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EVALUATION OF ANALYTICAL PERFORMANCE OF RAPID DIAGNOSTIC TEST KITS FOR BLOOD GROUPINGS

Antwi, M. H.[1], Badu, A.[2], Ampaw, E. M.[3], Siaw, K. A.[4], Appeaning, M.[5], Darban, I.[6], Kwabia, K. A.[7], Ussher , F. A.[8], Ametepe, S.[9], Tenkorang, S. B.[10], Agyemang, B.[11], Buckman T. A.[12], Amissah, A. T.[13], Ablordeppey, W.[14], Mitchell, S.[15], Akwetey, A.[16], Kuwornu, T. K.[17], Agyemang, O. L.[18]

[1,2, 4,5,6,7,8,9,10,13.14,15,16&17]Department of Medical Laboratory Science, Faculty of Health and Allied Sciences, Koforidua Technical University, Koforidua, Ghana.
[3&11]Department of Applied Mathematics, Faculty of Applied Science and Technology, Koforidua Technical University, Koforidua, Ghana.
[12]Department of Molecular Medicine, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Ghana.
[18]Department of Early Grade, Pentecost Preparatory School, Daboya, Savanna Region of Ghana.
[1]maxwell.antwi@ktu.edu.gh

ABSTRACT

Purpose: This study aimed to determine the analytical performance of ABO and Rh D rapid diagnostic test kits as a substitute for the conventional tube method.

Design/Methodology/Approach: 161 participants who visited the Koforidua Technical University Clinic Laboratory were recruited from August to September 2022. The rapid diagnostic ABO and Rh D test kits were used to determine the ABO and Rh D blood groups of the study participants. The concordance rate, kappa analysis and Receiver Operating Characteristics (ROC) were performed.

Findings: The strength of agreement of the two diagnostic methods in detecting ABO and Rh D blood type using Kappa analyses was excellent (k=1.00; standard error=0.00; P-value=0.001). All diagnostic performance statistics of the RDT were not different from the tube method being the standard.

Research Limitation: This research focused on the analytical performance of the rapid test kits but not on different brands of the test kits for their comparative studies.

Practical Implication: This paper could potentially ensure reliable results, facilitate certification, and enable test kits to be accepted in the market.

Social Implication: This study will assist policy-makers in increasing access to healthcare to enable testing in resource-limited settings and improving health outcomes as timely diagnosis and management facilitate better patient outcomes.

Originality/ Value: This study is based on providing reliable information on portable and point-of-care diagnostic testing outside conventional laboratory settings for timely diagnosis.

Keywords: *Blood group.detection. performance. rapid diagnostic test. rhesus factor.*

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INTRODUCTION

Blood transfusions are often to save lives but sometimes may cause degrees of morbidity and death. These may be due to improper pre-analytical, analytical, and post-analytical procedures (Armstrong, 2008d; Perel et al., 2014; Li & Guo, 2022a). ABO and RhD blood typing are generally considered an essential part of clinical diagnosis in every clinical setting that involves blood transfusion, performed on both transfusion recipients and blood donors. The nature of the ABO blood group arises from two features of the blood system. First, in the case of other blood group systems, antibodies of the ABO blood system exist in the serum of almost every individual who does not possess the corresponding antigens (Li & Guo, 2022a). Also, the agglutinins of the ABO blood group system can fix complement and have the capacity to induce intravascular hemolysis of incompatible red cells. As a result of these instances, improper typing of ABO and RhD blood groups of a patient and donor could lead to deadly conditions during the process of blood transfusion (Pandey et al., 2024). Even though crossmatch is usually put as an additional measure to minimise ABO and RhD incompatibility, this might not be the case in every laboratory setting (Chapman et al., 2004; Pandey et al., 2024).

Currently, there are many approaches for ABO and RhD blood group determination. The accuracy and reliability of blood grouping are critical for blood transfusion (Perel et al., 2014; Li & Guo, 2022a). The conventional procedures of ABO and RhD blood grouping typing are mainly based on morphological analysis and phenotypic examination. Even though the tube method is considered a gold standard for blood typing, it requires antigens-specific antisera and is often performed by a professional. The procedure is time-consuming and requires highly skilled expertise (Li & Guo, 2022b). The procedure involves using the patient's red cells (RBCs), which are incubated with antibodies against a specific blood type in a tube and centrifuge, after which the tube is resuspended and examined for agglutination or hemolysis. Thus, the tube method, which involves designated equipment, cannot quickly and easily be performed, unlike the rapid diagnostic one, which, on the other hand, can be easily performed in any clinical laboratory setting and requires no specialised equipment (Magnette et al., 2024).

