WHAT IS ALREADY KNOWN ON THIS TOPIC

Rapid HIV tests provide timely, point of care methods for screening and diagnosis, but interpretation of positive bands is subjective

WHAT THIS STUDY ADDS

Weak positive bands on rapid HIV tests are mainly false positives and should be confirmed by enzyme immunoassay and western blotting before providing a diagnosis

by enzyme immunoassay and western blotting to maintain quality control in programmes using rapid tests.[3] This is of particular importance given the proliferation of rapid tests and given the potential social and psychological consequences of a false positive HIV diagnosis. The proportion of samples yielding weak positive bands was relatively low in our study (5.8%), so retesting of weak positive results would not impose a heavy burden on most programmes. It would, however, require laboratory backup which could lead to delay in disclosure of some results.

We conclude that weak positive bands on rapid tests cannot be interpreted as positive in serum from Ugandan populations.

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Diagnostic accuracy and clinical utility of a simplified low cost method of counting CD4 cells with flow cytometry in Malawi: diagnostic accuracy study

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ABSTRACT

Objectives To assess the diagnostic accuracy and clinical utility of a simplified low cost method for measuring absolute and percentage CD4 counts with flow cytometry.

Design A CD4 counting method (Blantyre count) using a CD4 and CD45 antibody combination with reduced blood and reagent volumes. Diagnostic accuracy was assessed by measuring agreement of the index test with two other assays (TruCount and FACSCount). Clinical utility was investigated by comparing CD4 counts with the new assay with WHO clinical staging in patients with HIV.

Setting Research laboratories and antiretroviral therapy clinic at a medical school and large government hospital in southern Malawi.

Participants Assay comparisons were performed on consecutive blood samples sent for CD4 counting from 129 patients with HIV. Comparison of CD4 count with staging was conducted on 253 consecutive new patients attending the antiretroviral therapy clinic.

Main outcome measures Limits of agreement with 95% confidence intervals between index test and reference standards.

Results The limits of agreement for Blantyre count and TruCount were excellent (cell count −48.9 to 27.0 $\times 10^9$/l for absolute counts in the CD4 range <400$\times 10^9$/l and −2.42% to 2.37% for CD4 percentage). The assay was affordable with reagent costs per test of $0.44 (£0.22, €0.33) for both absolute count and CD4 percentage, and $0.11 for CD4 percentage alone. Of 193 patients with clinical stage I or II disease, who were ineligible for antiretroviral therapy by clinical staging criteria, 73 (38%) had CD4 counts <200$\times 10^9$/l. By contrast, 12 (20%) of 60 patients with stage III or IV disease had CD4 counts >350$\times 10^9$/l.

Conclusions This simplified method of counting CD4 cells with flow cytometry has good agreement with established commercial assays, is affordable for routine clinical use in Africa, and could improve clinical decision making in patients with HIV.

INTRODUCTION

CD4 counting could improve appropriate allocation of antiretroviral therapy for people infected with HIV.[1] Despite initiatives to reduce the price of the necessary reagents for developing nations to $3-6 (£1.5-3.0; €2.2-4.4) per test,[2] this cost is still high for Africa.[3] CD4 counting with flow cytometry is perceived by many to be too complex for use in Africa. WHO guidelines state that where CD4 counting is available, adults and children over 5 years with HIV should start

antiretroviral therapy as soon as their CD4 counts drop below 200×10⁹/l, regardless of clinical staging. In children under 5 years CD4 percentage of total lymphocyte count (CD4 percentage) is recommended to help decide on initiation of antiretroviral therapy.

There are two main approaches for making CD4 counting more widely available in Africa. Firstly, reduce the cost of and simplify flow cytometric CD4 counting. Secondly, develop alternative counting methods. Flow cytometry, however, is the ideal method and has high accuracy.[1 4] High throughput is possible as about 250 samples a day can be processed.[3] Effective external quality assurance schemes are available in Africa.[5 6] Finally, flow cytometers can measure CD4 percentage as well as absolute counts.

