Evaluation of the Accuracy of CD4 Tests performed in five Public Health laboratories in Kisumu County-Kenya

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*Abstract-*The quantification of CD4 T-lymphocytes, an important marker of HIV disease progression, signals initiation of or regulation of antiretroviral therapy. The study assessed the accuracy of CD4 tests results generated at five public health laboratories in Kisumu County- Kenya. Samples from HIV positive patients that had been tested for CD4 at five high volume (>1000 tests/month), high proximity (within 100km radius of Kisumu county) laboratories were retested at the reference laboratory. Results were entered into a Microsoft excel sheet and analyzed to determine the percentage coefficient of variation (CV), the mean difference and the concordance correlation coefficient between results generated at the public health laboratories and the local reference laboratory. A coefficient of variation of ≤ 13 %, a mean difference of ± 50 cells/µl and a concordance correlation coefficient of ≥ 0.90 were considered to be within acceptable limits. Overall, repeat test results had a mean CV of 10.5% (Range: 8.9%-11.4%) and a mean difference of -15 cells/µl (Range: -3.3; -47.2). Laboratory A had the least coefficient of variation of 8.9%, the least bias; -3.33 cells/ µl (-119.59; -112.93) and the highest concordance correlation coefficient of (0.98) hence most accurate of all. The CD4 results at all laboratories were comparable to the reference laboratory results. The CD4 Proficiency Testing (PT) program is a feasible alternative to formal costly External Quality Assessment (EQA) programs for CD4 testing. Scale up of repeat testing PT could encourage continuous quality improvement in CD4 testing among laboratories in Kenya.

Index Terms: Accuracy, CD4 count, HIV/AIDS, Repeat test

I. INTRODUCTION

In Kenya, the human immunodeficiency virus (HIV) prevalence in adults aged 15-64 years was estimated to be 5.6% in 2012 (1). Of those infected, 63% were on antiretroviral treatment (ART) by the year 2012 (1). The "cluster of differentiation 4"(CD4) T-lymphocytes are a subset of the white blood cells that are the main target of the HIV(2). In Africa, the average healthy HIV-free adult CD4 count has been estimated to be 1067 cells/µl of blood (3). The quantification of CD4 T- lymphocytes is therefore an important marker of HIV disease progression and signals the time of initiation or regulation of antiretroviral therapy (ART) (3, 4).

In 2011, the Kenya Ministry of Health recommended ART initiation in HIV-infected adults with a CD4 count of ≤ 350 cells/µl (5). Since the procedure of CD4 testing requires specialized equipment, reagents and well trained personnel, it historically has been performed in tertiary level laboratories which have most of the required infrastructure (6, 7). The process of quality assurance of CD4 tests, and therefore accuracy (defined as the extent to which an experiment yields similar results upon repeated trial), has been identified as a key challenge in many resource poor settings (6, 8). This has hugely been as a result of technical laboratory factors that include the use of electronic versus manual pipettes, testing equipment maintenance, gating strategy and staff skills which may influence the accuracy of the test results (9).

The following four formal external quality assessment (EQA) schemes are prominent in sub-Saharan Africa:1) United Kingdom National External Quality Assessment Services, 2) Quality Assurance Systems International, 3) College of American Pathologist, and 4) African Regional Quality Assessment Scheme (8). However, the costs of these schemes make them inaccessible to most public health laboratories in resource limited-settings hence the need for innovative quality assurance strategies. Sample split-testing PT is an alternative to these formal EQA schemes(10).

To assess the clinical usefulness of CD4 test results in patients' ART management, this study aimed to provide data on the accuracy of CD4 test results produced in five public health laboratories in Kisumu County while using the test results from the local reference laboratory; Kenya Medical Research Institute (KEMRI) HIV Research Laboratory in Kisumu, as a 'gold standard'.

II. RESEARCH ELABORATIONS

A. Study design and sites

We conducted this laboratory-based cross-sectional study between 2011 and 2014. Blood samples from HIV positive patients tested for CD4 at five public health laboratories (also referred to as study laboratories) were collected and retested at KEMRI HIV Research

Laboratory (also referred to as the Index laboratory) (Figure 1). The study compared test results obtained at each study laboratory with the results of the same samples obtained at the index laboratory; as well the results obtained at all study laboratories with the results of the same samples obtained at the index laboratory. Both the study laboratories and the reference laboratory utilized flow cytometry single platform techniques for their CD4 enumeration; all of the study laboratories utilized the single platform Becton Dickinson (BD) Facscount CD4 machine while the reference laboratory utilized the BD FACScalibur machine for all its testing.