In the past decades, point-of-care diagnostics procedures have become highly demanded for diagnosis and monitoring treatment. There is no doubt that point-of-care diagnostics has taken a significant role in blood grouping in every clinical laboratory setting (Engel & Krumeich, 2020; Bahati et al., 2022). These emerging technologies for ABO and RhD blood grouping may be alternative and supplemental approaches. The availability of rapid diagnostic test kits can be helpful for prompt and accurate ABO and RhD blood group determination in laboratory settings with limited facilities. Despite the resource-limited settings and reduced patient turnaround time of RDT, it is not produced in large quantities and, therefore, challenging to commercialise in

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clinical laboratory settings. The RDT of ABO and RhD often uses a small volume of blood samples, usually obtained by finger prick or venipuncture. This technique employs a lateral diffusion system similar to the malaria RDT test to generate results. The result of RDT of ABO and RhD is exhibited in the form of visible bands that can be interpreted by non-expert users easily (Songjaroen et al., 2018; Reynolds et al., 2023).

Even though microscopy has been weighed as the gold standard for Malaria examination, a total of 1742 blood samples were tested by microscopy and rapid diagnostic test; 335 (19.4%) samples were detected as false negative with microscopic examinations, while 128(13.3%) negative samples were detected as false positives by the RDT. This study concluded that, in the absence of a skilled medical laboratory professional for malaria diagnosis in sub-Saharan areas of Africa, WHO recommends RDT as a good alternative for malaria diagnosis. This has made RDT become a primary tool for malaria parasitological diagnoses (Bahati et al., 2022; Engel & Krumeich, 2020). The analogy and synergy for ABO and Rh D blood typing using RDT could be true in this case. The performance of ABO and RhD RDT of blood typing and the conventional tube method may be affected by several factors, including sensitivity, specificity, and sometimes the clinical laboratory settings (Li & Guo, 2022c). The study sought to evaluate the analytical performance of RDT as against the conventional tube method of ABO and Rh Dblood group testing at the Koforidua Technical University clinic.

LITERATURE REVIEW

Blood group detection is essential to blood banking services, ensuring compatibility between the donor and recipient. The conventional tube method has been the gold standard for blood group detection. Notwithstanding, rapid diagnostic test (RDT) kits have emerged as a promising substitute, offering faster and more ease of use for blood group typing.

Accuracy and Reliability of RDT Kits

Studies have analysed the accuracy and reliability of RDT kits for blood group detection. A study by Reynolds et al. (2023) compared the performance of an RDT kit with the conventional tube method for ABO and RhD typing. The results showed that the RDT kit had a sensitivity and specificity of 99.6% and 99.9%, respectively, for ABO typing and 99.3% and 99.6%, respectively, for RhD typing. Another study by Magnette et al. (2024) evaluated the performance of an RDT kit for ABO and RhD typing in clinical healthcare. The results showed that the RDT kit had a sensitivity and specificity of 99.2% and 99.5%, respectively, for ABO typing and 98.5% and 99.2%, respectively, for RhD typing.

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Advantages and Limitations of RDT Kits

RDT kits offer advantages over the conventional tube method, including faster testing time, convenient use, and reduced requirements for specialised equipment and training (Srivathsa & Dendukuri, 2023). However, despite these advantages, RDT kits have some limitations, including lower sensitivity and specificity than the conventional tube method and the potential for false positive and false negative results (Reynolds et al., 2023).