Over recent years several technological developments have shown that flow cytometric CD4 counting could be more straightforward (see bmj.com). We investigated whether these technologies could be miniaturised to reduce costs and applied them to the FACSCalibur flow cytometer. We developed a single platform method (the Blantyre count) that could be performed with reduced reagent costs and could accurately determine both absolute and percentage CD4 with increased simplicity compared with existing flow cytometric methods. We compared our method with the existing TruCount and FACSCount CD4 counting assays for diagnostic accuracy and assessed the potential impact on clinical decision making.

METHODS

The study was conducted at the Malawi-Liverpool-Wellcome Trust Research Programme and Queen Elizabeth Central Hospital in Blantyre. The estimated prevalence of HIV infection among adults in Blantyre district is 22%.[7] We used a FACSCalibur flow cytometer.

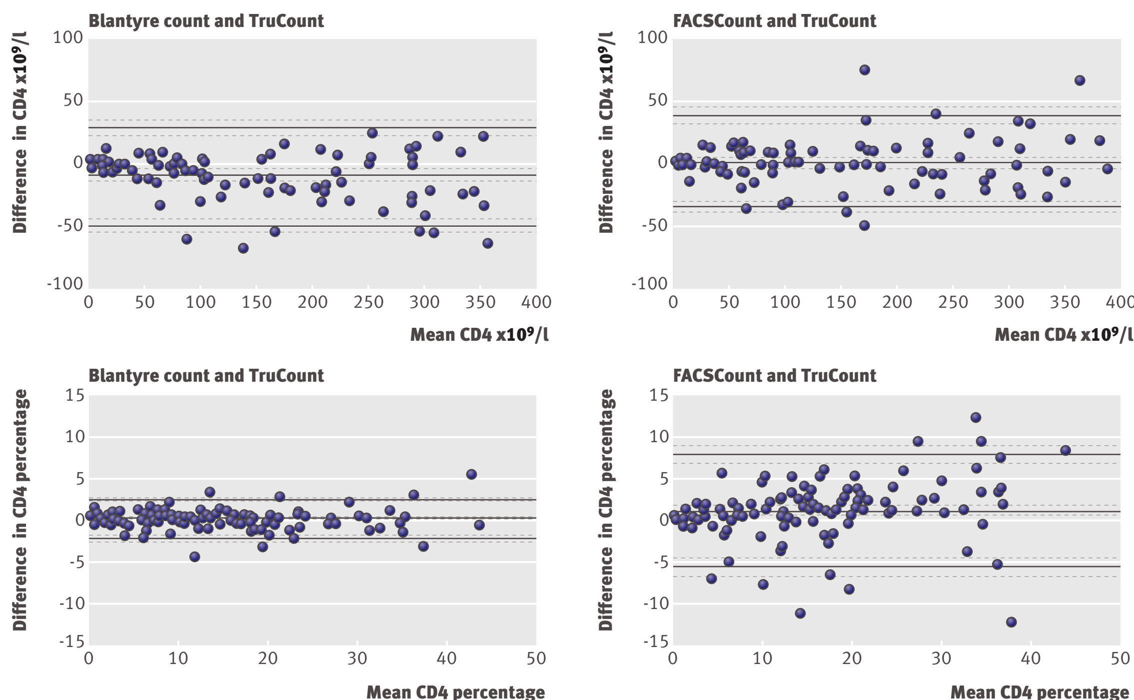
Reference standards (TruCount and FACSCount assays)

We used TruCount[8] and FACSCount[9] assays for CD4 counts. We chose TruCount as the reference standard because it is a commercial CD4 counting method that was developed to be used on the same instrument as the index test and generates both absolute and percentage CD4. We used FACSCount as a second reference standard because it is one of the most widely used CD4 counting technologies in Africa but requires a lymphocyte count from a haematological analyser to generate CD4 percentages.

