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Review Article

Microplate Reader versus Manual Water-Bath (37°C) Methods for Clot-Based Coagulation Testing: A Comparative Review

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Abstract

Background and Objective: Clot-based testing is a series of assays carried out to ascertain the functionality of the clotting proteins or enzymes. Over the years, the tilt tube method (grounded technique) using a water bath has been available in our local setting and in Nigeria as a whole. This review aimed to provide information on the usefulness of the microplate reader compared to the manual method (use of a water bath) in the clot-based testing method. **Materials and Methods:** This review adopted a narrative review approach to provide a comprehensive overview of the use of microplate readers and manual water bath (37°C) methods in clot-based coagulation testing. Relevant literature was systematically searched from major electronic databases, including PubMed, Scopus, Web of Science and Google Scholar. The search covered publications from January 2000 to December 2025, with emphasis on studies evaluating coagulation testing methodologies, laboratory automation and analytical performance of clot-based assays. **Results:** The reviewed literature indicates that manual water-bath methods are prone to variability arising from subjective clot endpoint determination, timing inconsistencies and potential temperature fluctuations that may influence coagulation enzyme activity. In contrast, microplate reader-based assays provide automated optical detection through absorbance, fluorescence, or luminescence measurements, thereby reducing observer bias and improving standardisation. Studies reviewed also demonstrated that microplate readers facilitate simultaneous analysis of multiple samples, improve workflow efficiency, reduce reagent consumption and enhance result reproducibility. These advantages make microplate reader technology a promising alternative to conventional manual methods for clot-based coagulation testing. **Conclusion:** From the review so far, the findings have shown that microplate reader-based coagulation testing offers significant advantages over manual water-bath methods in terms of automation, analytical consistency, sample throughput and reproducibility.

Key words: Microplate reader, water bath, clot-based testing, microplate, haemostasis, prothrombin time, activated partial thromboplastin time

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Clot-based coagulation testing is fundamental to the diagnosis and monitoring of haemostatic disorders. Assays such as Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Thrombin Time (TT) and fibrinogen estimation are routinely employed to evaluate the integrity of the coagulation cascade and support clinical decision-making in patients with bleeding disorders, thrombotic diseases, liver dysfunction and those receiving anticoagulant therapy. Because treatment decisions frequently depend on these results, analytical accuracy, precision and reproducibility are essential requirements in coagulation laboratories^{1,2}. Historically, coagulation testing relied predominantly on manual techniques involving visual observation of clot formation after incubation at 37°C, the physiological temperature required for optimal coagulation enzyme activity. The water bath became an important laboratory instrument for maintaining the temperature required for manual coagulation testing. However, manual clot detection is susceptible to operator-dependent variability, inconsistencies in reaction timing and subjective determination of the clotting endpoint^{3,4}. These limitations have contributed to the development and adoption of automated approaches that provide more objective methods of clot detection. In routine coagulation analysers, optical and mechanical clot-detection systems represent two major technological approaches, each with specific analytical characteristics and limitations. Optical and mechanical detection methods can produce comparable coagulation results while differing in their principles of clot detection and analytical performance¹.

The development of microplate-based analytical systems has provided another approach to coagulation testing. Standardised multiwell microplates permit simultaneous analysis of multiple samples while requiring relatively small volumes of plasma and reagents⁵. The incorporation of kinetic optical detection has enabled changes in absorbance associated with fibrin formation to be monitored continuously. Pratt and Monroe demonstrated the feasibility of microplate-based PT and APTT assays using a kinetic microplate reader to monitor changes in absorbance at 405 nm during plasma coagulation. Their findings showed that the microplate format could support simultaneous testing of multiple samples and could be adapted for specific coagulation applications².

Subsequent studies have further demonstrated the potential of microplate technology for coagulation factor assessment. Tilley *et al.*^{3,4} developed a microplate coagulation

assay for Factor V activity based on kinetic measurement of absorbance during fibrin clot formation. The method was capable of measuring clotting time, initial clot formation rate and extent of clot formation, while allowing multiple samples to be analysed simultaneously^{3,5}. This approach demonstrated that microplate-based optical measurements can provide quantitative information about clot formation and can potentially be adapted to the assessment of other coagulation factors.