Unlike the conventional method, few alternative studies have been reported for detecting blood groups. Srivathsa & Dendukuri (2023) presented a novel automated method for ABO, Rh D blood typing using simple morphological smartphone image processing algorithms in conjunction with a fabric strip-based rapid diagnostic test. Then et al. (2023) also used paper diagnostic methods to detect ABO and Rh D blood groups as an alternate method. A paper-based device for rapid typing of human blood groups has also been reported by Li et al. (2023). The optical sensor method for rapid ABO, Rh D blood group typing has also been reported as an alternative by Li et al. (2024). Dielectrophoretic characterisation of red blood cells as an investigative tool to identify blood type for medical diagnostic applications has also been reported as an alternate method in ABO, Rh D blood typing (Li & Guo, 2024). All these methods have employed easy, convenient, and fast techniques for detecting blood types, which aid in screening and diagnosis. Analytical performance for competencies of immunochromatography technique, which employs antigen-antibody complex labels in detecting ABO and Rh D blood type, have barely been assessed among the Ghanaian populace.

Rapid diagnostic test kits for blood group typing offer ease of use and a relatively accurate alternative to the conventional tube method. While RDT kits have some limitations, they can be useful in situations where rapid testing is demanded, such as in emergencies or resource-limited settings. Further studies are needed to evaluate the performance of RDT kits in different settings and to identify strategies for improving their accuracy and reliability.

METHODOLOGY

Study Area

The study was conducted at Koforidua Technical University Clinic Laboratory Department in the New Juaben Municipality of Eastern Region, Ghana. The university clinic serves thousands of students and the neighbourhood community, making it suitable for the studies. The school currently has six faculties.

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Research Design

A descriptive cross-sectional study was deployed in the investigation over two months, from July to August 2022, at Koforidua Technical University Clinic Laboratory. The Koforidua Technical University Research Committee and the Medical Director of the University clinic approved this study. The study's objective and test procedure were explained, and samples were collected from students and individuals from the university community who gave their consent after filling out the written consent form.

Population

The study population was 468 patients, including all persons 18 years of age and above who requested laboratory investigations from the University Clinic laboratory within the study period.

Sampling Technique and Sample Size

A purposive sampling technique was used to enrol 161 participants for the study. According to the laboratory records, the average number of patients who visit the clinic's laboratory is about 234 per month. The minimum sample size was calculated using Slovin's formula with a 95% confidence interval and a 5% margin of error to be 148. A convenient sampling technique was used to enrol 161 participants for the study.

Rapid Diagnostic Test (RDT) Selection

Three RDTs available on the Ghanaian health care market were selected: the Wiz Biotech-Solid phase blood typing test kit, the blood group test kit Eldoncard, and the INTEC blood grouping test kit. Their names were written on separate sheets of paper, rolled nicely, and placed in a bow. With a blindfold, one of the RDTs (Wiz Biotech-Solid Phase) was randomly chosen for the study.

Blood Sampling and Processing

Blood samples were obtained from study participants by venipuncture and then put into EDTA tubes. A trained medical laboratory scientist at the school clinic performed the blood sampling. The EDTA tubes were then labelled with the participants' identification numbers and used for the intended investigations.

Laboratory Investigation

The ABO and RhD blood types were determined using the rapid diagnostic kit (Solid phase, InTec Products, NC located at Haicang 361022, Xiamen Wiz Biotech, Fujian, P.R China) method following the standard procedures as stated by the manufacturer. Glass test tubes were used to perform the conventional tube method and the forward typing procedure was carried on each blood sample using monoclonal Anti-A, anti-B and anti-D reagents (Solid phase, InTec Products, NC located at Haicang 361022, Xiamen, Fujian, P.R China).

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Data Management and Statistical Analysis

Microsoft Excel sheet was used to record the result, and the concordance rate between the conventional tube method and rapid diagnostic kits technique of ABO and RhD blood typing was calculated. Again, the specificity and sensitivity of both techniques were computed and the IBM SPSS Version 22 was used to analyse the results. Kappa analysis was done to test the strength of agreement between the two diagnostic methods in detecting ABO and Rh D blood types. The Receiver Operating Characteristics (ROC) curve to test for the performance of the RDT method for ABO and Rh D blood type detection was also calculated. Statistical significance was assumed at P values less than 0.05.

RESULTS AND DISCUSSION

Table 1 shows ABO Blood type results using both the Tube testing method and the Rapid Diagnostic Test kits. The blood groups of the study participants were determined using these two different methods for variations in their results. For each of the blood types determined by each method, the same blood type results were recorded under each method. 51(31.7%) study participants tested blood type A for both the tube, conventional standard methods, and the newly introduced rapid diagnostic tests. The same results were recorded for the other blood types AB 7(4.3%), B 45(28.0%) and O 58(36.0%) in each of the two test methods, which are the tube method and the RDT.

