, L.L.; Wu, Y.; Yang, L.; Chen, J.Y.; Xia, N.S. Rapid fluorescent lateral-flow immunoassay for hepatitis B virus genotyping. *Anal. Chem.* **2015**, *87*, 5173–5180. [CrossRef] [PubMed]
24. Leivo, J.; Kivimäki, L.; Juntunen, E.; Pettersson, K.; Lamminmäki, U. Development of anti-immunocomplex specific antibodies and non-competitive time-resolved fluorescence immunoassay for the detection of estradiol. *Anal. Bioanal. Chem.* **2019**, *411*, 5633–5639. [CrossRef]
25. Figuero, E.; Sánchez-Beltrán, M.; Cuesta-Frechoso, S.; Tejerina, J.M.; del Castro, J.A.; Gutiérrez, J.M.; Sanz, M. Detection of periodontal bacteria in atheromatous plaque by nested polymerase chain reaction. *J. Periodontol.* **2011**, *82*, 1469–1477. [CrossRef]
26. Cui, C.; Shu, W.; Li, P. Fluorescence in situ hybridization: Cell-based genetic diagnostic and research applications. *Front. Cell Dev. Biol.* **2016**, *4*, 89. [CrossRef]
27. Yin, G.; Bie, S.; Gu, H.; Shu, X.; Zheng, W.; Peng, K.; Jiang, M. Application of gene chip technology in the diagnostic and drug resistance detection of Helicobacter pylori in children. *J. Gastroenterol. Hepatol.* **2020**, *35*, 1331–1339. [CrossRef]
28. Palomaki, G.E.; Deciu, C.; Kloza, E.M.; Lambert-Messerlian, G.M.; Haddow, J.E.; Neveux, L.M.; Canick, J.A. DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: An international collaborative study. *Genet. Med.* **2012**, *14*, 296–305. [CrossRef]
29. Banu, S.; Rahman, S.M.; Khan, M.S.R.; Ferdous, S.S.; Ahmed, S.; Gratz, J.; Houpt, E.R. Discordance across several methods for drug susceptibility testing of drug-resistant Mycobacterium tuberculosis isolates in a single laboratory. *J. Clin. Microbiol.* **2014**, *52*, 156–163. [CrossRef]
30. Camesano, T.A.; Natan, M.J.; Logan, B.E. Observation of changes in bacterial cell morphology using tapping mode atomic force microscopy. *Langmuir* **2000**, *16*, 4563–4572. [CrossRef]
31. Budding, A.E.; Hoogewerf, M.; Vandenbroucke-Grauls, C.M.; Savelkoul, P.H. Automated broad-range molecular detection of bacteria in clinical samples. *J. Clin. Microbiol.* **2016**, *54*, 934–943. [CrossRef] [PubMed]
32. Fathima, S.N. Classification of blood types by microscope color images. *Int. J. Mach. Learn. Comput.* **2013**, *3*, 376. [CrossRef]
33. Acharya, V.; Kumar, P. Identification and red blood cell automated counting from blood smear images using computer-aided system. *Med. Biol. Eng. Comput.* **2018**, *56*, 483–489. [CrossRef] [PubMed]
34. Szczepański, T.; van der Velden, V.H.; van Dongen, J.J. Flow-cytometric immunophenotyping of normal and malignant lymphocytes. *Clin. Chem. Lab. Med.* **2006**, *44*, 775–796. [CrossRef]
35. Hu, J.; Cui, X.; Gong, Y.; Xu, X.; Gao, B.; Wen, T.; Xu, F. Portable microfluidic and smartphone-based devices for monitoring of cardiovascular diseases at the point of care. *Biotechnol. Adv.* **2016**, *34*, 305–320. [CrossRef]
36. Zhang, Z.; Ohta, S.; Shiba, S.; Niwa, O. Nanocarbon film electrodes for electro-analysis and electrochemical sensors. *Curr. Opin. Electrochem.* **2022**, *35*, 101045. [CrossRef]