B. Definitions

Accuracy was defined as how similar the results obtained for individual samples at the study laboratories under study were to the results obtained from the index laboratory. The accuracy of these test results pairs were assessed based on three parameters; coefficient of variation, mean difference (bias), and the concordance correlation coefficient (CCC).

The coefficient of variation (CV) is the ratio of the standard deviation to the mean, expressed as a percentage. The bias is the measure of how much a CD4 count that is obtained by the study laboratories differs from the CD4 count obtained by the reference laboratory expressed as cells/µl of whole blood. The concordance correlation coefficient is the measure of agreement of the measurement pairs.

C. Sample collection

Whole blood samples were used in the study if they were either collected, in an ethylenediaminetetraacetic acid (EDTA) or Becton Dickinson (BD) CD4 stabilization tube, from persons aged 5 years and older, had an accompanying CD4 result from the each of the study laboratories and were within the tube manufacturer's recommended period of collection (48 hours for those collected in EDTA tubes and 7 days for those collected in BD CD4 stabilization tubes). Samples were excluded or rejected if poorly labeled or clotted. Samples from the study laboratories were transported at room temperature by courier or delivered in person to the reference laboratory.

D. Sample processing

All study laboratories were provided with standard operating procedures that entailed performing tests within 48 hours if collected in EDTA tube or within 7days if collected in CD4 Stabilization tube, after drawing the blood specimen, pipetting blood samples accurately and in a reproducible manner using the reverse pipetting technique, incubating all tubes in darkness during staining and using the lyse/no-wash method of testing according to the manufacturer's instructions.

The reference laboratory accordingly retested the samples using the same standard operating procedures and recorded the corresponding results for each study laboratory on the specific samples and results submission form.

E. Data analysis

The data from the laboratories' test results pairs were entered into a Microsoft Excel sheet and subjected to results' comparison analysis. Bias and limits of agreement (LOA) were calculated using the Bland-Altman method (11). The CV was calculated to assess the extent of dispersion of the results in relation to the mean for each study laboratory and for all the study laboratories combined. The concordance correlation coefficient, to determine the linearity of the results, was calculated through linear regression analysis. Data analysis was conducted using STATA vs.12 statistical software.

F. Ethical considerations

Approval to conduct this study was granted by the KEMRI Ethical Review Committee (SSC number 2485) and the CDCs Associate Director for Science (ADS). Analysis was conducted according to the Clinical Laboratory Standards Institute (CLSI) measurement procedures for comparison and bias estimation using patient samples; approved guidelines EP09 (12).

III. RESULTS

A total of 285 samples from all the five study laboratories were tested. Study laboratories contributed between 40 and 91 samples each.

The median CV of the results was 10.5% with the individual laboratory results ranging from 8.9% to 13.4%. The ranking in accuracy based on the percentage coefficient of variation was: 1 for laboratory A (8.9% CV), 2 for laboratory E (10.1% CV), 3 for laboratory B (10.6% CV), 4 for laboratory D (11.4% CV) and 5 for laboratory C (13.4% CV) (Table 1).

The mean difference (bias) ranged from, -3.3 cells/µl to 13.1 cells/µl. The mean bias for all study laboratories was 15 cells/µl of whole blood. The ranking of the testing laboratories according to bias (and LOA) was: 1 for laboratory A -3.3 cells/µl (-119.6;112.9), 2 for

laboratory E -4.7 cells/μl (-130.2; 120.8),   3 for laboratory B 13.1 cells/ μl (-99; 125.2); 4 for laboratory C -14.8 cells/μl (178.6-149.1); and 5 for laboratory D -47.2 cells/μl (-168.5-74.2) (Table 2).

The overall CCC (and 95% CI) was 0.95 (0.94, 0.96).   Individual CCCs (and 95% CI) at study laboratories were; Lab A 0.98 (0.96-0.99), Lab B 0.96 (0.94-0.99), Lab C 0.91 (0.88-0.95), Lab D 0.96 (0.94-0.98), Lab E 0.96 (0.94-0.99). (Figure.2).

IV. CONCLUSIONS

The overall results for all of the five laboratories were within the acceptable range of the predefined measures of CD4 accuracy. All the three parameters used to assess accuracy of CD4 results in the study pointed at the accuracy of the study laboratories' results compared to the reference laboratory results. The results of this study strongly suggest that CD4 tests performed by public health laboratories in Kisumu County, Kenya, are comparable to those of the reference laboratory and can therefore be relied upon by clinicians for patients' ART management.

Laboratory A had the least variation in results and therefore was the most accurate according to both percentage coefficient of variation and bias. The laboratory had initiated quality management structures at the time of the study which could be a possible contributor to their good results.