Microplate readers therefore offer several potential advantages over conventional manual methods, including objective optical detection, simultaneous processing of multiple samples, reduced sample and reagent requirements and the ability to generate continuous kinetic measurements. However, microplate-based coagulation testing is not necessarily equivalent to conventional clinical coagulation testing. Differences in reagent concentrations, sample volumes, reaction temperatures, optical detection principles and definitions of clotting endpoints may influence assay performance and limit direct comparison between methods. In addition, optical measurements of fibrin formation may be affected by the heterogeneous nature of the clot, demonstrating the importance of appropriate assay design and interpretation of optical signals³⁻⁵.

Despite the demonstrated feasibility of microplate-based coagulation assays, there remains a need for a clear synthesis of the similarities and differences between microplate reader-based clot detection and the conventional manual water-bath (37°C) approach⁶⁻⁸. In particular, limited consolidated evidence addresses how these methods compare with respect to analytical performance, endpoint detection, reproducibility, temperature requirements, reagent consumption, throughput and practical applicability in haemostasis laboratories^{9,10}. This gap is particularly relevant to laboratories in resource-constrained settings where fully automated coagulation analysers may not be readily available, but there is a need for methods that improve objectivity, efficiency and reproducibility.

Therefore, this review comparatively evaluates microplate reader and manual water-bath (37°C) methods for clot-based coagulation testing, focusing on their operating principles, analytical performance, temperature requirements, endpoint detection, reproducibility, sample and reagent requirements, advantages, limitations and potential applications in haemostasis laboratories. The review further considers the potential role of microplate reader technology as an alternative or complementary approach to conventional manual clot-based coagulation testing.

MATERIALS AND METHODS

Review design: This study employed a structured narrative literature review to compare microplate reader-based versus manual water-bath (37°C) methods for clot-based coagulation testing. The search covered publications from January 2000 to December 2025. The review focused on the principles of clot detection, analytical performance, temperature requirements, endpoint determination, reproducibility, sample and reagent requirements, advantages, limitations and potential applications of the two approaches. The review process was structured to enhance transparency in the identification, selection and synthesis of relevant literature.

Literature search strategy: A comprehensive literature search was conducted using the PubMed/MEDLINE, Scopus, Web of Science and Google Scholar databases. Additional relevant articles were identified by screening the reference lists of selected publications and relevant review articles.

Principles and applications of microplate reader and Manual Water-Bath methods in Clot-Based coagulation testing

Types of microplate: The choice of microplate colour and base material is usually determined by the assay readout and the need to maximise the signal-to-background ratio. Microplates generally come in four colour types: Clear, white, black and grey^{11,12}.

Clear microplates: Clear microplates are typically used for absorbance (colorimetric) assays. Most conventional polystyrene microplates are suitable for visible light measurements, while ultraviolet (UV)-transparent microplates are often made from cyclic olefin copolymer (COC) or cyclic olefin polymer (COP), allowing measurements at wavelengths as low as 230 nm¹³.

White microplates: White microplates are commonly used for luminescence-based assays because their reflective surfaces enhance signal intensity and reduce well-to-well crosstalk. They may also be useful in fluorescence assays involving fluorophores with longer half-lives, where background autofluorescence is minimized¹⁴.

Black microplates: Black microplates are particularly suitable for fluorescence-based assays because they reduce background autofluorescence and minimise optical interference between adjacent wells. Their use improves assay sensitivity and signal specificity¹⁵.

Other microplates: Less commonly used colours include grey and yellow microplates, which are designed for specific assay applications. In addition, some microplates are manufactured with clear-bottom wells to facilitate optical measurements and microscopic examination when required¹².

The appropriate choice of microplate is important because an unsuitable plate type may adversely affect assay sensitivity, signal detection and overall analytical performance.