Table 1:. shows ABO Blood type results using both the Tube testing method and the Rapid Diagnostic Test (RDT) kits.

DETERMINATION OF ABO BLOOD TYPE USING RDT AND TUBE METHOD						
		TUBE METHOD				Total (%)
		A	AB	B	O	
RDT	A	51	0	0	0	51(31.7)
	AB	0	7	0	0	7(4.3)
	B	0	0	45	0	45(28.0)
	O	0	0	0	58	58(36.0)
Total		51	7	45	58	161(100)

Table 2 shows the strength of agreement of the two diagnostic methods in detecting ABO blood type using Kappa analyses was excellent (k=1.00; standard error=0.00). The Kappa value of 1 with a P-value significance of 0.001 addresses the issue of consistency of the implementation of the RDT test as an excellent method in the determination of ABO blood type.

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Table 2: Shows the Strength of Agreement of the Two Diagnostic Methods (the Tube and RDT) in Detecting ABO Blood Type using Kappa analyses.

Symmetric Measures					
		Value	Asymptotic Standard Error[a]	Approximate T[b]	Approximate Significance
Measure of Agreement	Kappa	1.000	.000	19.314	.001
N of Valid Cases		161			

Table 3 shows the Diagnostic performance of RDT compared to Tube testing in ABO blood group detection. The RDT method detected blood group A, 100% sensitivity (93-100 CI), 100% specificity (93-100 CI), 100% positive predicting value, 100% negative predicting value and 100% accuracy (96.5-100 CI). For blood group B, 100% sensitivity (92-100 CI), 100% specificity (92-100 CI), 100% positive predicting value, 100% negative predicting value, and 100% accuracy (96-100 CI) were detected. For blood group AB, 100% sensitivity (59-100 CI), 100% specificity (59-100 CI), 100% positive predicting value, 100% negative predicting value, and 100% accuracy (76.8-100 CI) was also recorded. For blood group O, 100% sensitivity (93.8-100 CI), 100% specificity (93.8-100 CI), 100% positive predicting value, 100% negative predicting value, and 100% accuracy (96.9-100 CI) were determined. All diagnostic performance statistics were not different from the tube method being the standard.

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Table 3: Shows Diagnostic performance of RDT compared to Tube testing in ABO blood group detection.

Statistic	**ABO TYPING WITH RDT**							
	A		**B**		**AB**		**O**	
	Value	95% CI	value	95% CI	Value	95% CI	value	95% CI
Sensitivity	100	93-100	100	92.1-100	100	59-100	100	93.8-100
Specificity	100	93-100	100	92.1-100	100	59-100	100	93.8-100
NLR	0.00		0.00		0.00		0.00	
PPV	100		100		100		100	
NPV	100		100		100		100	
ACC	100	96.5-100	100	96-100	100	76.8-100	100	96.9-100

Sensitivity, specificity, Negative predictive value and Positive predictive value were calculated with cross-tabulation and confirmed with MedCal statistical software online (https://www.medcalc.org/calc/diagnostic_test.php)

Table 4 shows the results of the Rhesus D blood type using both the tube testing method and the Rapid Diagnostic Test kits. The Rhesus D blood groups of the study participants were determined using these two different methods for variations in their results. For each of the Rhesus D blood types determined by each method, the tube method detected 85 (52.8%) Rh D negative results against the RDT method, which detected 83 (51.6%) Rh D negative results. Out of the total study participants, 161 (100%), the tube method detected 76 (47.2%) Rhesus D positive results, while the RDT detected 78 (48.4%) Rhesus D positive results.

