Application of Mathematical Model-Latent Class Model in Methodological Evaluation of Diagnostic Algorithms and Imperfect Reference Standard of Selected Index Test Techniques in Parasitology

Azuonwu Obioma[1,], Ihua, Nnenna[1], Adebayo-Olajide Testimonies Chikanka[2] Onwuli Donatus[3], Reuben E[4], Orabueze, Ifeoma Celestina[5], Azuonwu Goodluck[6], Poplong Natasha Angel[7], Tamuno-Boma[8], Odinga, Omaegbu Ebere Elizabeth[9], John-AmadiVictory Sorbari[10], Ekwuozor, Ikem Kris Eloka[10], Wokem Gloria Ngozika[1], Vetty Agala[11]*

[1]Department of Medical Laboratory Science, Medical Bacteriology/Virology/Parasitology Unit, Rivers State University, Nkpolu–Oroworukwo, Port Harcourt, Rivers State, Nigeria.

[2] Department of Microbiology and Biotechnology, Caleb University, Lagos, Nigeria

[3]Department of Medical Laboratory Science, Chemical Pathology Unit, Rivers State University, Nkpolu–Oroworukwo, Port Harcourt, Rivers State, Nigeria

[4]Department of Human Physiology, College of Medicine, Rivers State University, Nkpolu–Oroworukwo, Port Harcourt, Rivers State, Nigeria

[5]Faculty of Pharmacy, Department of Pharmacovigilance, University of Lagos, Nigeria

[6]Department of Nursing, University of Port Harcourt, Choba, Nigeria

[7] Departments of Biological Science, Faculty of Science, Federal University of Kashere, Gombe State, Nigeria

[8]Department of Biochemistry, Rivers State University, Nkpolu–Oroworukwo, Port Harcourt, Rivers State, Nigeria

[9]Department of Computer Science, Rivers State University, Nkpolu–Oroworukwo, Port Harcourt, Rivers State, Nigeria

[10]Department of Animal and Environmental Biology, Rivers State University, Nkpolu–Oroworukwo, Port Harcourt, Rivers State, Nigeria

[11]Department of Community Medicine, University of Port Harcourt, Choba Nigeria

***Corresponding author:**

Azuonwu Obioma

Rivers State University, Port Harcourt, Nkpolu–Oroworukwo, Port Harcourt, Rivers State, Nigeria

E-mail: bimajacobs@yahoo.co.uk

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ABSTRACT

Background: Disease diagnosis cannot be made with certainty thus, choosing the best diagnostic strategy is basic for understanding patient management outcomes. This requires substantiation of the comparative performance of diagnostic algorithms. The use of a single index test in parasitic detection has been invalid and had also proven unacceptable among critical professionals. The aim of this study anchors on Methodological Evaluation of Diagnostic Algorithms and Imperfect Composite Reference Standard of Selected Index Test Techniques in Parasitology using the application of mathematical models-Latent class model. It will

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also compare the diagnostic performance of three index test techniques in the detection of parasites, using Extrapolated-composite Imperfect Reference Standard and Bayesian Latent Class Model. **Study Protocol:** This study was carried out in Rivers State, Nigeria. Laboratory investigation of the index test techniques for direct microscopy, Brine microscopy and Diethyl Ether Microscopy followed the routine parasitological methods with a sample size of eighty. The imperfect reference (gold) standard was extrapolated from a combination of the three index test techniques. All tests were categorically analysed as binary outcomes (positive or negative). Statistical analysis was performed using SPSS version 21 to test for inter-rater agreement and other concordance indices. Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value, Prevalence, Likelihood Ratio Positive and Negative, False Discovery Rate, False Omission Rate, and Diagnostic Odd Ratio, Kappa, Kendall's Coefficient of Concordance, average Spearman Correlation and Cochran Q were the test statistics used in this study. An alpha level of 5% was set for decision. Also, Bayesian latent class Model was performed with Modelling of Infectious Disease Centre (MICE) Model Code MODEL103. **Results:** For detection rate, Direct Microscopy was the least while Diethyl Ether Microscopy was the highest. Strong concordance was observed showing good inter-rater agreement. The study generally recorded low sensitivity irrespective of the technique or model used. Composite reference standard did not differ statistically (p>0.05) from the Latent Class Model only for sensitivity, others showed marked variation (p<0.05). **Conclusion:** This current study has been able to bear out the significance of LCM as a useful tool.

Keywords: Diagnostic, Algorithms, Imperfect, Reference, Standard, Parasitology, Methodological, Evaluation, Index, Test, Techniques, Extrapolated, Bayesian, Latent, Class, Model

INTRODUCTION

Intestinal parasites are even today major contributors to the global burden of disease burden, affecting especially the population living in regions, in the developing countries according to the report of Alum and colleagues (Alum *et al*., 2010) [1]. Neglected tropical diseases have gained global attention despite the fact that it is a major problem of the developing nations thus, lymphatic filariasis and soil-transmitted helminths like *Ascaris lumbricoides, Hookworm* and *Trichuristrichiura* have contributed to increased disease burden of unimaginable proportion (Harhay *et al*., 2011) [2]. Furthermore, in recent time; zoonotic helminths have been increasingly reported as a cause of spurious infection in human beings (Gonçalves *et al*., 2012) [3]. Nevertheless, general public health safe practices cum personal hygiene among other factors like potable water availability, socio-economic conditions, education, temperature, humidity and the survival of the environmental different stages of the parasites are some of the factors that determine and promote the transmission of intestinal parasites (Alum *et al*., 2010) [1].

