Validation and Comparism Of Cyscope Microscope, Quantitative Buffy Coat And Rapid Diagnostic Kit For Malaria Diagnosis Among Clinic Attendees in SouthWest Nigeria

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Introduction

- Malaria in Nigeria, is responsible for 60 percent of outpatient visits to health facilities; 30 percent of childhood deaths; 25 percent of deaths in children under one year; and 11 percent of maternal deaths
- Furthermore, financial loss from malaria was estimated to 132 billion Naira per year (approximately $838,564,000 USD)*
  - *FMoH

Introduction

- Unavailability of accurate, rapid, reliable and cost effective malaria diagnostic instruments constitute a major challenge to malaria elimination campaign
- Light microscopy is the conventional method of laboratory diagnosis of malaria but associated with labour intensiveness, long Turnaround Time (TaT), lack of expert microscopists among others
- Alternative diagnostic instruments like Cyscope, QBC and CareStart™ Rapid diagnostic Kit developed but their validity and cost effectiveness have not been determined in Nigeria, a resource limited and endemic setting

Objectives

General objective:

To evaluate and compare the diagnostic performance of Cyscope microscope, QBC and CareStart in malaria parasite detection in Ibadan, Southwest , Nigeria, using light microscopy as the gold standard

- Specific objectives:
  - Determine the performance characteristics of Cyscope, QBC and CareStart™ in malaria diagnosis
  - Assess the TaT for each of the aforementioned malaria diagnostic instruments under normal laboratory working condition
  - Determine the cost effectiveness of the diagnostic instruments

Methodology

- Study Area

  The study area was the municipal area of Ibadan in southwest Nigeria, characterized by low level of environmental sanitation, poor housing, overcrowding, lack of potable water and improper management of wastes especially in the indigenous core areas characterized by high density and low income populations

  – Study site: University College Hospital Ibadan

  Study design: Cross sectional facility based-study

  Study period: January to April, 2014

Methodology

- Eligibility criteria
  - Patients of any age with fever; having axillary body temperature above 37°C, and/or wherein the clinician suspects malaria, presenting in the selected clinics in Ibadan, south west ,Nigeria, and consented were included in the study
- Sample size : $n_x = \frac{(1.96 + 1.28)^2 \times P(1 - P)}{(P - P_0)^2}$
- $N = n_x/P_x$ 194
- Using P_x : 76.4% malaria in children < 5 yrs*

* Tiddi & Akogun (2005)

Methodology

- Sampling technique
- A two-stage sampling technique was used
  - Stage 1: one tertiary health facility (HF), one secondary HF and two primary HFs were selected by simple random sampling
  - Stage 2: at each HF, participants were selected by systematic random sampling until the sample size was reached.

Methodology

- Sample and data collection:
  - Patients with laboratory request form sent in by the requesting physician for malaria parasite test recruited
  - Certified phlebotomists collected one milliliter of blood sample by venepuncture from each participant into an EDTA anticoagulant specimen bottle

Methodology

- Biologic testing
- Test done using the diagnostic instruments following the SOP and manufacturer's instructions (appendix)

Definition of variables

- Turnaround Time (TaT): Defined here as time taken from when sample was received by the laboratory personnel who processed it, and the total time taken for completing all stages of the laboratory procedure and results was generated

- Stop watch was used and TaT separately recorded in another structured data collection register/ 'time sheet' and then compared

Methodology

- Cost effective analysis: defined as an economic study in which the costs are expressed in monetary units, here in U.S Dollars, and the results in non-monetary units, here in number of tests done by the instruments.
  - It is the ratio between the resources used and the related effects, classified by comparison of the costs /input and consequences/outcome*

*The standard Guidelines on Health Economic Evaluation as put together in 2006 by Evelyn Walter et. al., of the Institute for Pharmaeconomic Research, Vienna, was used for this analysis.

Methodology

- **COST/ INPUT**
  - costs were divided into machine or equipment cost, reagents /consumables cost, manpower/personnel, electricity or other miscellaneous
- ***Assumptions***
- That machine cost is per unit time of use, assuming uniform depreciation over time / lifespan of the equipment (fixed lifespan is 3 years for the entire machine)
- That period of use was fixed at 8 hours per day

Methodology

- Acquisition cost was divided by total life span of equipment (in real use days i.e. excluding weekends) to get machine cost per unit time of use. Cost of reagents/consumables was calculated per session of use (8hours/day)
- Manpower/Personnel cost was calculated using standard monthly wage of basically qualified staff to operate each diagnostic tool; expressed in wage/hour

- **CONSEQUENCES/OUTPUT**
  - The yield per procedure over the work/ allotted time was calculated as output

Methodology

- Data analysis : Microsoft Excel (2008) was used for data entry, data cleaning, and analysis. Quantitative data were summarized using proportions and means

- Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Values were calculated
- Kappa statistics was used to analyse inter-rater agreement

