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Research Article

Validation of Diagnostic Accuracy and Performance of NIMR-made Electrophoretic Machine in Determination of Haemoglobin Phenotype in Nigeria

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Abstract

Introduction: The sensitivity and specificity of a test is key in diagnostic accuracy, performance assay and analytical quality management. It is a fulcrum in which quality control (QC) procedures viz: quality design and planning rest. NIMR-made electrophoresis machines for the determination of the Haemoglobin (Hb) phenotype were tested against the standard (imported/foreign electrophoretic machines used in evaluation centres for routine work). The objective was to test the validity, usefulness and application in line with the Standards for Reporting of Diagnostic Accuracy Studies (STARD) protocol. STARD is a protocol that seeks to improve the completeness and transparency of reports of diagnostic accuracy studies in testing new equipment, fabricated equipment, new or modified method, reagents and or test kits.

Methods: NIMR-made haemoglobin electrophoretic machines were sent to eight selected secondary and tertiary laboratory centres for validation using standard test protocol.

Results: The sensitivity and specificity congruence between foreign-made brands used by these centres and NIMR-made electrophoretic machine was 100%, while the time of separation of the bands was also better with the NIMR-made electrophoretic machine (6.69 minutes) compared to foreign-made brands (11.54 min) (P = 0.001) However, neatness of band separation, the accuracy of band separation, the consistency of band separation, the ease of use of machine and the percentage agreement were not significantly different.

Conclusion: This study shows a 100% diagnostic agreement in terms of sensitivity and specificity based on STARD guidelines.

Keywords: *Validity; Diagnostic Accuracy; Performance; NIMR-Made Electrophoretic Machine; Sensitivity; Specificity*

Introduction

The goal of validation (new method, product, reagents, services etc.) is to provide empirical evidence for reproducibility and accuracy to be clinically acceptable and applicable according to international standards [1]. Besides, it is necessary to determine the acceptability of the data generated for successive test validation of the product, routine usage, test sensitivity and specificity with diagnostic precision, accuracy and clarity [2]. Also, the involvement of an external validator on test validation gives credibility, confidence and reliability to methods under validation as a way of allowing for independent assessment of diagnostic accuracy, performance and quality assessment of the product or method developed by an inventor and or researcher.

Laboratory test method validation typically refers to the multi-tiered process of evaluating the performance of a new instrument or test methodology, often concerning an instrument or methodology that is currently in use. In its broadest sense, method validation comprises the evaluation of test performance following a change in reagents, instruments and methodology [3,4].

The World Health Organisation (WHO)[5] states that analytical aspects that will be evaluated should include at least the following performance characteristics:

- Precision of measurement
- Intra-assay variation (within-run if applicable),
- Inter-assay variation (within days)
- Inter-instrument variation (for point of care technologies with very low throughput)
- Limit of detection (LOD), the lower limit of quantification (LLOQ) and robustness.

An unbiased and non-conjectural evaluation of clinical performance should seek to compare assay's result among two independent analysts from different centres and test conditions. It should also include an assessment of the assays' operational characteristics.

To facilitate vivid completeness and transparent reporting of diagnostic accuracy studies, the STARD statement was developed: Standards for Reporting of Diagnostic Accuracy Studies [1]. It is also a quality assessment tool for diagnostic accuracy studies smartly named QUADAS [3]. This system allows researchers to adhere strictly to the use of index terminologies and parameters such as sensitivity, specificity and other standard variables as a guide in test evaluation and validation.

Haemoglobin electrophoresis remains one of the cheapest and most reliable basic laboratory tests for sickle cell diagnosis. The cost of diagnosis is influenced by cost of equipment, consumables and manpower. The impact of importation duties on the eventual cost of equipment in an upcoming economy may escalate cost-per-test, with eventual late or non-diagnosis. About 300,000 children are born with sickle cell anaemia in Sub-Saharan Africa annually. Ramping up of community-based screening programs and use of a cheap and sensitive equipment is focal to the success of such early diagnosis programs. The NIMR-made haemoglobin electrophoresis machine is well placed to provide a cheap and efficient means of achieving this aim.

Objective of the Study

The objective of this study was to compare the performance of NIMR-made electrophoretic machine (NMEM) for haemoglobin phenotypes determination using routine machines (imported brands) currently in use in different study centres. Our study was aimed to determine band separation time, accuracy, consistency, sensitivity, specificity and to validate the efficiency of the electrophoretic capacity of the machine using STARD protocol.