However, intestinal parasitoses are common parasitic infections and hence, various techniques have been used for examination of intestinal parasitic diseases (Mulat *et al*., 2015) [4]. However, direct wet mount brine and Formol ether concentration techniques have been used as a means of diagnosis for several years in Africa (Moges *et al*., 2010) [5]. Although other diagnostic methods are available including immunoassay and molecular technique, direct microscopy is commonly used as a diagnostic method in parasitology (Knopp *et al*., 2009) [6]. Direct microscopy is most commonly used technique due to the fact that it is fast, it does not consume time, cheap and permits the concurrent detection of the various classes of parasites either helminths or protozoans (Camacho *et al*., 2012; Canavate *et al*., 2012; Ribeiro and Furst, 2012; Tello *et al*., 2012) [7-10]. Based on these, in some low and mid economic settings, the use of direct microscopy is preferred and seldom used over the tests like Brine Microscopy and Diethyl-Ether Microscopy. Although, techniques based on centrifugation have demonstrated to be better (Canavate *et al*., 2012) [8]. This is not without exceptions anyway [(Devera *et al*., 2008; Tello *et al*., 2012) [10,11]. The quest of what index test techniques possess the best diagnostic accuracy is still unclear.

Selecting the most effective diagnostic method is essential for good patient management and public health interventions. This requires substantiation of the comparative performance of alternative tests or diagnostic algorithms. As a result, there is a need for diagnostic test accuracy. Also, another barrier is that the diagnostic accuracy of the tests is usually determined through the comparison of the index test results with those of a reference standard. These reference standards are presumed to be perfect, i.e. allowing the classification of diseased and non-diseased subjects without error. In practice, this assumption is however rarely valid and most reference standards show false-positive or false-negative results. When an imperfect reference standard is used, the estimated

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accuracy of the tests of interest may be biased, as well as the comparisons between these tests.

The importance of accurate diagnosis cannot be overemphasized. Accurate disease diagnosis is the first step in the appropriate treatment and management of patients. Often, the issues of diagnostic conflicts and in-conclusive results seem the outcome of studies assessing the diagnostic accuracy of test algorithms including findings of some meta-analysis. Performance evaluation of diagnostic tests is critical in the search for accurate diagnoses. A gold standard test is usually absent in parasitology, thus rendering satisfactory assessment of diagnostic accuracy difficult.

Assessments of the presence or absence of a condition cannot constantly be made with confidence (Sullivan & Holly, 2007) [12]. Latent class analysis has been proposed as a statistical technique that allows disease assessment in the absence of a gold standard or the presence of an imperfect gold reference standard from earlier studies (Dawid & Skene 1979; Walter & Irwig, 1988) [13,14]. Latent class analysis (LCM) is used to assess diagnostic test accuracy when a gold standard assessment of disease is not available but results of multiple imperfect tests are or when it is impossible to perform in field conditions such as seen in parasitology. The latent class model has two latent classes, indicating diseased and non-diseased. In its basic format, latent class analysis requires the observed outcomes to be statistically independent conditional on the disease status.

In this model, the disease status is an unobserved, or latent, variable, and a probabilistic model is assumed for the relationship between results of several imperfect diagnostic tests results and the latent disease status (Pepe, 2007) [15]. Estimation of the Latent Class Analysis model is either through maximum likelihood (Goetghebeur *et al*., 2000; Black & Craig 2002) [16,17] or Bayesian methods (Goetghebeur *et al*., 2000; Dendukuri & Joseph, 2001; Bernatsky *et al*., 2005) [16,18,19] to achieve diagnostic accuracy of the tests. This present study utilized the later method.

This study considered the basis, where three index tests were observed and conditional independence (CI) assumed. Concordance and inter-rater agreement were noted. The study showed explicitly how observed two and three-way associations between test results are used to infer disease prevalence and diagnostic accuracy of test true and false positive rates as well the achievement of the study purpose based on some rationales.

Diagnostic investigation is key in the search for accurate diagnostic techniques to provide adequate patient care, assess drug efficacy, monitor the effectiveness of control programs and obtain proper knowledge of the parasitic epidemiology (Tarafder *et al*., 2010; Harhay *et al*., 2011) [2,20].

Assumption of a perfect reference standard in practice is rarely valid. Nevertheless, less than perfect reference standards which differ between studies may have been used. The use of latent variables model in diagnostic studies is to adjust for the use of imperfect reference standards. Many diagnostic studies are small and give imprecise estimates (Bachmann *et al*., 2006) [21]. Also, in the field of parasitology, no validated gold reference standard has been established. Besides, composite reference (gold) standard and LCM have not been applied within the locale of this study.

The purpose of this study was Methodological Evaluation of Diagnostic Algorithms and Imperfect Composite Reference Standard of Selected Index Test Techniques in Parasitology; to compare the diagnostic performance of three index test techniques in the detection of parasites, using Extrapolated-composite Imperfect Reference Standard and Bayesian Latent Class Model. This was specifically handled via;

1. Determining diagnostic accuracy of selected index test techniques (Direct Microscopy, Brine Microscopy and Diethyl-Ether Microscopy) using an extrapolated-composite reference (gold) standard.

2. Calculating the inter-rater agreement between any two index test techniques using Kappa.

3. Measuring trend of agreement and intra-test homogeneity of the selected three index test techniques (Direct Microscopy, Brine Microscopy and Diethyl-Ether Microscopy) using Kendall's Coefficient of Concordance, average Spearman Correlation and Cochran's Q.