Laboratory C had the most variation in results and therefore was least accurate of the laboratories according to the percentage coefficient of variation and the second last accurate according to bias. Laboratory D had the least accurate results according to bias and the second least accurate according to percentage bias. However all these difference were not clinically significant to alter ART treatment decision making.

Similar to a study by Hutlin et al that found a median inter-laboratory variation of 9.9% coefficient of variation, the CD4 results from all the study laboratories varied from those of the reference laboratory by a median of 10.5% coefficient of variation (13). The mean percent coefficient of variation was within the prescribed CV limit of agreement for the study (defined here as ≤ 13% CV). The variation in the results was acceptable according to a previous study by Mlawanda et al (14) .

The CCC revealed a strong liner relationship between all result pairs (0.95) (Figure 3). The current finding is consistent with reported results of other previous studies of inter-laboratory inter-method agreement of CD4 results (15).The strong correlation is an indicator of the agreement of the results from the public health testing laboratories to those of the local reference laboratory.

The mean difference (bias) of the result pairs was 15 cells/μl. This bias was within that reported in previous CD4 inter-laboratory measurement studies (15). Bland-Altman analysis revealed that the majority of measurements were within the 95% confidence interval of test result generated. The 5% of outliers across all measurements could be attributed to possible random pipetting errors (16). All the study laboratories utilized manual pipettes; compared to electronic pipettes, manual pipettes usually fail to deliver accurate volumes of samples or reagents unless when used by technicians experienced in reverse pipetting. The equipment maintenance status in the test laboratories could also not be verified (17).

Among the limitations of this study was the unequal contribution of samples by different laboratories to the overall sample size. The spread of data was therefore different across the five testing laboratories, which could have possibly contributed to the high variance in the results of laboratory C, which contributed the highest number of samples to the overall sample size. The laboratories under assessment were at different levels in terms of capacity in quality systems, with some already in the process of undergoing World Health Organization (WHO) step-wise accreditation. The predefined acceptable limits for accuracy were based on available literature, however, actual inter-laboratory or inter-method variability acceptable limits for CD4 testing remain debatable (14).

The study demonstrates that participation in a CD4 repeat Proficiency Testing (PT) program can be useful in monitoring the accuracy of CD4 testing. Conducting repeat testing performed at public health laboratories at a reference laboratory can help in the assessment of staff competency, condition of reagents and equipment. With structured systems in place, the repeat test PT program can be used as an alternative to the formal external quality assessment (EQA) programs for CD4 that are usually not accessible to most public health laboratories. We recommend that the PT program be scaled up to cover all CD4 laboratories in the region in an effort to assure the quality of CD4 testing in public health laboratories in Kenya.

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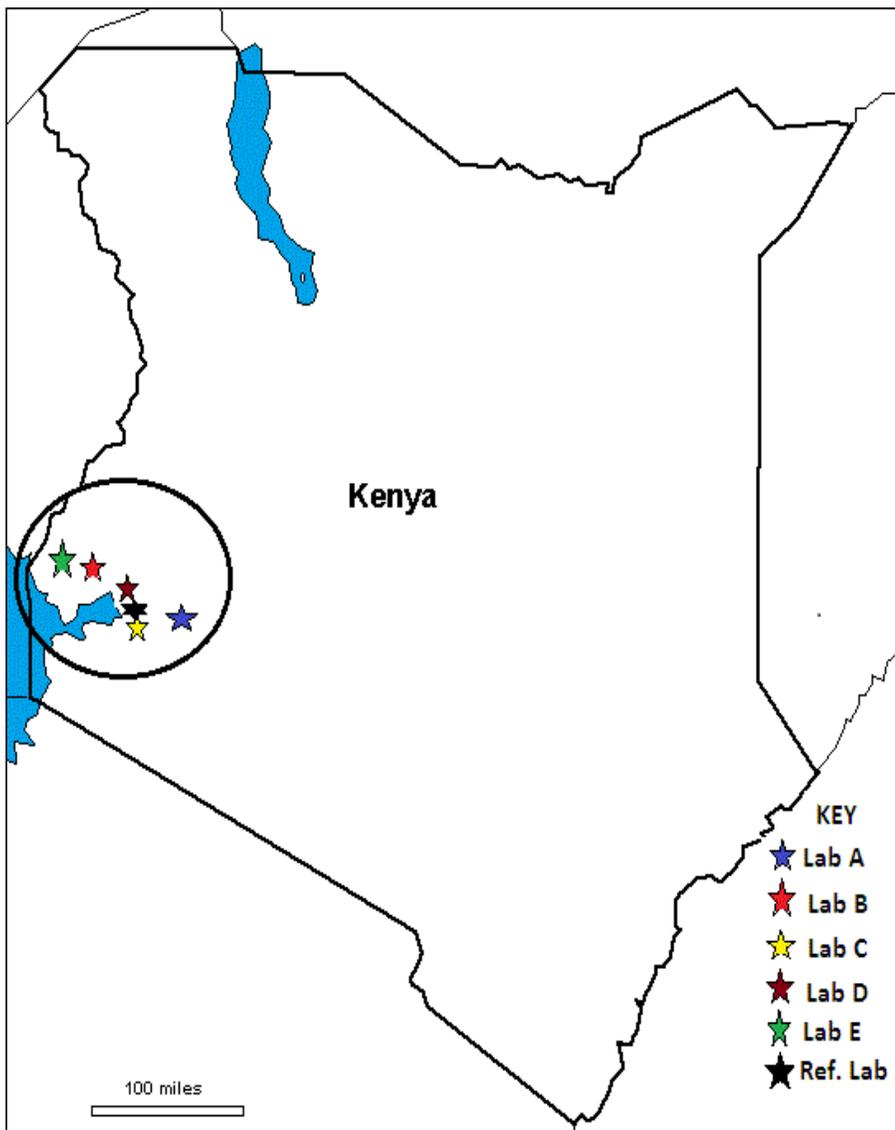Ng'ayo Navies Newton Frodwa,BSc (Biotechnology), MscS,Email: nngayo@yahoo.co.uk Telephone: +254724362598