- McNemar Chi-square was used to assess the statistical significance of difference between the results of the instruments at 95% confidence interval and level of significance set at $p \leq 0.05$

Methodology

- Quality control
  - SOPs were developed and validated for every clinical and laboratory procedures ensuring compliance with international practicing standard
  - Laboratory procedures were repeated by another experienced professional and a tie breaker observer was engaged where necessary to ensure agreement before results were entered
  - Double data entry and confirmation was done to ensure data integrity
  - All laboratory personnel were blinded to the result of tools they did not use

Methodology

- Ethical approval for the study was obtained from the University of Ibadan/University College Hospital Ethics Committee
  - Informed consent and confidentiality of participants information was ensured
  - The participant's benefit of participation was presentation of their results to the requesting clinicians and advise on treatment

Results

- A total of 502 blood samples were tested for malaria parasite
- Malaria prevalence in the tested samples was 19.5% (CareStart™), 21.7% (light microscopy), 30.7% (Cyscope) and 32.7% (QBC) respectively
- Sensitivity of the instruments compared with light microscopy was 76% (CareStart™), 95% (Cyscope) and 98.1% (QBC)
- Specificity was 85.5% (QBC), 87.3% (Cyscope) and 96% (CareStart™)

Results

- Positive Predictive Value for the instruments were 65.2% (QBC), 67.5% (Cyscope), and 84.7% (CareStart™)
- Negative Predictive Value were 93.6% (CareStart™), 98.6% (Cyscope) and 99.4% (QBC)

- McNemar : OR= 28.5 (CI=7.54 - 241.01) for QBC; 10.0 (CI= 4.01 - 32.13) for Cyscope and 0.6 (CI= 0.3 - 1.13) for CareStart™

Results

- The turnaround time was 5minutes (Cyscope), 10minutes (QBC), 20minutes (CareStart™) and 45 minutes (light microscopy)
- Average cost per hour of use for the instruments was $2.04 (Cyscope), $5.61 (RDT), $5.89 (QBC) and $10.77 (light microscopy)

Comparison Of The Diagnostic Performance Of Carestart, Cyscope and QBC Using Light Microscopy As Gold Standard

Diagnostic Instrument	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
CareStart	76 (72.26 - 79.74)	96 (94.29 - 97.71)	84.7 (81.44 - 87.76)	93.6 (91.46 - 95.74)
Cyscope	95 (93.09 - 96.91)	87.3 (84.39 - 90.21)	67.5 (63.4 - 71.6)	98.6 (97.57 - 99.63)
QBC	98.1 (96.91- 99.29)	85.5 (82.42 - 88.58)	65.2 (61.03 - 69.37)	99.4 (98.72 - 100.08)

Comparison of Agreement Index amongst CareStart, Cyscope and QBC Using Light Microscopy as Gold Standard

Diagnostic Instrument	Kappa Value	95% Confidence Interval	Standard Error
CareStart	0.71	0.64 - 0.77	0.034
Cyscope	0.72	0.65 - 0.78	0.035
QBC	0.75	0.68 - 0.82	0.037

Comparison of the operational characteristics of all the diagnostic tools

Parameters	Light Microscope	Cyscope	QBC	CareStart
Average time/test	45 minutes	5 minutes	8 minutes	20 minutes
Blood qty./test	10 ul	8 ul	50 ul	3 ul
Electric current by standby battery	No	Yes	No	NA
Average cost of equipment	$1,197	$1,155	$14,970	NA
Ease of Use	High	Medium	Medium to High	Low
Number of test/hour	1	12	7	3

Comparison Of The Cost Effectiveness Analysis For The Diagnostic Performance Of Carestart, Cyscope, QBC And Light Microscopy

INPUT	LIGHT	CARESTART	CYSCOPE	QBC
Machine/Equipment cost ($)	1,197	NA	1,155	14,970
Cost per unit time of use ($/hr)	0.2	NA	0.2	2.5
Reagent/consumables ($)/test/hr	0.12	4.32	7.2	11.4
Personnel ($)/hr	21.14	12.5	17.10	21.14
Electricity ($)/hr	0.08	NA	0.03	0.23
Total Cost per test ($)	10.77	5.61	2.04	5.89

Discussion

- The prevalence rates of malaria parasites detection were 32.7% (QBC), 30.7% (Cyscope) and 19.5% (CareStart) when compared with 21.7% detection rate with light microscopy
- These rates are lower compared to findings by Badaru et.al. of 76.3%, 76.4%, and of 84.7% at Maiduguri, Yola, and Ota respectively in Nigeria (Bell and Peeling, 2006; WHO, 2000; Badaru, 2010)
- This could be due to the fact that the present study was largely carried out in the low transmission season

Discussion

- This finding is in concordance with earlier studies on sensitivity and specificity of diagnostic tools for malaria parasite detection
- Previous studies conducted using *P. falciparum* sensitive HRP2 rapid diagnostic kits in north-eastern Tanzania and in Uganda, showed sensitivities of 95.4%, 97.2% and 97.6% for Parachek, Parachek Pf and and ParaHIT *f,* respectively (Pekins *et al.,* 1999, Jeremiah *et al.,* 2007)