Index test (Blantyre count)

We used venous blood from healthy adults anticoagulated with EDTA to develop our assay (see bmj.com for laboratory details). We calculated absolute CD4 counts (×10⁹/l) and CD4 percentage and assessed repeatability of our assay and examined stability of results over five days.

We modified our assay to reduce costs further when only an absolute CD4 count (Blantyre count



Comparison of CD4 counts determined with three flow cytometric methods using 96 blood samples from patients with HIV with CD4 counts <400×10⁹/l for absolute CD4 cell counts (×10⁹/l) and 129 blood samples for CD4 cell counts as a percentage of total lymphocyte count (CD4 percentage). FACSCount CD4 percentage was obtained from FACSCount absolute CD4 counts and total lymphocyte counts from a haematological analyser. Black lines depict bias and upper and lower limits of agreement. Grey broken lines denote 95% confidence intervals for these values

(absolute)) or percentage CD4 count (Blantyre count (percentage)) is required.

CD4 counting comparison studies

In the main CD4 counting comparison study we included consecutive blood samples from patients with HIV sent to our laboratory for full blood count and CD4 count determination from 27 January to 17 February and from 18 April to 9 May 2006 (n=134). We measured CD4 and CD4 percentage for each sample using Blantyre count, Blantyre count (absolute), TruCount, and FACSCount assays.

We carried out a smaller study on consecutive blood samples from patients with HIV sent to the laboratory in June 2006 to compare CD4 percentages generated by Blantyre count and Blantyre count (percentage) assays (n=28). Samples were not used if they exhibited clots, were sent from outside Queen Elizabeth Central Hospital, were received after the day of blood collection or if insufficient blood was available to complete all tests. There were no other selection criteria. All blood samples from all participants meeting the inclusion criteria underwent the index and reference standard tests.

Two authors performed and read the FACSCount assay and full blood count. Two other authors performed and read TruCount and Blantyre count assays together within six hours of the FACSCount assay. We subsequently trained local laboratory technicians over two to three days to perform the Blantyre count method. Manual gating of events acquired with Blantyre count was performed blind to other results. For absolute CD4 counts, we assessed agreement only for samples with a TruCount CD4 count below 400×10^9/l as this is the relevant range for clinical decision making. For comparisons of CD4 percentage we used all samples.

Clinical utility study

We tested a further 253 EDTA anticoagulated venous blood samples from new patients attending the adult antiretroviral therapy clinic from May to July and from September to October 2006 for CD4 count using Blantyre count. CD4 counts and clinical staging were compared for each patient. Clinical staff in the antiretroviral therapy clinic performed staging blind to CD4 count results.

External quality assurance

CD4 results were determined on six NEQAS (United Kingdom national external quality assessment scheme) stabilised blood samples from the UK between January and May 2006.

Statistical analysis

We examined agreement between each pair of methods by estimating bias and limits of agreement (=bias plus or minus 1.96×SD) with 95% confidence intervals. Repeatability was assessed with coefficients of variation obtained from five repeats of assays.

RESULTS

Refinement of Blantyre count—We used 20 µl blood and 10 µl of counting beads for our assay as twice the lowest volume at which optimal assay repeatability was maintained, and 0.5 µl of each antibody. See bmj.com. Using these quantities, the costs of reagents per assay were \$0.44 (£0.22, €0.33) for both absolute and percentage counts, \$0.40 for an absolute CD4 count, and \$0.11 for CD4 percentage alone. See bmj.com for characteristics of patients.

Absolute CD4 counts in agreement studies—The median CD4 count was 193×10^9/l (range 0 to 1884×10^9/l) with TruCount. The mean bias when we used Blantyre count rather than TruCount for samples with a CD4 count of <400×10^9/l was -11.0×10^9/l for Blantyre count. Similarly, low biases were found for other assay comparisons (table). Limits of agreement were −48.9 to 27.0×10^9/l for Blantyre count and TruCount and were within the range −55 to 40×10^9/l for all other assay comparisons (table, figure).