Methodology

Study sites and settings: NIMR-made electrophoretic Machines were evaluated in 8 secondary and tertiary medical facilities located in Nigeria. The choice of these facilities was based on the high standard of services and volume of clients. These were:

1. Federal Medical Centre Ebute Metta, Lagos state
2. Clinical Sciences Department Laboratory, Nigerian Institute of Medical Research, Lagos
3. General Hospital Ijede, Lagos state
4. Clinical Diagnostic laboratory, NIMR, Lagos
5. God's Victory Medical Laboratory, Shomolu
6. Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife
7. Usmanu Danfodiyo University Teaching Hospital, University of Sokoto
8. University of Nigeria Teaching Hospital, Enugu.

Study design: NIMR-made electrophoretic machines were evaluated against other electrophoretic machines used in the respective centres for the determination of haemoglobin phenotypes as a gold standard.

Study procedure: Each participating centres were informed on the core objectives of the study, procedure and protocols. Samples collected for evaluation of the electrophoretic machine were: AA, AS, AC, SC and SS (standard) from previously confirmed phenotypes from twenty individuals and twenty unknown randomly collected samples from the general population. These were analysed in comparison with the machines used by the various centres.

Materials provided to each centre by the NIMR Research team were: Each centre was provided with the following: Genotype machine (NIMR made), Tris-EDTA Borate Buffer, Whatman number three paper**,** Cellulose acetate paper, A roll of a paper napkin, Hemolysate and known haemoglobin genotypes (AA, AS, AC, SC, CC and SS) controls.

Running of HB phenotypes assay

Haemoglobin phenotypes were determined generally by the centres using the cellulose acetate electrophoresis paper technique. Five (5 µl) microlitres of whole blood were placed in a test tube and one ml of 10% normal saline added and spun for 2 minutes at 1000 rpm. The supernatant was decanted and red cells subsequently lysed with 2 - 3 drops of lysing solution into the test tube. Using an applicator, the hemolysate was applied on cellulose acetate paper that was previously soaked in buffer, brought out and wiped with a tissue napkin to remove the excess buffer. With the use of forceps, the cellulose paper was lifted and placed horizontally on the electrophoretic tank containing Tris EDTA buffer solution for 15 - 20 minutes at 220V. Hemolysate from known samples was used as control. For the NIMR machine, participants were advised to watch out for separation time strictly between is 3 - 5 minutes and to record the quality of separation observed.

The following parameters were evaluated: 1. Time of band separation, 2. The neatness of band separation 3. Accuracy of band separation 4. Consistency of band separation 5. Ease of use of machine.

Statistical analysis

Statistical analysis was done using SPSS version 20.0. The diagnostic performance characteristics (index test) were assessed to include sensitivity, specificity and predictive values with 95% confidence intervals (CI). The study was performed in compliance with the updated version of Standards for Reporting of Diagnostic Accuracy (STARD), 2015.

Calculation of sensitivity and specificity

Sensitivity: Sensitivity was calculated as the number of true positive results compared to true positives by the known result. Sensitivity = expressed as %.

Specificity: Specificity will be calculated as the number of true negative specimens identified by the index method compared to true negatives by the known result.

Specificity==expressed as %.

	Result of Reference Testing		
Result under Evaluation	+	_	
+	a True positives	B False positives	a + b
-	C False negatives	D True negatives	c + d
	a + c	b + d	

Confidence intervals: The 95% confidence intervals were calculated to assess the level of uncertainty introduced by sample size. Exact 95% confidence intervals for binomial proportions were calculated from the F-distribution [6].

Discrepant results: Discrepant results are defined as results that vary from one analyst to the other. Those specimens with results that were consistent with analyst results did not undergo further testing. Specimens with discrepant test result among analyst were re-tested.

Results

There were 8 facility-based laboratories included in this study. A total of 100 samples were analyzed across the laboratories in a ratio of 50:50 responses from the Laboratory Scientists/Consultant Haematologists. These samples included: HbAA 71 (127.8%), HbAS 23 (41.4%) and HbAC 6 (10.8%).