37. Shahid, M.; Sami, H.; Sharma, S.; Kumar, A.; Khan, P.A.; Khan, H.M. Comparative analysis of Electro-Chemiluminescence Immunoassay (ECLIA), ELISA and Rapid Diagnostic Test (RDT) for detection of Hepatitis B Surface Antigen (HBSAG). *Pathology* **2020**, *52*, S126–S127. [CrossRef]
38. Topal, S.; Şaylan, M.; Zaman, B.T.; Bakırdere, S. Determination of trace cadmium in saliva samples using spray assisted droplet formation-liquid phase microextraction prior to the measurement by slotted quartz tube-flame atomic absorption spectrophotometry. *J. Trace Elem. Med. Biol.* **2021**, *68*, 126859. [CrossRef]
39. Erarpat, S.; Bodur, S.; Günkara, O.T.; Bakırdere, S. Combination of high performance liquid chromatography and flame atomic absorption spectrophotometry using a novel nebulizer interface supported T shaped slotted quartz tube for the determination of Vitamin B12. *J. Pharm. Biomed. Anal.* **2022**, *217*, 114855. [CrossRef]
40. Strzelak, K.; Koncki, R. Nephelometry and turbidimetry with paired emitter detector diodes and their application for determination of total urinary protein. *Anal. Chim. Acta* **2013**, *788*, 68–73. [CrossRef]
41. Hui, X.; Tian, J.; Wang, X.; Zhang, Z.; Zhao, Y.; Gao, W.; Li, H. Overall analyses of the reactions catalyzed by acetohydroxyacid synthase/acetolactate synthase using a precolumn derivatization-HPLC method. *Anal. Biochem.* **2023**, *660*, 114980. [CrossRef] [PubMed]
42. Theiner, S.; Schoeberl, A.; Schweikert, A.; Keppler, B.K.; Koellensperger, G. Mass spectrometry techniques for imaging and detection of metallodrugs. *Curr. Opin. Chem. Biol.* **2021**, *61*, 123–134. [CrossRef] [PubMed]

43. Jain, R.; Jain, B.; Chauhan, V.; Deswal, B.; Kaur, S.; Sharma, S.; Abourehab, M.A.S. Simple determination of dichlorvos in cases of fatal intoxication by gas Chromatography-Mass spectrometry. *J. Chromatogr. B* **2023**, *1215*, 123582. [CrossRef] [PubMed]
44. Famiglini, G.; Palma, P.; Termopoli, V.; Cappiello, A. The history of electron ionization in LC-MS, from the early days to modern technologies: A review. *Anal. Chim. Acta* **2021**, *1167*, 338350. [CrossRef]
45. Gugten, J.G. Tandem mass spectrometry in the clinical laboratory: A tutorial overview. *Clin. Mass Spectrom.* **2020**, *15*, 36–43. [CrossRef]
46. Okai, G.G.; Cardozo, K.H.M.; Ferrer, C.M.; Goldman, M.M.; Clarke, N.J.; Vieira, J.G.H. LC-MS/MS reduces interference by high levels of 25(OH)D and its metabolites on measured 1,25(OH)2D. *Steroids* **2022**, *187*, 109095. [CrossRef]
47. Bhimwal, R.; Rustandi, R.R.; Payne, A.; Dawod, M. Recent advances in capillary gel electrophoresis for the analysis of proteins. *J. Chromatogr. A* **2022**, *1682*, 463453. [CrossRef] [PubMed]
48. Wang, Y.; Wu, J.; Sun, P.; Yu, J.; Pu, Q. A fully functional palmtop microchip electrophoresis analyzer with laser-induced fluorescence detection. *Sens. Actuators B Chem.* **2022**, *372*, 132645. [CrossRef]
49. Devanesan, S.; AlQahtani, F.; AlSalhi, M.S.; Jeyaprakash, K.; Masilamani, V. Diagnosis of thalassemia using fluorescence spectroscopy, auto-analyzer, and hemoglobin electrophoresis—A prospective study. *J. Infect. Public Health* **2019**, *12*, 585–590. [CrossRef]