CD4 percentage in agreement studies—The median CD4 percentage was 13.0% (range 0.0-44.0%) using TruCount. Agreement between CD4 percentage

Estimated bias and limits of agreement, with 95% confidence intervals for pairs of flow cytometric methods used to measure absolute and percentage CD4 cell counts

Assay comparison	Bias (95% CI)	Limits of agreement: Lower limit (95% CI)	Limits of agreement: Upper limit (95% CI)
Absolute CD4			
Blantyre count and TruCount	−11.0 (−14.9 to −7.1)	−48.9 (−55.7 to −42.1)	27.0 (20.2 to 33.8)
FACSCount and TruCount	1.2 (−2.6 to 4.9)	−35.4 (−41.9 to −28.8)	37.7 (31.2 to 44.3)
Blantyre count and FACSCount	−12.1 (−16.6 to −7.7)	−54.8 (−62.4 to −47.1)	30.5 (22.8 to 38.1)
Blantyre count and Blantyre count (absolute)	−5.8 (−9.3 to −2.3)	−39.3 (−45.3 to −33.3)	27.7 (21.7 to 33.7)
CD4 percentage			
Blantyre count and TruCount	−0.03 (−0.24 to 0.19)	−2.42 (−2.78 to −2.05)	2.37 (2.00 to 2.73)
FACSCount* and TruCount	0.92 (0.32 to 1.52)	−5.83 (−6.87 to −4.79)	7.66 (6.62 to 8.70)
Blantyre count and FACSCount*	−0.94 (−1.53 to −0.35)	−7.56 (−8.57 to −6.54)	5.67 (4.66 to 6.69)
Blantyre count and Blantyre count (percentage)	0.01 (−0.26 to 0.28)	−1.35 (−1.82 to −0.88)	1.37 (0.90 to 1.84)

*CD4 percentages with FACSCount calculated from absolute CD4 count by FACSCount and total lymphocyte count from haematological analyser.

generated by Blantyre count and TruCount was excellent over the full range of values, with a bias of −0.03% and limits of agreement −2.42% to 2.37%. Comparison of either Blantyre count or TruCount CD4 percentage with values generated using FACSCount showed poorer agreement. Blantyre count and the Blantyre count (percentage) variant could be used interchangeably for CD4 percentage with excellent limits of agreements (table, figure).

Repeatability of Blantyre count—We calculated coefficients of variation on five repeats of our assay on four blood samples with mean CD4 values 718, 712, 260, and 191×10^9/l and mean CD4 percentage 40.3%, 42.9%, 15.0%, and 13.8%. Mean (SD) coefficients of variation were 5.2% (2.7%) for absolute CD4 count and 2.5% (0.8%) for CD4 percentage.

Accuracy of Blantyre count—In tests on six stabilised blood samples (CD4 count 117-1269×10^9/l and percentage 7.28%-60.73%) from NEQAS with our assay, five of six absolute CD4 counts and five of six CD4 percentages were within 1 SD of the NEQAS value, with one result of six between 1 and 2 SD of this value for each test. Blantyre count values were on average 95% of the absolute NEQAS CD4 count and 97% of the CD4 percentage.

Stability of aged samples—CD4 T cell and lymphocyte populations could be clearly distinguished and gated over the five days of the stability study, with a blood sample with day 1 CD4 count of 487×10^9/l and CD4 percentage of 36.1%. Daily coefficients of variation for absolute counts remained below 6% and for CD4 percentage below 2.5%. The mean absolute CD4 count stayed within 10% and the CD4 percentage within 5% of the day 1 values.