Assessment of diagnostic agreement based on Standards for Reporting of Diagnostic Accuracy Studies (STARD) statement and guidelines 2015

Table 1 and figure 1 summarizes the result of the diagnostic agreement between Foreign-made electrophoretic machines (FMEM) used by the respective study centres (Helena Bioscience, Dy300 Med-friend, England, ULT) and NIMR-made electrophoretic machine (NMEM). Diagnostic agreement between FMEM and NMEM concerning sensitivity and specificity agreement was 100%.

Also, agreement among other parameters (FMEM vs NMEM) are elaborated in table 1 and figure 1 to include: Time of band separation [11.54 mins: 6.69 mins with P-value of 0.001], Neatness of band separation [92.03%: 92.43% with P-value of 0.702], Accuracy of band separation [92.68%: 92.68% with P-value of 1.00], Consistency of band separation [93.24%: 93.24% with P-value of 1.00], Ease of use of machine [96.19%: 97.19% P-0.166] and % agreement at 100% respectively.

S/N	Test parameters for validation	Foreign-made genotype machines (mean ratings)	NIMR- made genotype machine (mean ratings)	P-value
1.	Time of band separation (in mins)	11.54mins	6.69mins	0.001
2.	Neatness of band separation	92.03%	92.43%	0.702
3.	Accuracy of band separation	92.68%	92.68%	1.00
4.	Consistency of band separation	93.24%	93.24%	1.00
5.	Ease of use of machine	96.19%	97.19%	0.166
6.	Sensitivity	NA	100%	NA
7.	Specificity	NA	100%	NA
8.	% Agreement	NA	100%	NA

Table 1: *Showing test parameters of validation for foreign-made and NIMR-made genotype machine. Not Applicable=NA.*

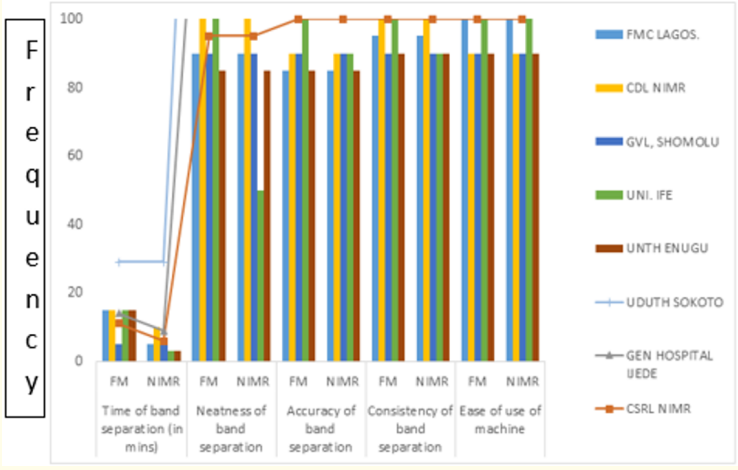

Figure 1: *Assessments of diagnostic accuracy and performance agreement.*

Assessment of various haemoglobin phenotypes in respective centres

Various haemoglobin types, HbAA, HbAS and HbAC were reported in the study from various laboratories. In all, 71, 23 and 6 blood samples tested were reported to be HbAA, HbAS and HbAC respectively.

Discussion

The search for a cheap, durable and reliable diagnostic tool for sickle cell disease (SCD) has been on for several years now. Haemoglobin electrophoresis has remained the most widely available instrument for the diagnosis of SCD in several resource-poor countries especially within the Sub-Saharan Africa. This study was targeted at production and validation of a cheap and affordable electrophoresis system for the diagnosis of SCD.

The NMEM tested across the various laboratories in the country compared to other similar machines produced by other manufacturers (mainly foreign) showed optimal sensitivity of 100%. The confounders and bias inherent in a single facility test run was obviated by the multiple concomitant methodology utilized in this work.

Time of band separation was less with NMEM compared to FEM. This implies a shorter run-time per test and will markedly reduce turnaround time. The reduction in the time taken for band separation was about 35%. The neatness of band separation was much better with the NMEM compared to FEM. This assessments though subjective was recorded as such from the various facilities in the different zones in Nigeria with varying temperature and humidity. Accuracy of band separation was found to be similar with the FEMs. This was observed despite the shorter time of band separation and therefore indicates better conductivity and band migration in the NMEM. With the consistency of band separation, which is an indication of reproducibility and ease of standardization, the NMEM was rated to be equal to the FEMs. The laboratory staff-haematologists and laboratory scientists involved in these tests ranked the ease of use of NMEM compared to FEM and the FEMs were not found to be better than NMEM.