Classification of microplate readers: Microplate readers may be classified according to wavelength-selection technology and detection capability.

Microplate readers by wavelength selection

Filter-based readers: Filter-based microplate readers use optical filters to select specific wavelengths. They are generally highly sensitive and provide rapid wavelength switching, making them suitable for assays such as Enzyme-Linked Immunosorbent Assays (ELISA). However, they require dedicated filters for each wavelength and do not permit wavelength scanning¹⁶.

Monochromator-based readers: Monochromator-based readers use diffraction gratings to select wavelengths. They offer greater flexibility because multiple wavelengths can be selected without changing filters, although sensitivity may be lower compared with filter-based systems for certain applications¹⁶.

Microplate readers by detection technology

Dedicated microplate readers: Dedicated microplate readers perform a single detection function, such as absorbance, fluorescence, or luminescence measurement. They often provide excellent sensitivity and reliability for specific applications¹⁶.

Multimode microplate readers: Multimode microplate readers combine two or more detection technologies within a single instrument. These systems offer greater versatility and can accommodate a wide range of laboratory applications, although they are generally more expensive than dedicated readers¹⁷.

Working principle of microplate reader: The microplate reader is a specialised spectrophotometric instrument designed to analyse samples contained within microplate wells. Unlike conventional spectrophotometers that operate over a broad wavelength range, microplate readers utilise optical filters or diffraction gratings to select specific

wavelengths appropriate for the assay being performed. Most readers operate within the visible spectrum, while some models also support ultraviolet measurements¹⁸⁻²⁰. Light is directed through or onto the sample contained within each well and the transmitted, absorbed, or emitted signal is detected by a photodetector. The signal is subsequently amplified, converted into digital data and analysed by dedicated software. In clot-based coagulation testing, changes in optical density can be monitored to detect clot formation and determine coagulation endpoints objectively^{18,21,22}.

Working principle of the water bath: A laboratory water bath is designed to maintain samples at a constant and controlled temperature. It consists of a water-filled chamber, a heating element, a temperature sensor and a control system. The sensor continuously monitors the water temperature and transmits information to the control unit, which regulates the heating element to maintain the desired temperature^{21,23,24}. In clot-based coagulation testing, the water bath is commonly maintained at 37°C, which represents the physiological temperature required for optimal activation of coagulation enzymes and proteins. Samples and reagents are incubated at this temperature before clot formation is manually assessed and timed²⁴.

Limitations of the microplate reader in clot-based testing: Although microplate readers offer several advantages, they also have limitations in clot-based coagulation testing. Studies assessing fibrin clot structure often utilise optical measurements based on light scattering and absorbance changes during clot formation. While microplate readers can monitor clot development and provide qualitative information regarding fibrin formation, they may not always accurately quantify certain structural characteristics of fibrin fibres compared with high-end spectrophotometric systems²⁵. Furthermore, optical measurements may not directly reflect the functional capacity of coagulation proteins or clotting factors. Consequently, microplate reader-based assays should ideally be interpreted alongside functional coagulation assays. Another limitation is the relatively high acquisition and maintenance cost of microplate readers, which may restrict their availability in resource-limited laboratories²⁶.

Limitations of the water bath in clot-based testing: The major limitations of water bath-based clot testing relate to temperature control and manual clot detection. Faulty temperature-regulating mechanisms may result in deviations from the required 37°C incubation temperature, potentially

affecting enzyme activity and leading to inaccurate results²⁷. Manual observation of clot formation is also subject to operator variability. During procedures such as Prothrombin Time (PT) testing, small clots may not be immediately detected by laboratory personnel, leading to errors in timing and endpoint determination. Such variability can negatively affect assay precision and reproducibility. In addition, the use of water baths may expose personnel to risks such as burns, electrical shock, or accidental splashing of heated liquids²⁷.