Discussion

- However, the result of this study disagrees with other studies which showed lower sensitivities Studies conducted in Yola, Enugu, Port-Harcourt; Nigeria and in Ethiopia which found a sensitivity of 69.7% for Global device rapid diagnostic kit, 42.3%

  - for a *P.f* rapid diagnostic kit, 47% for SD Bioline rapid diagnostic kit *pf/pv* and 47.5% for Parascreen, an HRP -2 and pLDH based rapid diagnostic kit respectively (Kadeshaw *et al.,* 2008, WHO 2010a)

Discussion

- Increasingly, countries and implementing partners have identified that limited diagnostic capacity represents a major barrier to implementation and sustainability of prevention, treatment and care programs for malaria (Maputo Declaration, 2008)

- Cyscope microscope has performance and features that makes it suitable for deployment into tertiary, secondary and primary health care facilities as compared with other tools

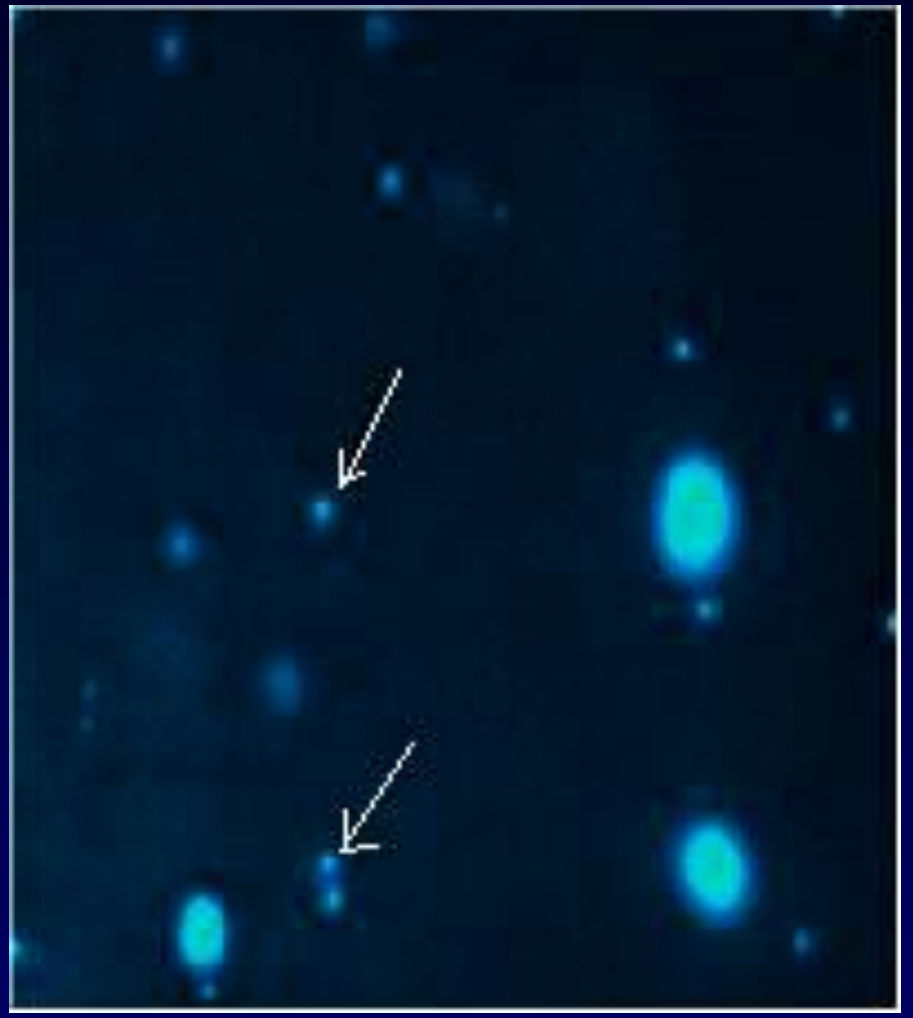
Conclusion

- This study showed that Cyscope fluorescent microscope had the least turnaround diagnostic time and it is the most cost effective of all the malaria laboratory diagnostic instruments evaluated

Recommendation

- In pursuance of the ongoing malaria elimination phase of the country and in line with the realization of WHO's malaria treatment policy of parasite-based diagnosis before treatment, targeted at reducing the scourge of anti-malarial drug abuse;

  – it is therefore recommended that Cyscope fluorescent microscopy be adopted by healthcare stakeholders for malaria parasite detection



Fluorescent nuclei of *Plasmodium* parasites (arrowed) within unstained peripheral erythrocytes beside the CyScope microscope. The large fluorescent round areas represent the nuclei of leukocytes. LED fluorescence light (365 nm), 1000-fold magnification. (Source: www.partec.com)