4. Computing Diagnostic accuracy using Bayesian Latent Class Model (LCM) amidst imperfect reference (gold) standard.

5. Comparing diagnostic accuracies of the extrapolated-composite reference (gold) standard and the Bayesian Latent Class Model (LCM)

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Study Protocol: This study was carried out in Rivers State, Nigeria. Laboratory investigation followed the conventional parasitology methods and carried at the Microbiology Laboratory of University of Port Harcourt Teaching Hospital, Rivers State, Nigeria. Three tests were performed for each subject. About eighty samples were used. The index test techniques used were direct microscopy, Brine microscopy and Diethyl Microscopy. The imperfect reference (gold) standard was extrapolated from a combination of the three index test techniques. All tests were categorically analysed as binary outcomes (test positive or negative).In addition, the composite reference standard method results were analysed SPSS. Statistical analysis was performed using SPSS version 21 to test for inter-rater agreement and other concordance indices. Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value, Prevalence, Likelihood Ratio Positive and Negative, False Discovery Rate, False Omission Rate, and Diagnostic Odd Ratio, Kappa, Kendall's Coefficient of Concordance, average Spearman Correlation, Cochran Q were the test statistics used in this study. An alpha level of 5% was set for decision.

A Bayesian latent class approach (Joseph *et al*., 1995) [22] was used to obtain estimates for the sensitivity, specificity, and prevalence of the two techniques and the proportion of positives for each intestinal parasite. The conditional dependence between the three tests was estimated using a fixed parameter (Gelman & Joseph, 2001) [23]. Modelling of Infectious Disease Centre (MICE)-Imperfect Gold Standard Models was used to estimate Bayesian Class Model. Model Code MODEL103, Model Name The 3-tests in 1-population Model (Simplified Interface) and Job ID 20191025165737363. MICE is funded by Li KaShing and Wellcome trust and initiated under collaboration between Mahidol-Oxford Tropical Medicine Research Unit (MORU) and Faculty of Tropical Medicine, Mahidol University, Thailand. The underlying principle of LCM is as follows; the Two Latent Class Model (2 LCM) was used in this study. In this model, the true disease/infection status of an individual is considered a latent variable, with two mutually exclusive categories (1 indicating. diseased/infected/positive and 0 means non-diseased/non-infected)/negative. The manifest binary variables that express the diagnostic test results, only give an indication of disease/infection status. The 2 LCM assumes that, given the true state of the disease or infection, the results of the diagnostic tests are independent. This assumption is known as the Hypothesis of Conditional Independence (HCI). In general, inferences were based on a number of iterations after discarding an initial burn-in of iteration numbers. Convergence is assessed by running multiple chains from various starting values according to Gelman , *et al*. (Gelman & Joseph, 2001) [23].

This study applied this principle by running dissimilar chains from different starting points to assess convergence while ensuring robust estimation. Model convergence was assessed using Gelman and Rubin convergence statistics. The total number of burn-in iterations was 2,000, this was the first 2000 iterations and was discarded as burn-in while the Total number of iterations used for analysis = 20,000 and this next 20,000 iterations by chain were used to parameterize the model via obtaining a sample of the marginal posterior density for each parameter (proportion of positive cases, sensitivity and specificity). Also, thinning intervals was 10 and used to assess the probability of observed frequencies, assuming the model was true. The median and the credible interval of these samples were used as point and interval estimation of the parameter. All parameters were estimated with 95% credible intervals (Bayesian version of the confidence intervals). Convergence was monitored using the standard diagnostic procedures based on a visual assessment of the long chains for each parameter and using the Gelman-Rubin and the Raftery-Lewis measures (Smith, 2007) [24]. Furthermore, the Bayesian p-value was calculated as described in detail by Nérette, *et al*. (2008) [25]. This version of Bayesian p-value suggests the lack of fit when p-values near 0 or 1 (Gelman & Joseph, 2001; Nérette et al., 2008; Neelon *et al*., 2011) [25,26]. Below are the results obtained.

RESULTS

A total of eighty (80) stool samples were subjected to the analysis of three replicates by each index test techniques.

Table 1, Frequency distribution of infection status using various index techniques for single Direct Microscopy =14 (17.5), Brine Microscopy=17 (21.3) and Diethyl-Ether Microscopy=19 (23.8). This report showed Diethyl-Ether Microscopy to be the technique with the highest number of parasite detection whereas, direct microscopy appeared to be the least. Comparatively, double combinations of index techniques showed no dissimilarity in the detection order like the single. The triple combination of all three index techniques used in this study revealed a detection frequency/rate of 50 (62.5). This triple index technique combination showed a synergic detection effect by implication was chosen to be the assumed

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gold reference standard in this study, otherwise referred to as imperfect reference gold standard or Extrapolated-Composite Gold Standard.

Table 1: Frequency Distribution of Infection Status using various Techniques/Combination.

Index Test Techniques	Index Technique/Combination	Number Tested (%)	Number Negative (%)	Number Positive (%)
Single	Direct Microscopy	80	66 (82.5)	14 (17.5)
	Brine Microscopy	80	63 (78.8)	17 (21.3)
	Diethyl-Ether Microscopy	80	61 (76.3)	19 (23.8)
Double	Direct Microscopy * Brine Microscopy	80	49 (61.3)	31 (38.8)
	Direct Microscopy * Diethyl-Ether Microscopy	80	47 (58.8)	33 (41.2)
	Brine Microscopy* Diethyl-Ether Microscopy	80	44 (55.0)	36 (45.0)
Triple	Direct Microscopy * Brine Microscopy* Diethyl-Ether Microscopy	80	30 (37.5)	50 (62.5)

Determine diagnostic accuracy of selected index test techniques (Direct Microscopy, Brine Microscopy and Diethyl-Ether Microscopy) using an extrapolated/combined reference (gold) standard.