Importance of microplate reader: The microplate reader has become an important instrument in modern laboratory practice because of its ability to improve efficiency, accuracy and standardisation. Its importance includes:

- Providing flexibility for a wide variety of microplate-based assays
- Facilitating simultaneous analysis of multiple samples, thereby increasing throughput
- Reducing manual errors associated with visual interpretation and timing
- Improving reproducibility and consistency of laboratory results
- Enhancing workflow efficiency through automation
- Supporting timely result generation for clinical and research applications
- Promoting greater accuracy and reliability in laboratory investigations

RESULTS AND DISCUSSION

The reviewed literature demonstrated that both conventional manual and microplate-based approaches can be used for clot-based coagulation testing, but they differ in endpoint detection, throughput and analytical standardisation.

Manual and optical clot detection: Manual water-bath methods rely principally on visual observation of clot formation following incubation at approximately 37°C. This approach is simple and accessible but is susceptible to operator-dependent variation in timing and endpoint recognition^{4,28}. Studies evaluating instrumental optical clot detection have demonstrated good analytical precision and strong agreement with mechanical clot-detection methods for PT and APTT, supporting the potential reliability of objective optical measurement²⁹.

Microplate-based coagulation testing: The literature demonstrated the feasibility of adapting microplate readers for clot-based coagulation assays. Pratt and Monroe developed microplate PT and APTT assays in which a kinetic microplate reader monitored changes in absorbance at 405 nm during plasma coagulation. The microplate format permitted multiple samples to be analysed simultaneously and required relatively small sample volumes²⁸.

Tilley *et al.*^{3,4} subsequently developed a microplate assay for Factor V activity. The method continuously measured changes in absorbance during fibrin formation and was able to determine the time, initial rate and extent of clot formation. Multiple samples could be analysed simultaneously, demonstrating the potential of the approach for quantitative coagulation-factor testing²⁹.

The present review highlights the comparative advantages and limitations of microplate reader-based and manual water-bath (37°C) methods for clot-based coagulation testing. Coagulation assays such as Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Thrombin Time (TT), fibrinogen estimation, platelet aggregation studies and coagulation factor assays are fundamental to the diagnosis and monitoring of haemostatic disorders. Consequently, the accuracy, precision and reproducibility of these assays are essential for ensuring appropriate clinical decision-making and patient management^{1,2,30}. The microplate reader is a versatile analytical instrument capable of simultaneously measuring absorbance, fluorescence and luminescence across multiple samples. This capability allows for high-throughput analysis and objective endpoint determination, thereby reducing the variability associated with manual clot detection. In contrast,

the water bath serves primarily as a temperature-regulating device that maintains samples at the physiological temperature of 37°C required for optimal coagulation enzyme activity. Although the water bath remains widely used because of its simplicity, accessibility and relatively low cost, it relies heavily on visual clot detection and manual timing, which may introduce operator-dependent variability and affect assay reproducibility³¹.

A major advantage of microplate-based coagulation testing is its ability to analyse multiple patient samples simultaneously while requiring relatively small reagent and sample volumes (Fig. 1). This feature is particularly beneficial when expensive reagents or factor-deficient plasmas are used, or when large sample numbers must be processed. Various clot-based assays have been successfully adapted to microplate formats, including PT, APTT, thrombin generation assays, platelet aggregation studies, activated protein C resistance testing, factor activity assays and fibrinolytic assessments. Studies have demonstrated that microplate readers provide reliable and reproducible measurements of clot formation through optical monitoring of absorbance^{1,2}, fluorescence, or luminescence changes associated with fibrin formation²⁸⁻³⁰. The ability of microplate readers to generate standardised optical measurements represents a significant improvement over the manual tilt-tube technique (Fig. 2). In conventional water-bath methods, clot formation is determined visually by laboratory personnel and the exact endpoint may vary depending on operator experience, reaction speed, lighting conditions and observational skills. Such variability can contribute to differences in clotting times between operators and laboratories. Automated optical



Fig. 1: Arrangement of platelet-poor plasma (PPP) samples in duplicate within a 96-well microplate before clot-based coagulation analysis

Courtesy of Thrombosis and Atherosclerosis Research Institute, McMaster University, Hamilton, Ontario, Canada