Table 4: Shows Rh D Blood Typing with Tube Testing Method And RDT Technique

DETERMINATION OF RH (D) BLOOD TYPE USING BOTH TUBE AND RDT METHOD					
			RH(D) RDT		
			Negative	Positive	Total (%)
RH(D) TUBE	Negative	Count	83	2	85(52.8)
	Positive	Count	0	76	76(47.2)
Total		Count	83	78	161(100)

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Table 5 shows the Diagnostic performance of RDT compared to Tube testing in Rhesus D blood group detection. The RDT method detected Rhesus D Negative results with 100% sensitivity (95.7-100 CI), 100% specificity (95.7-100 CI), 100% positive predicting value, 100% negative predicting value, and 100% accuracy (98-100 CI). The Rhesus D Positive results with the RDT recorded 97.4% sensitivity (92.1-100 CI), 97.4% specificity (92.1-100 CI), 97.4% positive predicting value, 97.4% negative predicting value and 97.4% accuracy (96-100 CI). The RDT diagnostic performance statistics for both Rhesus D blood types gave very high and good accuracy in detecting both negative (100%, 98-100 CI) and positive (97.4%, 96-100 CI) even though the Rhesus positive result could not give 100% accuracy. Nevertheless, the accuracy value met the 95% confidence interval since every test has an inherent error of <0.05%.

Table 5: shows the Diagnostic performance of RDT compared to Tube testing in RH (D) Detection.

Statistic	**RH(D) TYPING WITH RDT**			
	Negative		**Positive**	
	Value	95% CI	Value	95% CI
Sensitivity	100	95.7-100	97.4	92.1-100
Specificity	100	95.7-100	97.4	92.1-100
NLR	0.00	-	0.03	0.01-0.10
PPV	100	-	97.4	90.6-99.33
NPV	100	-	97.4	90.6-99.33
ACC	100	98-100	97.4	96-100

Figure 1 shows the Receiver Operating Characteristics (ROC) curve showing the performance of the RDT method for ABO blood type detection. The area under the curve for detection of ABO blood types using the RDT method was calculated and given 1.00, which indicates an accurate and excellent performing diagnostic test tool for the RDT method.

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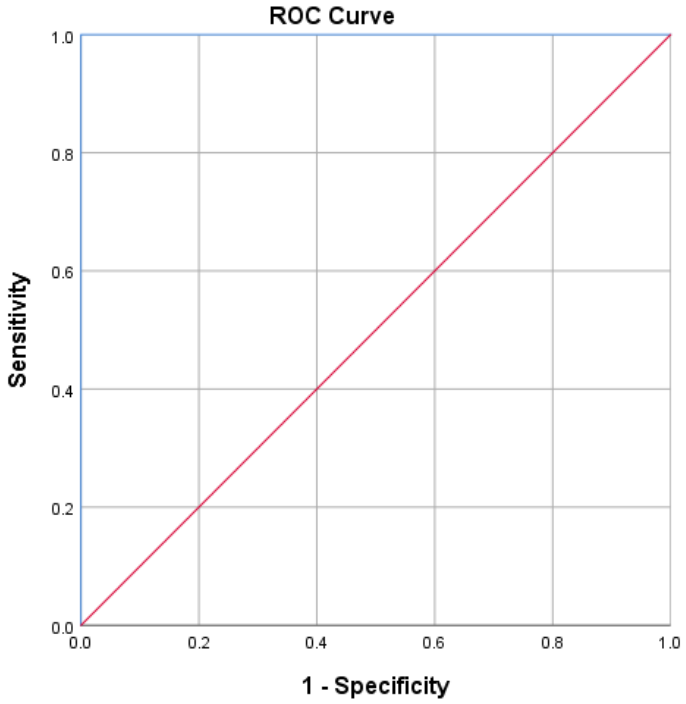

Figure 1: The Receiver Operating Characteristics (ROC) curve showing the performance of RDT method for ABO blood type detection

DISCUSSION

The new rapid diagnostic test kits have existed for a few years in several countries, including Ghana. The competency of these newly introduced test kits in detecting ABO and Rh D blood type has barely been assessed. This study was primarily conducted to assess the competency of rapid diagnostic test kits of ABO and Rh D blood typing.

All the study participants' blood samples were tested with the newly introduced rapid diagnostic method and compared with the conventional tube method, which was deemed the gold standard for the blood group. Forward typing was employed to test the two methods of typing the red cell antigens. The conventional tube method considered the gold standard, was used as a check control for each rapid diagnostic technique conducted with the same blood sample simultaneously.