Table2: Sensitivity, Specificity, PPV, NPV and Prevalence Estimated using Extrapolated/Combined Gold Standard (Imperfect). A prevalence of 63% was obtained in this study. For the index test technique used in this present study, the following results were reported based on diagnostic algorithm; Diethyl-Ether Microscopy (38%) was the most sensitive while Direct microscopy (28%). Correspondingly, the Positive Predictive Value shared equal rating order. On the other hand, Direct microscopy (35%) had the highest specificity and Diethyl-Ether Microscopy (32%) had the lowest. Similarly, this is in consonance with the outcome of the Negative Predictive Value (NPV).

Table 2: Sensitivity, Specificity, PPV, NPV and Prevalence Estimated using Extrapolated/Combined Gold Standard.

Index Tests (Techniques)	Sensitivity (%)	Specificity (%)	PPV(%)	NPV (%)	Prev (%)
Direct Microscopy	28	35	0.456	0.013	
Brine Microscopy	34	33	0.519	0.010	63
Diethyl-Ether Microscopy	38	32	0.555	0.009	

Prev=Prevalence, PPV= positive predictive value, NPV=Negative predictive value

Table 3 further illustrates diagnostic testing revealed more about the test accuracy using diagnostic indices like: Likelihood Ratio Positive and Negative, False Discovery Rate, False Omission Rate, and Diagnostic Odd Ratio. In this study, it was observed that Diethyl-Ether Microscopy showed the highest percentage for Likelihood Ratio Positive (1.230), Diagnostic Odd Ratio (1.422), and False Omission Rate (0.991). But recorded the lowest percentage for Likelihood Ratio Negative (0.865) and False Discovery Rate (0.445). Nonetheless, Direct Microscopy displayed contradictions to Diethyl-Ether Microscopy in all indices measured, Refer to table 2 for detail.

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Table 3: LR (Pos), LR(Neg), DOR, FDR and FOR.

Index Tests (Techniques)	LR (Pos)	LR(Neg)	DOR	FDR	FOR
Direct Microscopy	0.824	1.290	0.639	0.544	0.987
Brine Microscopy	1.030	1.000	1.030	0.481	0.990
Diethyl-Ether Microscopy	1.230	0.865	1.422	0.445	0.991

LR=Likelihood Ratio, Pos=Positive, Neg=Negative, FDR=False Discovery Rate, FOR=False Omission Rate, DOR= Diagnostic Odd Ratio

Evaluate inter-rater agreement between any two index test techniques using Kappa.

Table 4 showed Kappa index (Broemeling, 2009) [27] which is the inter-rater agreement was calculated to assess the diagnostic concordance between the Index test techniques. This study demonstrated very good agreement when weighed on Cohen's kappa classification scale. However, the diagnostic concordance between Brine Microscopy* Diethyl-Ether Microscopy had the highest rating (0.928) while Direct Microscopy * Diethyl-Ether Microscopy was the least (0.810) although it is on the lower limit of classification range termed very good.

Table 4: Inter-Rater Agreement using Kappa.

Index Tests/Techniques Combination	Kappa (k) value	p-value	Agreement Status
Direct Microscopy * Brine Microscopy	.880	0.00	Very Good
Direct Microscopy * Diethyl-Ether Microscopy	.810	0.00	Very Good
Brine Microscopy* Diethyl-Ether Microscopy	.928	0.00	Very Good

Classification of Cohen's kappa; < 0.20=Poor, 0.21-0.40=Fair, 0.41-0.60=Moderate, 0.61-0.80=Good, 0.81-1.00=Very Good

Measure trend of agreement and intra-test homogeneity of the selected three index test techniques (Direct Microscopy, Brine Microscopy and Diethyl-Ether Microscopy) using Kendall's Coefficient of Concordance, average Spearman Correlation and Cochran's Q.

Table 5; Kendall's Coefficient of Concordance was used to assess the trend of agreements (inter-rater)among the three techniques. A Kendall's W=.048, df=2 and p=.022 demonstrate a level of disagreement. Hence, no overall trend of agreement among the index test techniques and the detections may essentially be regarded as random. The outcome here shows that Diethyl-Ether Microscopy technique was rated high in terms of parasite detection as represented by the mean Rank = 2.04. In addition to the measure of concordance, the average Spearman Correlation over index test techniques was used to measure concordance over the three techniquesand extrapolated from Kendall's as -0.428.

Table 5: Kendall's Coefficient of Concordance.

Index Tests /Techniques	Rank	Kendall's W	Chi-Square	Df	p-value
Direct Microscopy	1.95				
Brine Microscopy	2.01	.048	7.600	2	.022
Diethyl-Ether Microscopy	2.04				

Kendall's W value 0= Perfect Disagreement, 1= Perfect Agreement. Average Spearman Correlation=kw-1/k-1=3(0.048)-1/3-1= -0.428.

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Equally, the Cochran Q test was used to test intra-test homogeneity, that is, that assessed if the percentage of positive results was the same among the three replicates of each index test techniques. The result here showed Cochran's Q =7.600, df=2 and p=0.02. This implies that assuming the three techniques are similar as hypothesized, there is still a 0.2% chances of finding the differences the researcher observed in this sample. Since this chance is smaller than 5%, the researcher rejects the null hypothesis. See table 6.