50. Farmer, J.R.; DeLelys, M. Flow Cytometry as a Diagnostic Tool in Primary and Secondary Immune Deficiencies. *Clin. Lab. Med.* **2019**, *39*, 591–607. [CrossRef]
51. Sorigue, M.; Cañamero, E.; Miljkovic, M.D. Systematic review of staging bone marrow involvement in B cell lymphoma by flow cytometry. *Blood Rev.* **2021**, *47*, 100778. [CrossRef] [PubMed]
52. Liu, J.; Mengel, K.; Friedberg, K.D. In vivo effects of fluconazole on lymphocyte subpopulations of the thymus and spleen in mice: Flow cytometry analysis. *Int. J. Immunopharmacol.* **1996**, *18*, 449–457. [CrossRef] [PubMed]
53. Calidonio, J.M.; Schifferli, K.H. Biophysical and biochemical insights in the design of immunoassays. *Biochim. Biophys. Acta BBA Gen. Subj.* **2023**, *1867*, 130266. [CrossRef]
54. Marini, S.; Fasciglione, G.F.; Giardina, B. Production and characterization of monoclonal antibodies against l-carnitine: Radioimmunologic assays for l-carnitine determination. *Clin. Chim. Acta* **1996**, *249*, 93–108. [CrossRef] [PubMed]
55. Pan, W.; Jiang, T.; Lu, T.; Jin, Q.; Xi, Y.; Zhang, W. Biomimetic-mineralized bifunctional nanoflowers for enzyme-free and colorimetric immunological detection of protein biomarker. *Talanta* **2022**, *238*, 123001. [CrossRef]
56. Zhang, C.; Liu, Z.; Bai, M.; Wang, Y.; Liao, X.; Zhang, Y.; Wang, P.; Wei, J.; Zhang, H.; Wang, J.; et al. An ultrasensitive sandwich chemiluminescent enzyme immunoassay based on phage-mediated double-nanobody for detection of Salmonella Typhimurium in food. *Sens. Actuators B Chem.* **2022**, *352*, 131058. [CrossRef]
57. Bastarache, J.A.; Koyama, T.; Wickersham, N.E.; Ware, L.B. Validation of a multiplex electrochemiluminescent immunoassay platform in human and mouse samples. *J. Immunol. Methods* **2014**, *408*, 13–23. [CrossRef]
58. Chen, S.; Sun, Y.; Fan, F.; Chen, S.; Zhang, Y.; Zhang, Y.; Meng, X.; Lin, J. Present status of microfluidic PCR chip in nucleic acid detection and future perspective. *TrAC Trends Anal. Chem.* **2022**, *157*, 116737. [CrossRef]
59. Pearson, L.A.; Agostino, P.M.; Neilan, B.A. Recent developments in quantitative PCR for monitoring harmful marine microalgae. *Harmful Algae* **2021**, *108*, 102096. [CrossRef]
60. Tillati, S.; Pati, I.; Delle Donne, M. Horiba Micros ES 60 Blood Cell Analyzer in Blood Donor Eligibility: A Validation Study. *Diagnostics* **2022**, *12*, 2586. [CrossRef]
61. Tang, S.S.; Liang, C.H.; Liu, Y.L. Intermittent hypoxia is involved in gut microbial dysbiosis in type 2 diabetes mellitus and obstructive sleep apnea-hypopnea syndrome. *World J. Gastroenterol.* **2022**, *28*, 2320. [CrossRef]
62. Altunok, İ.; Aksel, G.; Eroğlu, S.E. Correlation between sodium, potassium, hemoglobin, hematocrit, and glucose values as measured by a laboratory autoanalyzer and a blood gas analyzer. *Am. J. Emerg. Med.* **2019**, *37*, 1048–1053. [CrossRef]
63. Karadag Gecgel, S. Comparison of HBV-DNA Levels with Biochemical and Microbiological Parameters for Chronic Hepatitis Evaluation, Bursa, Turkey. *J. Med. Microbiol. Infect. Dis.* **2021**, *9*, 17–24.