Figure 1: Map of Kenya highlighting Kisumu County and identifying the approximate location of the study laboratories in 2014
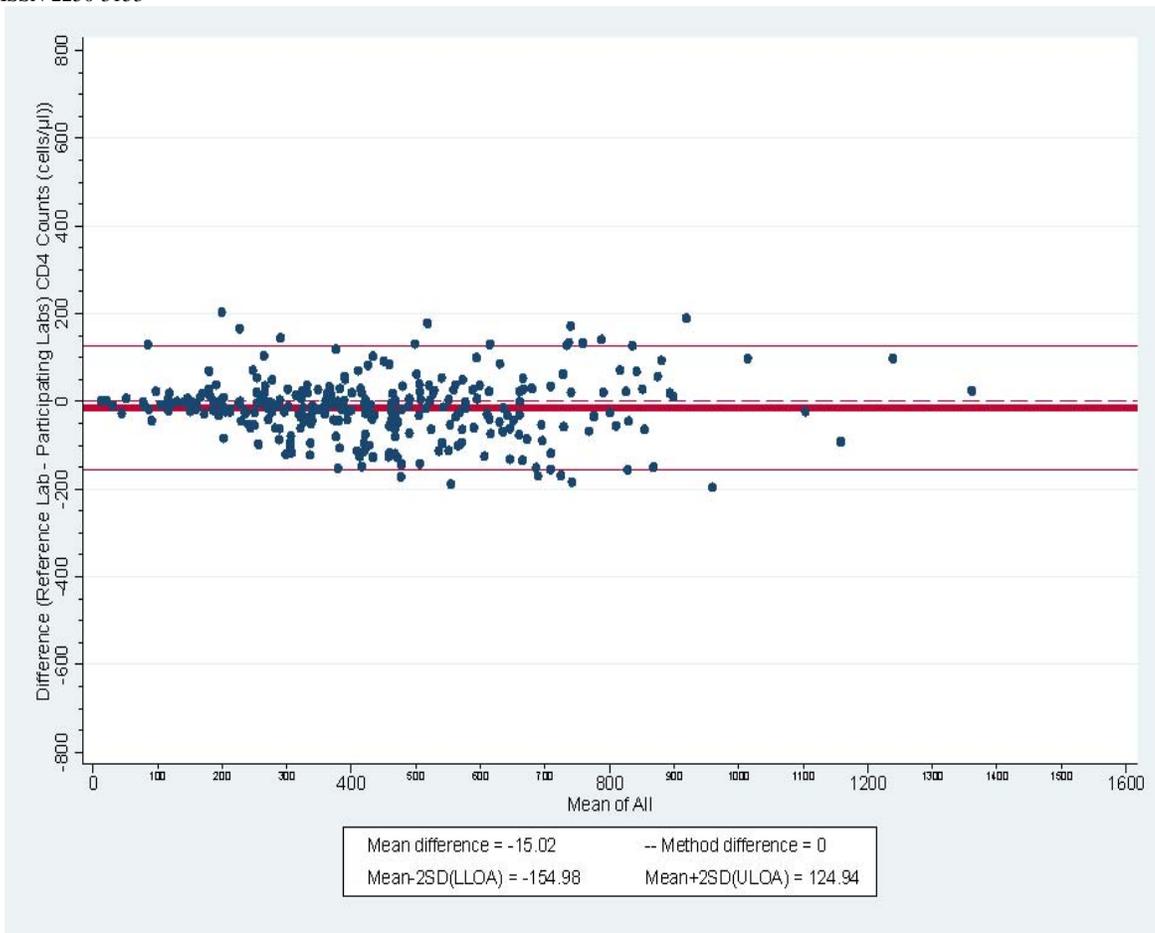
Figure 2: Difference plot of the CD4 results of the five public health laboratories versus the results of the reference laboratory in Kisumu 2011- 2014
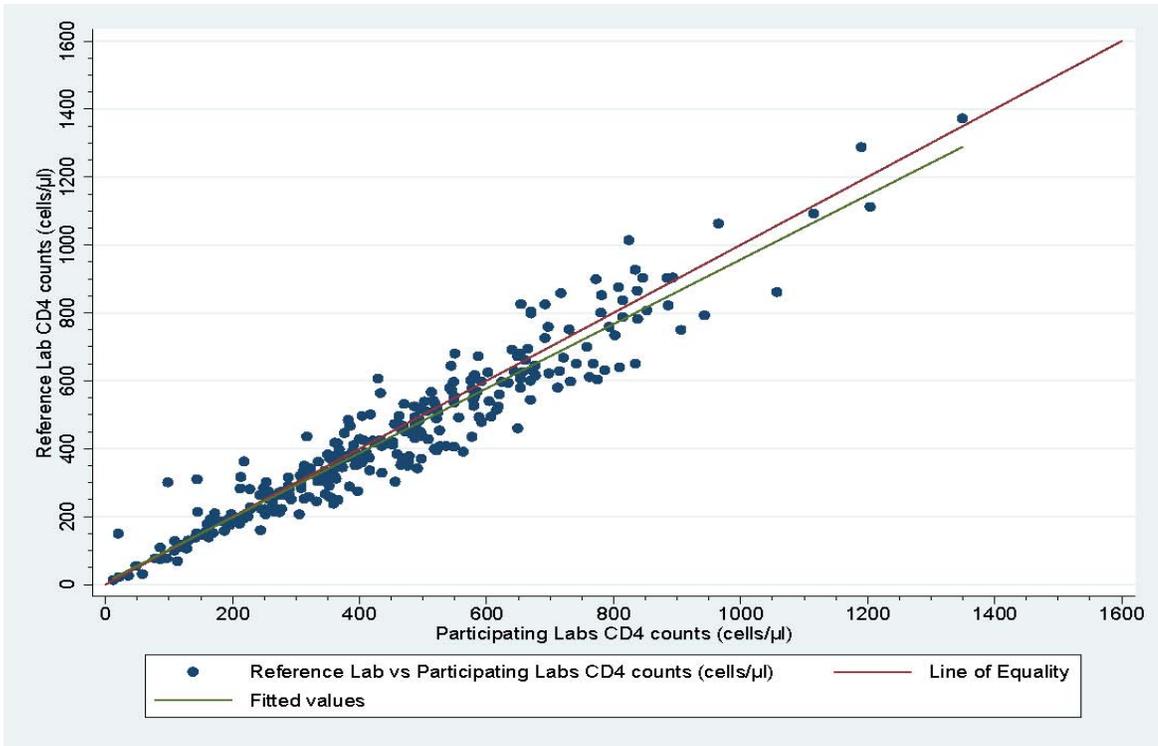
Figure 3: A scatter plot of the CD4 results of the five public health laboratories versus the results of the reference laboratory Kisumu 2011-2014

Table 1: Individual performance in CD4 testing by individual five public health laboratories according to Percentage Coefficient of Variation of results, Kisumu 2011-2014

Laboratory Identity	Number of samples	(CV)*%	Rank
A	45	8.9	1
B	40	10.6	3
C	91	13.4	5
D	66	11.4	4
E	43	10.1	2
Median		**10.5**	N/A
Mean		**10.9**	N/A

Table 2: Individual performance in CD4 testing by individual five public health laboratories according to Mean Difference (bias) of results, 2011-2014

Laboratory Identity	Bias 95%CI[†] (cells/µl)	Rank
A	-3.3(-119.6-112.9)	1
B	13.1(-99.0-125.2)	3
C	-14.8(-178.6-149.1)	4
D	-47.2(-168.5-74.2)	5
E	-4.7(-130.1-120.8)	2
Mean	**-15. (-155.0-124.9)**	N/A