Table 6: Cochran's Q (intra-test homogeneity) of the selected Techniques.

Infection Status						
Index Tests /Techniques	**Positive**	**Negative**	**Total**	**Cochran's Q**	**Df**	**p-value**
Direct Microscopy	14	66	80			
Brine Microscopy	17	63	80	7.600	2	0.02
Diethyl-Ether Microscopy	19	61	80			

Compute Diagnostic accuracy using Bayesian Latent Class Model (LCM) amidst imperfect reference (gold) standard

Table 7: The study exploited a novel model known as Bayesian latent class model (LCM). Prevalence, sensitivities and specificities, positive and negative predictive values (PPV and NPV) were estimated using Bayesian latent class model (LCM).The bayesian latent class model assumed that all tests evaluated are imperfect. Values shown are estimated median with 95% credible interval (CrI 95%).LCM revealed a prevalence (%) of 6.0 (0.0 - 99.1). The sensitivity result in LCM showed Diethyl-Ether Microscopy as the most sensitive -41.4 (0.4 - 99.5 and Direct Microscopy as the least -39.9 (0.3 - 99.6). Likewise, the positive predictive value followed a comparable pattern of rating. However, specificity rates showed that Direct Microscopy had the highest specificity-61.0 (46.6 - 92.1) while Diethyl-Ether Microscopy had the least specificity-58.8 (46.8 - 91.0). The NPV is equivalent to the specificity rating, Direct Microscopy been highest and Diethyl-Ether Microscopy the least.

Table 7: Bayesian latent class model (%) Analysis of the Three Techniques.

Parameters	**Direct Microscopy Median (CrI 95%)**	**Brine Microscopy Median (CrI 95%)**	**Diethyl-Ether Microscopy Median (CrI 95%)**
Prevalence (%)	6.0 (0.0 - 99.1)		
Sensitivity	39.9 (0.3 - 99.6)	41.0 (0.3 - 99.4)	41.4 (0.4 - 99.5)
Specificity	61.0 (46.6 - 92.1)	59.8 (46.5 - 93.6)	58.8 (46.8 - 91.0)
PPV	5.6 (0.0 - 99.7)	5.4 (0.0 - 99.8)	5.7 (0.0 - 99.8)
NPV	95.0 (1.1 - 100)	94.8 (1.0 - 100)	94.6 (1.1 - 100)

Cr=Credible Interval= Is the interval in which an unobserved parameter has a given probability

Table 8 presents the Bayesian Latent Class Model. Checking for fitness of Bayesian LCM,three conditionally independent index test techniques used produced eight (8) possible outcome patterns (111=+ + +, 110=+ + –, 101= + – +, 011=– + +, 100= + – –, 010= – + –, 001= – – +, 000=– – –).This assesses the agreement between "frequency observed" and "frequency predicted" using Bayesian p-value and posterior predictive distribution of each profile. This model allows for imperfect reference standards.

The bayesian p-value is the probability that replicate data (predicted frequency) from the Bayesian model were more extreme than the observed data. A Bayesian p-value close to 0 or 1 indicates that the observed result would be unlikely to be seen in a replication of the data if the mode was true. This means that when Bayesian p-value is close to 0.5 or exactly 0.5, the Bayesian model describes the observed data very well

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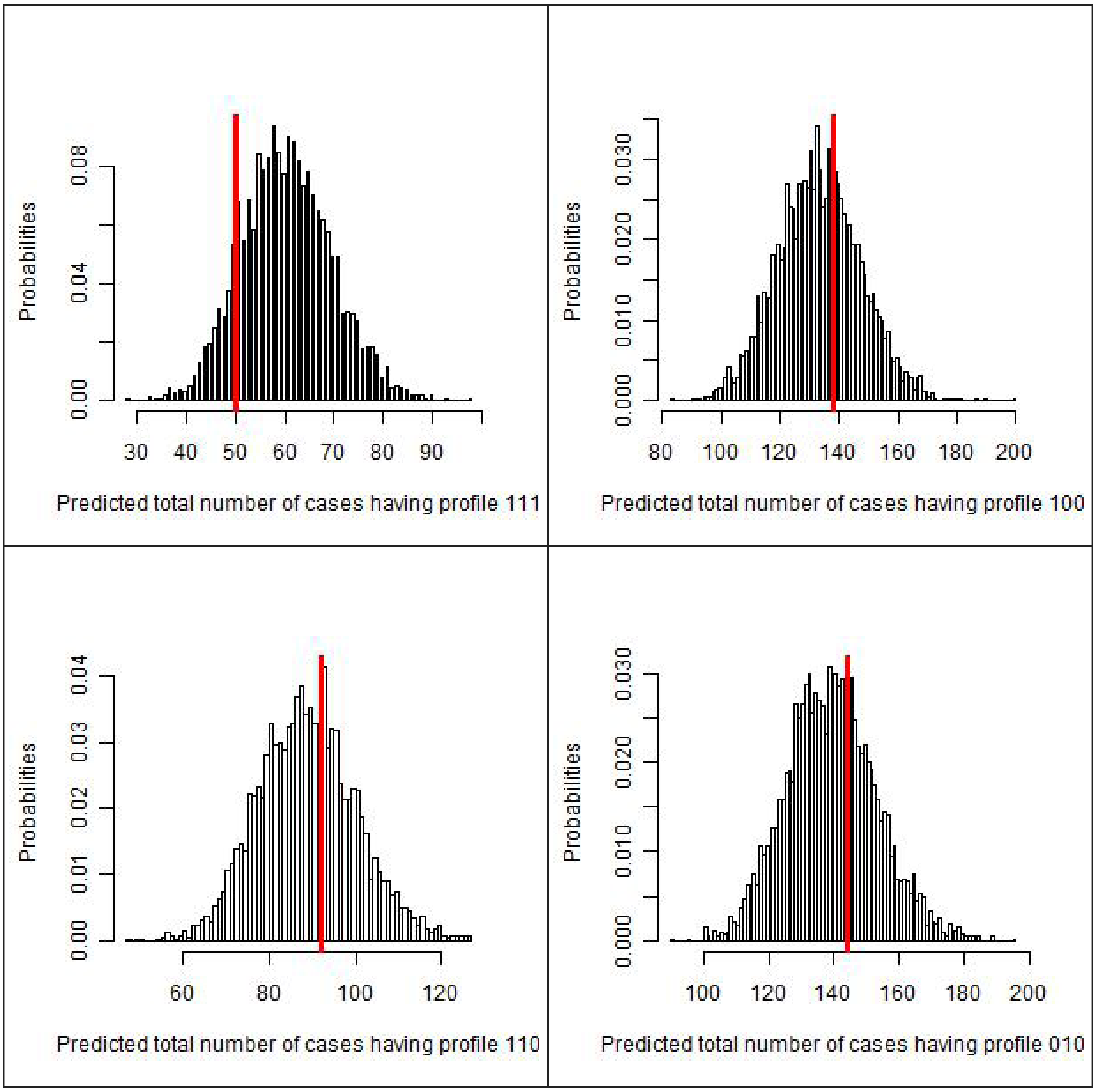
Table 8: Bayesian Latent Class Model Fitness Check.