64. Hu, F.; Li, J.; Zhang, Z. Smartphone-based droplet digital LAMP device with rapid nucleic acid isolation for highly sensitive point-of-care detection. *Anal. Chem.* **2019**, *92*, 2258–2265. [CrossRef] [PubMed]
65. Barbé, B.; Corsmit, E.; Jans, J. Pilot Testing of the "Turbidimeter", a Simple, Universal Reader Intended to Complement and Enhance Bacterial Growth Detection in Manual Blood Culture Systems in Low-Resource Settings. *Diagnostics* **2022**, *12*, 615. [CrossRef] [PubMed]
66. Badawi, R.D.; Shi, H.; Hu, P. First human imaging studies with the EXPLORER total-body PET scanner. *J. Nucl. Med.* **2019**, *60*, 299–303. [CrossRef]
67. Higuchi, S.; Kamishiro, Y.; Ishihara, M. Evaluation of a radon air monitor in the measurement of radon concentration in water in comparison with a liquid scintillation counter. *Radiat. Prot. Dosim.* **2019**, *184*, 426–429. [CrossRef] [PubMed]
68. Kim, H.; Kim, Y.O.; Kim, Y. Small red blood cell fraction on the UF-1000i urine analyzer as a screening tool to detect dysmorphic red blood cells for diagnosing glomerulonephritis. *Ann. Lab. Med.* **2019**, *39*, 271. [CrossRef]
69. Yao, Q.; Li, Y.; Tang, X. Separation of petroleum ether extracted residue of low temperature coal tar by chromatography column and structural feature of fractions by TG-FTIR and PY-GC/MS. *Fuel* **2019**, *245*, 122–130. [CrossRef]
70. Masindi, V.; Fosso-Kankeu, E.; Mamakoa, E. Emerging remediation potentiality of struvite developed from municipal wastewater for the treatment of acid mine drainage. *Environ. Res.* **2022**, *210*, 112944. [CrossRef]

71. Hooghvorst, I.; Torrico, O.; Nogués, S. *Doubled Haploid Parthenogenetic Production of Melon 'Piel de Sapo' Doubled Haploid Technology*; Humana: New York, NY, USA, 2021; pp. 87–95.
72. Hermilasari, R.D.; Rizal, D.M.; Wirohadidjojo, Y.W. Effect of Platelet-Rich Plasma (PRP) on testicular damage in streptozotocin-induced diabetic rats. *Bali Med. J.* **2020**, *9*, 351–355. [CrossRef]
73. Fadeyi, E.A.; Wagner, S.J.; Goldberg, C. Fatal sepsis associated with a storage container leak permitting platelet contamination with environmental bacteria after pathogen reduction. *Transfusion* **2021**, *61*, 641–648. [CrossRef] [PubMed]
74. Chizh, N.A. Cryogenic Equipment in Minimally Invasive Surgery. *Probl. Cryobiol. Cryomed.* **2018**, *28*, 200–211. [CrossRef]
75. Weaver, D.T.; McElvany, B.D.; Gopalakrishnan, V. UV decontamination of personal protective equipment with idle laboratory biosafety cabinets during the COVID-19 pandemic. *PLoS ONE* **2021**, *16*, e0241734. [CrossRef] [PubMed]
76. Fan, Q.; Qian, S.; He, Z.; Zheng, J.; Zheng, F.; Jiang, X. *Clinical Laboratory Instruments and Technologies*; People's Medical Publishing House: Beijing, China, 2015; pp. 1–5.