Fig. 2: Fluorescence microplate reader connected to a computer workstation for acquisition and analysis of clot-based coagulation assay data

Courtesy of Thrombosis and Atherosclerosis Research Institute, McMaster University, Hamilton, Ontario, Canada

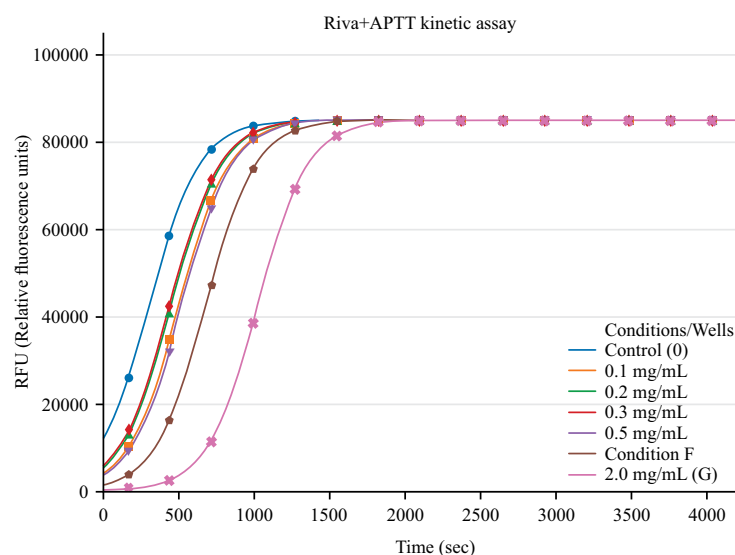


Fig. 3: Representative graphical output obtained from an activated partial thromboplastin time (APTT)-based clotting assay showing diluted plasma mixtures analysed in duplicate wells. They are carried out in duplicate e.g. A5 A6, B5 B6 etc

Courtesy of Thrombosis and Atherosclerosis Research Institute, McMaster University, Hamilton Ontario, Canada

detection minimises subjectivity and provides more objective endpoint determination, thereby improving inter-observer consistency and overall analytical reliability³¹⁻³².

Temperature control is another important consideration in clot-based coagulation testing. The coagulation cascade consists of a series of enzymatic reactions that are highly dependent on maintaining physiological conditions. Water baths are designed to maintain samples at 37°C; however, fluctuations in temperature resulting from equipment malfunction, inadequate calibration, or poor maintenance may affect enzyme activity and alter clotting times. Even small

deviations from the required incubation temperature can potentially influence assay performance and lead to inaccurate results (Fig. 3). Automated systems incorporating integrated temperature regulation and optical monitoring may reduce these sources of analytical variation³³. The findings of this review are consistent with previous studies³¹, who demonstrated that microplate coagulation assays provide reliable PT and APTT measurements while requiring smaller sample volumes and permitting simultaneous analysis of multiple specimens. Similarly, Tilley *et al.*³ reported that microplate-based coagulation assays are rapid, economical,

Table 1: Contrast between microplate reader and water bath in clot-based test

Microplate reader	Water bath
It is chemically, biologically and optically compatible with assay reagents and does not denature proteins	Measures the functional activity of the test being assayed for the possibility of protein denaturing due to fluctuations and in temperature of the faulty water bath
Has many wells, so it can be used to run and analyse many samples within a short period of time	One sample at a time is analysed because it is cumbersome to jiggle many samples in test tubes at the same time
It is used in a large laboratory setting that handles many samples a day	Use in under resourced cause of it cost effectiveness and good at handling a few samples
There is less concern about anomalous results arising from contamination cause it is a closed system	Contamination is common, which can come from the water in the bath
Results obtained from it are more accurate because it is automated and uses a computer for its analysis and has higher chances of reproducibility anywhere in the world	The result is prone to human error and has lower chances of reproducibility

sensitive and reproducible, making them suitable for the assessment of coagulation factor activity and clot formation. Additional studies have shown that microplate-based methods improve workflow efficiency, reduce turnaround times and enhance standardisation in haemostasis laboratories^{34,35}. These characteristics are increasingly important as laboratories strive to meet growing demands for timely and accurate diagnostic services.