Profiles	Direct Micros-copy	Brine Micros-copy	Diethyl-Ether Microscopy	Frequency observed	Frequency predicted	Bayesian p-value
111	Positive	Positive	Positive	50	60	0.898
110	Positive	Positive	Negative	92	89	0.414
101	Positive	Negative	Positive	96	93	0.402
011	Negative	Positive	Positive	102	98	0.384
100	Positive	Negative	Negative	138	133	0.387
010	Negative	Positive	Negative	144	140	0.386
001	Negative	Negative	Positive	148	144	0.411
000	Negative	Negative	Negative	190	201	0.759

Positive=1, Negative=0.



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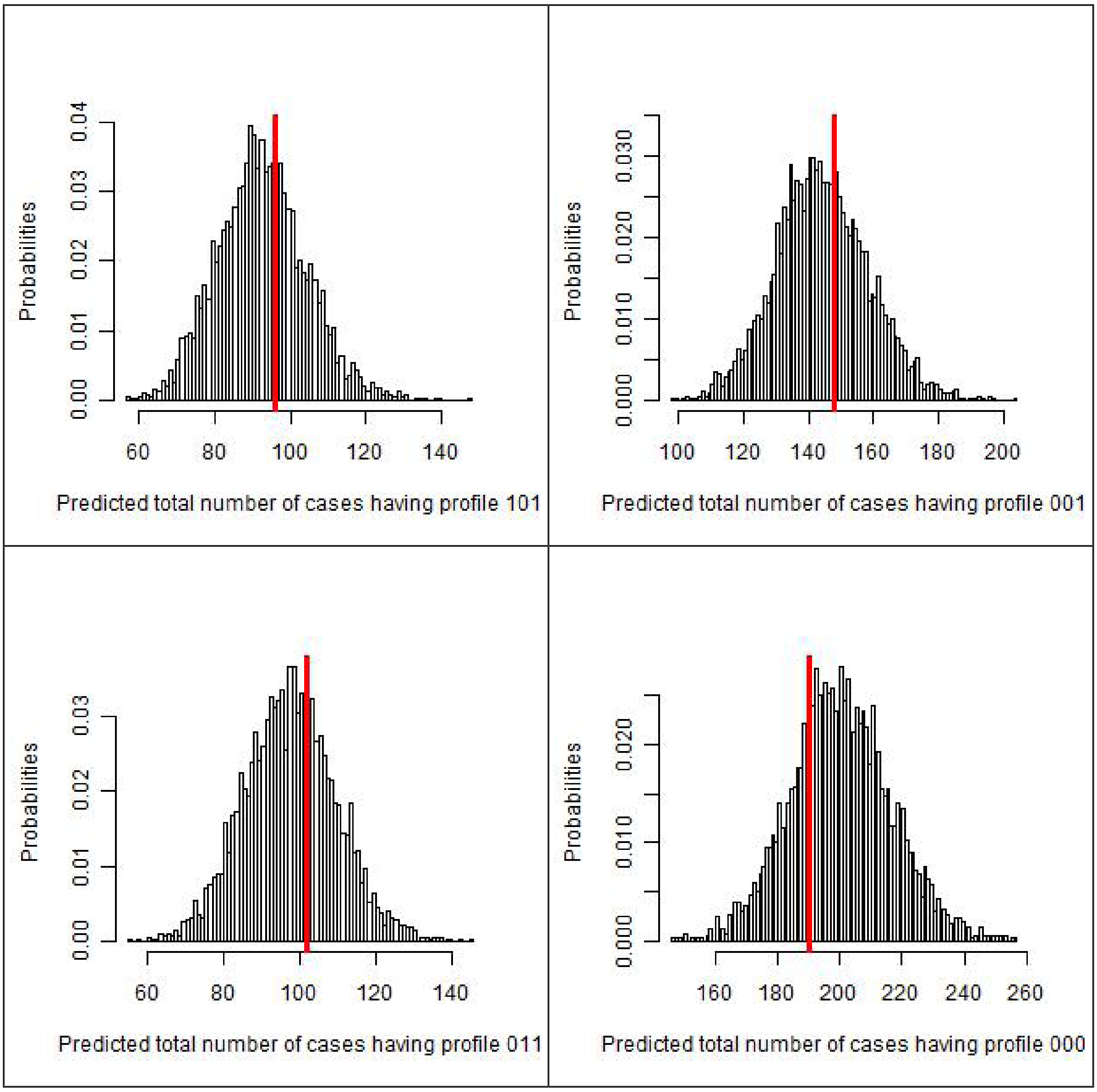