77. Seger, C.; Salzmann, L. After another decade: LC–MS/MS became routine in clinical diagnostics. *Clin. Biochem.* **2020**, *82*, 2–11. [CrossRef]
78. Lan, T.; Zhang, J.; Lu, Y. Transforming the blood glucose meter into a general healthcare meter for in vitro diagnostics in mobile health. *Biotechnol. Adv.* **2016**, *34*, 331–341. [CrossRef] [PubMed]
79. Lough, F.; Perry, J.D.; Stanforth, S.P.; Dean, J.R. Detection of exogenous VOCs as a novel in vitro diagnostic technique for the detection of pathogenic bacteria. *TrAC Trends Anal. Chem.* **2017**, *87*, 71–81. [CrossRef]
80. Vashist, S.K.; Schneider, E.M.; Luong, J.H.T. Rapid sandwich ELISA-based in vitro diagnostic procedure for the highly-sensitive detection of human fetuin A. *Biosens. Bioelectron.* **2015**, *67*, 73–78. [CrossRef]
81. Xiao, F.; Wang, L.; Duan, H. Nanomaterial based electrochemical sensors for in vitro detection of small molecule metabolites. *Biotechnol. Adv.* **2016**, *34*, 234–249. [CrossRef]
82. Kumánovics, A.; Akha, A.A.S. Flow cytometry for B-cell subset analysis in immunodeficiencies. *J. Immunol. Methods* **2022**, *509*, 113327. [CrossRef]
83. Song, H.; Zhu, Y. The in vitro diagnostics industry in China. *View* **2020**, *1*, e5. [CrossRef]
84. Pandith, A.; Kim, H.; Shin, T.; Seo, Y.J. Unprecedented green-emissive boranyl-hydrazone supramolecular assemblies and their in vitro diagnostic applications. *J. Photochem. Photobiol. B Biol.* **2019**, *197*, 111553. [CrossRef]
85. Perruca-Foncillas, R.; Davidsson, J.; Carlquist, M.; Gorwa-Grauslund, M.F. Assessment of fluorescent protein candidates for multi-color flow cytometry analysis of Saccharomyces cerevisiae. *Biotechnol. Rep.* **2022**, *34*, e00735. [CrossRef] [PubMed]
86. Dundon, W.G.; Settypalli, T.B.K.; Spiegel, K.; Steinrigl, A.; Revilla-Fernández, S.; Schmoll, F.; Naletoski, I.; Lamien, C.E.; Cattoli, G. Comparison of eleven in vitro diagnostic assays for the detection of SARS-CoV-2 RNA. *J. Virol. Methods* **2021**, *295*, 114200. [CrossRef] [PubMed]
87. Zhao, F.; Koo, B.; Liu, H.; Jin, C.E.; Shin, Y. A single-tube approach for in vitro diagnostics using diatomaceous earth and optical sensor. *Biosens. Bioelectron.* **2018**, *99*, 443–449. [CrossRef] [PubMed]
88. Akizawa, H.; Zhao, S.; Takahashi, M.; Nishijima, K.; Kuge, Y.; Tamaki, N.; Seki, K.; Ohkura, K. In vitro and in vivo evaluations of a radioiodinated thymidine phosphorylase inhibitor as a tumor diagnostic agent for angiogenic enzyme imaging. *Nucl. Med. Biol.* **2010**, *37*, 427–432. [CrossRef]
89. Seo, S.B.; Hwang, J.; Kim, E.; Kim, K.; Roh, S.; Lee, G.; Lim, J.; Kang, B.; Jang, S.; Son, S.U.; et al. Isothermal amplification-mediated lateral flow biosensors for in vitro diagnosis of gastric cancer-related microRNAs. *Talanta* **2022**, *246*, 123502. [CrossRef]