Despite these advantages, microplate readers are not without limitations. The initial cost of acquisition, maintenance, software integration and personnel training may limit their availability in some laboratories, particularly in low- and middle-income countries. Furthermore, although microplate readers provide valuable information regarding clot formation and fibrin structure, some optical measurements may not fully reflect the functional activity of coagulation proteins and therefore should be interpreted alongside established functional assays²⁶. In contrast, the manual water-bath method remains attractive because of its affordability and minimal technological requirements, making it accessible to many laboratories with limited resources. From a practical perspective, the choice between microplate reader-based and water-bath methods may depend on laboratory workload, available resources, technical expertise and quality assurance requirements^{36,37}. Laboratories with high sample throughput and a need for enhanced standardisation may benefit considerably from adopting microplate reader technology. Conversely, smaller laboratories may continue to rely on water-bath-based methods, provided appropriate quality control measures, calibration procedures and operator training are maintained.

Overall, the evidence reviewed suggests that microplate reader-based coagulation testing offers significant advantages in terms of automation, reproducibility, sample throughput and objective result interpretation (Table 1).

While the conventional water-bath method remains useful and accessible, the increasing emphasis on laboratory standardisation and quality improvement supports the broader integration of microplate reader technology into modern haemostasis testing³⁸⁻⁴⁰. Future studies should focus on further validation of microplate-based coagulation assays against established reference methods and explore their applicability across diverse clinical and laboratory settings.

Microplate reader technology should be adopted where feasible to improve standardisation, reproducibility and efficiency in clot-based coagulation testing. Laboratories currently using water-bath methods should strengthen their quality control procedures, while continuous training of laboratory personnel and students in automated coagulation testing methods should be encouraged. Public and private laboratories should progressively invest in automation technologies to enhance the efficiency and reliability of haemostasis testing. Further comparative studies are recommended to evaluate the diagnostic performance, cost-effectiveness and clinical applicability of microplate reader-based coagulation assays across different laboratory settings. In addition, national and institutional laboratory policies should support the modernisation and standardisation of haemostasis laboratories.

CONCLUSION

This review has highlighted the comparative strengths and limitations of microplate reader-based and manual water-bath (37°C) methods in clot-based coagulation testing. While the conventional water bath remains an important and widely used tool for coagulation assays, particularly in resource-limited settings, its reliance on manual clot detection and timing introduces the potential for operator-dependent variability and analytical inconsistencies.

In contrast, microplate readers offer several advantages, including automated optical detection, objective endpoint determination, reduced observer bias, improved reproducibility and the ability to process multiple samples simultaneously. Their capacity to perform absorbance, fluorescence and luminescence measurements enhances analytical flexibility and supports a wide range of haemostasis investigations. Furthermore, the high-throughput nature of microplate-based assays improves laboratory workflow efficiency, reduces reagent consumption and shortens turnaround times.

The evidence reviewed suggests that microplate reader-based methods represent a promising alternative to conventional water-bath techniques for clot-based coagulation testing. However, factors such as equipment cost, infrastructure requirements, maintenance and personnel training should be considered before implementation, particularly in low-resource environments.

SIGNIFICANCE STATEMENT

This review highlights the potential value of microplate reader technology in clot-based coagulation testing by providing automated, objective and reproducible measurements of clot formation. Compared with conventional water-bath and manual tilt-tube methods, microplate-based assays offer improved standardisation, reduced operator-dependent variability, enhanced sample throughput and more efficient utilisation of reagents. The ability of microplate readers to simultaneously analyse multiple samples using absorbance, fluorescence, or luminescence detection supports greater analytical consistency and workflow efficiency. Consequently, the integration of microplate reader technology into haemostasis laboratories may contribute to improved accuracy, reproducibility and standardisation of coagulation testing, while supporting the growing demand for automation in modern laboratory practice.

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