Figure1: Histogram showing frequency distribution of the three test results

*Red line represents the observed frequency of each test result profile, while the histograms illustrate the predictive posterior distribution of predicted frequency. In each of the figures, a dataset was replicated for **20,000** times and selected only 2,000 times (thin sampling equals to 10) to assess the probability of observed frequencies, assuming the model was true.*

Compare diagnostic accuracies of the extrapolated/ combined reference (gold) standard and the Bayesian Latent Class Model (LCM)

Table 9, *Gold standard model assumed that the gold standard test is perfect (100% sensitivity and 100% specificity; all patients with gold standard test positive are diseased and all patients with gold standard test negative are non-diseased). However, the study tested the index test techniques against the extrapolated composite gold standard (which was obtained from a combination of all index test techniques).

** Bayesian latent class model assumed that all tests evaluated are imperfect.

Comparison of Gold Standard and Bayesian Class Models revealed considerable discrepancies ($p<0.05$) in all the parameters (Prevalence, Sensitivity, Specificity, PPV and NPV) investigated via the index test technique except for the sensitivity of Diethyl-Ether Microscopy ($p>0.05$). See the table below.

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Table 9: Comparison of Gold Standard and Bayesian Class Model.

Index Tests (Techniques)	Parameter	*Composite Gold Standard Model (%)	**Bayesian Class Model (%)	p-value
Direct Microscopy	Prevalence	63	6.0	P<0.05
	Sensitivity	28	39.9	P<0.05
	Specificity	35	61.0	P<0.05
	PPV	0.456	5.6	P<0.05
	NPV	0.013	95.0	P<0.05
Brine Microscopy	Sensitivity	34	41.0	P<0.05
	Specificity	33	59.8	P<0.05
	PPV	0.519	5.4	P<0.05
	NPV	0.010	94.8	P<0.05
Diethyl-Ether Microscopy	Sensitivity	38	41.4	P>0.05
	Specificity	32	58.8	P<0.05
	PPV	0.555	5.7	P<0.05
	NPV	0.009	94.6	P<0.05

P<0.05=Significant. P>0.05=Not Significance

DISCUSSION

Accurate diagnosis of diseases is essential in health care in developing countries where infections are the most common causes of death and ill health (Peeling *et al*., 2007) [28]. Modern medicine has scaled up from mere observation (clinical signs and symptoms) to more empirical and evidence-based and is currently in the precision arena. Observable signs and symptoms are often not adequately accurate and may perhaps lead to issuing inappropriate treatment and inducing resistance as there are cases of anti-helminth resistance (Peeling *et al*., 2007) [28]. Molecular detection is not readily available and may be costly, insufficiently sensitive, and difficult or dangerous to perform under field conditions. This is a common case in parasitic infections like visceral leishmaniasis (Boelaert *et al*., 2007) [29].

Parasitology has been challenged by the unavailability of a gold reference standard thus, this study adopted the use of a composite reference standard extrapolated via a combination of the results of the three index test techniques as a standard test (imperfect gold standard). This is in agreement with earlier studies such as a study done by Mulat *et al*. (2015) [4] and another done in Gondar according to Endris and colleagues (Endris *et al*., 2013) [30]. In this study, Diethyl-Ether Microscopy outperformed the other two techniques. In comparison to parasite detection, findings here confirmed that Diethyl-Ether Microscopy was high as compared to the other two techniques. This result agrees favourably with other similar studies done previously (Moges *et al*., 2010) [5]. Besides, the detection rate of intestinal parasites with direct microscopy was lower than the Diethyl-Ether Microscopy in the present study. This result is in agreement with the Ethiopian study (Mulat *et al*., 2015) [4] and another study conducted in Nigeria (Sheyin *et al*., 2013) [31].

This present study is in consonance with the study of Santos, Luciano, Cerqueira and Soares in 2005 (Santos *et al*., 2005) [32] in that, Diethyl-Ether Microscopy demonstrated higher sensitivity than the other index test techniques used in the study. In addition, evidence from this study proved that Diethyl-Ether Microscopy recovery efficiency for parasites is greater than the direct microscopy and Brine techniques. This particular finding has been proven over the years even observed in an age-long study (Ritchie, 1984) [33]. Similarly, Levecke *et al*. (2009) [34] also recorded low sensitivity of the direct microscopy technique in the detection of low intensity of infection as observed in this study. And another study established an equivalent finding (Endris *et al*., 2013) [30]. This indicates that the use of direct microscopy as a confirmatory test will extensively amplify under-diagnosis of false-negative test results.

Apparently, diagnostic accuracy in parasitology, especially in this era of Neglected Tropical Diseases, necessitates a supplementary quick simple and sensitive index test technique. Conventional direct microscopy as the method of choice for stool examination has shown to have a low parasite detection rate, therefore, greatly limited owing to poor sensitivity. Consequently, the likelihood of false-negative results will be on the increase. Subsequently, the under-diagnosis of parasitic infections will be misleading.