Normal hemoglobin (HbAA) prevalence ranges from 55-75% while the sickle cell trait (HbAS) is 20- 30% in Nigerian population [1,3]. In the study also, hemoglobin variants of HbSS which is reported to be high in Nigeria years ago [4] were not reported in the study in all the centers. Of special interest is the very high frequency of HbAA in the study from all centers. This implies that in order to keep the trend of high frequency of HbAA gene in Nigeria, then Scientist and relevant stakeholders should continue to educate Nigerian populations most especially as it involves premarital counseling [7].

The Standards for Reporting of Diagnostic Accuracy Studies (STARD) ensure completeness and transparency of reports of diagnostic accuracy studies. STARD contains a list of essential items that can be used as a checklist by evaluators and validators of new products, methods and services.

Conclusion

Diagnostic accuracy studies are important in the estimation of sensitivity and specificity of the test that is compared against the reference standard to evaluate diagnostic deviation, determine the accuracy and other standard conformities using a component of QUADAS, a quality assessment tool for diagnostic accuracy studies. It allows for the elimination of study bias and conjecture while creating room for quality, acceptability and reproducibility most especially with the involvement of external assessors in evaluation in all or most cases. In consideration of STRAT and QUADAS standard, our NIMR Biotech electrophoretic machine was tested in 9 different reputable medical centres in Nigeria. It is 98% diagnostically accurate in terms of sensitivity and specificity as compared to the imported ones used in these centres.

What is Already Known on this Topic

Haemoglobin electrophoresis remains one of the cheapest and most reliable basic laboratory tests for sickle cell diagnosis. The cost of diagnosis is influenced by cost of equipment, consumables and manpower.

What this Study Adds

The NIMR-made haemoglobin electrophoresis machine is cheap, sensitive, reliable and efficient for early diagnosis of haemoglobin genotype.

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Competing Interests

The authors report no competing (commercial/academic) interests.

Authors' Contributions

Nkiruka Nnonyelum Odunukwe, conceptionalised, designed, interpreted the data, drafted and revised the article critically for important intellectual content.

Akwaowo Bassey Orok was involved in the conception, design of the study and writing of the protocol and drafting the article.

All authors were involved in the data collection and Olakiigbe Abiodun Kanmi performed the statistical analysis. Jane Okwuzu, Adeniyi Adeneye, Aigbe Ohihoin, Esther Ohihoin and Ngozi Otuonye were involved in the interpretation of the data. A Madu, L Salawu, AU Musa, OT Kayode, Samuel Akindele, J O Sulaiman were involved in validity testing of NIMR electrophoretic machine in different centers. Babatunde Salako revised the article critically for important intellectual content and approved the final manuscript. All the authors are in agreement to be accountable for all aspects of the work.

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Bibliography

1. Adeyemo OA and Soboyejo OB. "Frequency distribution 0f ABO, RH blood groups and blood genotypes among the cell biology and genetics students of University of Lagos, Nigeria". *African Journal of Biotechnology* 5.22 (2006): 2062-2065.
2. Egesie OJ., *et al*. "Prevalence of Sickle Cell Trait and Glucose 6 Phosphate Dehydrogenase Deficiency among Blood Donors in a Nigerian Tertiary Hospital". *African Journal of Biomedical Research* 16 (2013): 143.
3. Cohen JF., *et al*. "STARD 2015 guidelines for reporting diagnostic accuracy studies: explanation and elaboration". *British Medical Journal* 6 (2016): e012799.
4. Erhabor OO., *et al*. "Effects of falciparum malaria on the indices of anaemia among pregnant women in the Niger Delta of Nigeria". *Journal of Clinical Medicine and Research* 2.3 (2010): 035-041.
5. Organization World Health. "Protocol for the laboratory evaluation of HCV molecular assays". WHO/EMP/RHT/PQT/2018 (2018): V 4.0.
6. JAC Kirkwood BR and Sterne. "Essential medical statistics". Malden, Massachusetts, USA.: Blackwell Publishing Company (2003).
7. Pennap GR., *et al*. "Frequency Distribution of Hemoglobin Variants, ABO and Rhesus Blood Groups among Students of African Descent". *British Microbiology Research Journal* 1.2 (2011): 33-40.

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