The diagnostic performance of both test methods was assessed, and each technique yielded the same results in their ability to detect and not to detect a particular ABO blood type. A comparison

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between the two testing methods indicated that both techniques' results are in 100% concordance for ABO blood types without any discrepancies. The efficient and effective detection of blood types by RDT techniques may be helpful as a point-of-care method for determining blood groups and again as a substitute for the conventional tube method technique since it fulfils point of care test benchmark for ABO blood typing. With the competency of these RDT kits, detection and screening of blood donors' blood type and minimising turnaround waiting time for clients' blood typing are enhanced and made very easy as kits are uncomplicated to use and to be interpreted by healthcare professionals with little training and support at any blood typing site.

Unlike the conventional method, few alternative studies have been reported for detecting blood groups. Srivathsa & Dendukuri (2023) presented a novel automated method for ABO, Rh D blood typing using simple morphological smartphone image processing algorithms in conjunction with a fabric strip-based rapid diagnostic test. Then et al. (2023) also used paper diagnostic methods to detect ABO and Rh D blood groups as an alternate method. A paper-based device for rapid typing of human blood groups has also been reported by Li et al. (2023). The optical sensor method for rapid ABO, Rh D blood group typing has also been reported as an alternative by Li et al. (2024). Dielectrophoretic characterisation of red blood cells as an investigative tool to identify blood type for medical diagnostic application has also been reported as an alternate method in ABO, Rh D blood typing. All these methods have employed easy, convenient and fast techniques for detecting blood types to aid in screening and diagnosis. The immunochromatography technique, which employs antigen-antibody complex labels in detecting the competencies of ABO and Rh D blood type, has barely been assessed.

The conventional tube method detected the Rh D blood type, identifying an additional 2 of the study participants' results whose RDT was detected as harmful to be positive, giving a sum number of 85 (52.8%) under the Rh D tube negative results against the RDT method 83 (51.6%) under its negative RH D results.

The RDT detection for Rh D blood types recorded two of the study participants' samples as a false negative compared to the conventional tube method, which could detect the Rh D antigens that the RDT could not. The discrepant cases among the Rh D blood group, where two of the study participants' samples were wrongly typed, could be due to an inherent error of the test method or procedural error. Even with the conventional method, Rh D blood typing detection is read carefully because of the weak D antigens, which mostly lead to false adverse reports. Developing RDT kits for Rh D antigen detection will likely face similar errors.

Despite both ABO and Rh D blood types having 100% sensitivity for the study participants, the conventional tube method has better specificity for Rh D blood grouping compared to the newly

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introduced test kit. Few studies have been conducted previously comparing the conventional tube technique and the RDT kit based on the ABO and RhD blood group testing, which indicated a 99.6% to 100% correlation between the two methods. The conventional tube method is more sensitive, showing agglutinins examined by the naked eye and sometimes with the microscope for confirmation when positive. At the same time, the rapid diagnostic test kits only indicate results in qualitative form, such as red colour when the reaction is positive and no colour formation when the reaction is adverse.

Again, the cost assessment between both techniques was analysed based on the cost of reagent and turnaround time for performing the tests. It has been shown that the conventional tube technique is less expensive than the RDT kit technique because the RDT has yet to be embraced by the Ghanaian populace regarding its diagnostic performance. However, comparatively, the RDT is convenient to use in saving turn-around time for clients. The unreadiness of embracing the newly introduced kits, the lesser expertise in its operation and interpretation, and the fear that comes with them still pose major challenges to its usage and integration.

CONCLUSION

The findings of this study underscore the reliability of rapid diagnostic test (RDT) kits for blood group typing, highlighting their potential as viable alternatives to the conventional tube method. By providing timely and accurate results, these kits facilitate certification processes and enhance accessibility to healthcare, particularly in resource-limited settings. Implementing RDT kits can improve health outcomes by enabling prompt diagnosis and effective patient management. Policymakers and healthcare providers should consider establishing guidelines for usage and integrating RDT kits into routine clinical practices to maximise their benefits in diverse healthcare environments. Regular quality checks and standardisation of RDT kits are necessary to maintain high diagnostic performance. Regulatory bodies should ensure that only high-quality kits are approved for use. Future studies should evaluate the performance of different brands and models of RDT kits under varied conditions to ensure consistent reliability across diverse populations and settings. Additionally, research into minimising procedural and inherent errors in RDT detection methods, particularly for Rh D typing, is recommended.

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