On the detection of different positive cases by the three index tests, the combined use of the three index tests provided a better diagnosis. However, despite the general improvement of the diagnosis in these cases, the low sensitivity reported for both tests indicates an important underestimation of the total number of positive cases.

Since this study was based on the analysis of a single stool sample collection, the day-to-day variation in the output of parasitic forms was not assessed, thus, the estimations of sensitivities could have been higher if more samples had been collected and analyzed. The low sensitivity of diagnostic tests for parasitic detection may be related to the rapid degeneration of some parasite ova eggs over time. Furthermore, sensitivity is influenced by delays between stool production and the analysis times in the laboratories (Dacombe *et al*., 2007; Knoop et al., 2009; Krauth *et al*., 2012) [6,35,36]. In this study, the detection rate (62.5%) for the composite gold reference standard extrapolated is less than the one reported in Ethiopia (Mulat *et al*., 2015) [4].

Comparative analysis of the composite gold reference standard extrapolated with the Bayesian Class Model shows that there is proximity between Bayesian class model and the composite reference standard proposed by Alonzo and Pepe (1999) [37]. Particularly for the sensitivities, as the sample size reduces because the discrepant results between the two reference tests are discarded, the 95% confidence intervals are wider. In this present study, the sensitivities of the composite gold standard and LCM shared statistical consistency while the other diagnostic indices of specificity, PPV and NPV, as well as prevalence, showed marked variation between the composite gold standard and LCM.

From the specific objectives which guided this study; the following were conclusively drawn.

Firstly, the diagnostic accuracies of selected index test techniques (Direct Microscopy, Brine Microscopy and Diethyl-Ether Microscopy) using an extrapolated/composite reference (gold) standard in this study generally were low. Nonetheless, the prevalence was a bit high. The use of composite gold standard as done in this study is similar in older studies however the diagnostic accuracies differ from this study. This imprecision of composite reference (gold) standard due to lack of perfect gold standard in parasitology is evident in previous studies (Devera et al., 2008; Knopp *et al*., 2009; Levecke *et al*., 2009; Brandelli *et al*., 2010; Dogruman-Al *et al*., 2010; Glinz *et al*., 2010; Steinmann *et al*., 2010; Inês *et al*., 2011; Carvalho *et al*., 2012) [6,11,34,38-43]. This practice has led to biased estimations of accuracy. This is consistent with some publications which posit that simpler alternatives to latent class analysis, such as the composite reference standards, are problematic (Schiller *et al*., 2018) [44].

Secondly, diagnostic concordance between the index techniques demonstrated very good inter-rater agreement for all three index test techniques. Remarkably, the result of the individual diagnostic accuracy influenced the diagnostic concordance for any two index tests as observed. This was seen as the two index test techniques with higher diagnostic accuracies had stronger agreement while the ones with lower diagnostic power demonstrated variation to a lesser extent although all showed good concordance.

Thirdly, the diagnostic accuracy using Bayesian Latent Class Model (LCM) amidst imperfect reference (gold) standard suggest some levels of diagnostic accuracy. In addition, the Statistical comparison of the composite reference (imperfect Gold) Standard with the Bayesian Class Model proves that LCM, as recorded, is a better tool than the imperfect gold reference standard from the study finding. This finding is in agreement with prior studies (Bachmann *et al*., 2006) [21]. A perfect diagnostic test otherwise known as gold standard or reference (with 100% sensitivity and specificity) is, for the most part, a theoretical concept. In practice, there are several diseases like tuberculosis, pneumonia, Alzheimer's disease including parasitic infection for which there is no perfect (gold standard) test that can detect the presence of the disease with certainty.

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This complicates estimation of disease prevalence as well as the evaluation of diagnostic tests accuracy (Lu *et al*., 2004) [45]. Notably, just a few studies have explored diagnostic accuracy of test techniques in parasitology using the concept of absence of a gold standard test or imperfect gold standard (Booth *et al*., 2003; Traub *et al*., 2009; Tarafder *et al*., 2010) [20,46,47]. This study has added to the number thereby, emphasizing the utilization of this mathematical model which possesses vast applications including malaria studies and other tropical diseases (Speybroeck *et al*., 2011; Canavate *et al*., 2011) [8,48]. Besides, a separate study presents several Bayesian latent class models for the diagnosis of visceral leishmaniasis (Menten *et al*., 2008) [49]. Limmathurotsakul, *et al*. explore some diagnostic tests for melioidosis (Limmathurotsakul *et al*., 2010) [50].

CONCLUSION/RECOMMENDATION

The single-use of direct microscopy for parasites identification as routinely done in the area of this present study is insufficient and may lead to false-negative results. Hence, it is preferable to use Diethyl-Ether Microscopy technique to complement other techniques including the direct microscopy. Also, the extrapolated-composite reference standard promises to be great instead of any single technique alone. However, all the combined results of the three diagnostic index test techniques are vastly influenced by parasite prevalence. Consequently, the same technique will have diverse values in different areas of prevalence. Hence, prevalence is a determinant factor.

Furthermore, the accuracy of diagnostic test algorithms for the diagnosis and detection based on solely use of any single microscopic technique as well as the use of imperfect gold standard have been criticized and alternative statistical approaches have emerged without wrongly assuming any of the diagnostic tests as a perfect gold standard. At this juncture, this present study addressed this issue with a novel Bayesian Class Model approach, in the helminth context to obtain valid outcome as observed in this study; thus recommended.

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