90. Staroverov, S.A.; Kozlov, S.V.; Brovko, F.A.; Fursova, K.K.; Shardin, V.V.; Fomin, A.S.; Gabalov, K.P.; Soldatov, D.A.; Zhnichkova, E.G.; Dykman, L.A.; et al. Phage antibodies against heat shock proteins as tools for in vitro cancer diagnosis. *Biosens. Bioelectron. X* **2022**, *11*, 100211. [CrossRef]
91. Wang, Y.; Li, B.; Tian, T.; Liu, Y.; Zhang, J.; Qian, K. Advanced on-site and in vitro signal amplification biosensors for biomolecule analysis. *TrAC Trends Anal. Chem.* **2022**, *149*, 116565. [CrossRef]
92. Nusbaum, K.B.; Korman, A.M.; Tyler, K.; Kaffenberger, J.; Trinidad, J.; Kaffenberger, B.H. In vitro diagnostics for the medical dermatologist. Part I: Autoimmune tests. *J. Am. Acad. Dermatol.* **2021**, *85*, 287–298. [CrossRef]
93. Yamamoto, S.; Whyte, T.; Toen, C.V.; Melnyk, A.; Shewchuk, J.; Street, J.; Cripton, P.; Oxland, T.R. The diagnostic precision of computed tomography for traumatic cervical spine injury: An in vitro biomechanical investigation. *Clin. Biomech.* **2022**, *92*, 105529. [CrossRef] [PubMed]
94. Rajpal, S.; Mishra, P. Next generation biosensors employing molecularly imprinted polymers as sensing elements for in vitro diagnostics. *Biosens. Bioelectron. X* **2022**, *11*, 100201. [CrossRef]
95. Braga, F.; Pasqualetti, S.; Panteghini, M. The role of external quality assessment in the verification of in vitro medical diagnostics in the traceability era. *Clin. Biochem.* **2018**, *57*, 23–28. [CrossRef]
96. Fatangare, A.; Glässner, A.; Sachs, B.; Sickmann, A. Future perspectives on in-vitro diagnosis of drug allergy by the lymphocyte transformation test. *J. Immunol. Methods* **2021**, *495*, 113072. [CrossRef]
97. Vieira, P.; Jesus, V.; Cândido, M.A.; Pacheco-Soares, C.; Castilho, M.; Raniero, L. Specific nanomarkers fluorescence: In vitro analysis for EGFR overexpressed cells in triple-negative breast cancer and malignant glioblastoma. *Photodiagnosis Photodyn. Ther.* **2022**, *39*, 102997. [CrossRef] [PubMed]
98. Juárez, M.J.; Ibáñez-Echevarria, E.; Rojas, D.H.F.; Maquieira, Á.; Morais, S. Multiplexed analytical approaches to beta-lactam allergy in vitro testing standardization. *Anal. Chim. Acta* **2021**, *1173*, 338656. [CrossRef]

99. Caputo, D.; Cascone, C.; Santo, R.D.; Digiacomo, L.; Quagliarini, E.; Pozzi, D.; Coppola, R.; Caracciolo, G. Fine-Tuning of Nanoparticle-Protein Corona Composition: A New Strategy for the Development of Efficient in Vitro Diagnostic Testing for Early Pancreatic Cancer Detection. *J. Am. Coll. Surg.* **2020**, *231*, e34.
100. Kim, K.; Kashefi-Kheyrabadi, L.; Joung, Y.; Kim, K.; Dang, H.; Chavan, S.G.; Lee, M.; Choo, J. Recent advances in sensitive surface-enhanced Raman scattering-based lateral flow assay platforms for point-of-care diagnostics of infectious diseases. *Sens. Actuators B Chem.* **2021**, *329*, 129214. [CrossRef]
101. Sufianov, A.; Begliarzade, S.; Ilyasova, T.; Xu, X.; Beylerli, O. MicroRNAs as potential diagnostic markers of glial brain tumors. *Non-Coding RNA Res.* **2022**, *7*, 242–247. [CrossRef]