Clinical staging and CD4 counts for new patients attending antiretroviral therapy clinic—Of the new patients attending the antiretroviral therapy clinic, 76% (193/253) were clinical stage I (n=77) or stage II (n=116), while 24% (60/253) had stage III (n=51) or stage IV (n=9) HIV/AIDS. Twenty five (32%) patients with stage I disease and 48 (42%) with stage II disease had a CD4 count $<200\times10^9$/l. Eleven (22%) patients with stage III and one (11%) patient with stage IV HIV/AIDS had a CD4 count $>350\times10^9$/l.

DISCUSSION

Within Malawi, we have developed an affordable accurate method of counting CD4 cells with flow cytometry by refining and miniaturising existing technology. Increasing affordability by reducing reagent costs is a critical step in making this available in countries with limited resources. Currently the reagent cost of a comparable commercially available flow cytometric assay in Africa is $5.04 (£2.52, €3.74). As we were able to reduce costs of reagents to $0.44 (£0.22, €0.33) per assay, there is the potential for 91% cost savings. This would increase to 98% if only CD4 percentage is required but would decrease if the costs of competing tests are reduced.

Cost reduction was not achieved at the expense of accuracy. Over the CD4 count range of 0-400×10^9/l, our assay showed minimal bias and excellent agreement compared with established CD4 counting methods (TruCount and FACSCount). Determination of CD4 percentage by Blantyre count and TruCount methods showed excellent agreement over the full range of CD4 percentages. The good performance of Blantyre count in the NEQAS immunophenotyping scheme further shows the accuracy of this method.

As well as reducing the assay price, the modifications in our assay have simplified CD4 counting with flow cytometry and it has proved straightforward to train technicians.

Strengths and weakness of study

We carried out this work in a country where affordability is of chief importance. We looked at both absolute and percentage CD4, which have previously been neglected. The limits of agreement are similar to those of previous comparison studies of flow cytometry. By miniaturising the present assay, we managed to reduce reagent costs further compared with previous studies.

Even with the simplifications introduced, however, CD4 counting with flow cytometry requires a level of technical skill not always present in resource poor settings,[10] a reliable power supply, and a cold chain for reagent supplies. A flow cytometer represents a large capital outlay, although donor funding is sometimes available to help provide such instruments.

The simplified nature of the Blantyre count method means that this technology could be operated on less complex instruments than the FACSCalibur. Such an instrument could be manufactured at lower cost and would be simpler and less expensive to maintain.

Blantyre count could make the greatest impact on the care of children under 5 with HIV. Appropriate determination of CD4 percentage has often been neglected by investigators seeking to produce affordable CD4 counting.[13] Determination of CD4 percentage alone by the Blantyre count (percentage) variant is not only much cheaper than determining absolute CD4 counts but also technically easier and is much more accurate than using a FASCount instrument and haematological analyser. CD4 percentages were also more stable than absolute counts over five days in the same sample.

The determination of CD4 counts with Blantyre count in the antiretroviral therapy clinic confirms that use of WHO clinical staging criteria alone for deciding who should start antiretroviral therapy is suboptimal.

What would it cost?

Consideration of the economic feasibility of using the Blantyre count in Malawi has to include the capital cost of the flow cytometer (about $100 000), the annual service contract (about $10 000), and the salary of a laboratory technician (typical monthly salary $500) as well as reagent prices. Use of the Blantyre count method would be most cost effective with a limited number of flow cytometers operating at high sample throughput in regional centres and a coordinated system for transporting samples to these centres from peripheral clinics.

WHAT IS ALREADY KNOWN ON THIS TOPIC

CD4 counting is the main laboratory investigation for monitoring people with HIV but is often deemed too expensive and too complex to perform in resource poor settings

CD4 counting with flow cytometry can be made more affordable by the use of simple technical modifications, but CD4 percentages required in children under 5 years and miniaturisation of blood and reagent volumes have received little attention.

WHAT THIS STUDY ADDS

Technical modifications of flow cytometry with miniaturisation can simplify and reduce the cost of absolute and percentage CD4 counts while maintaining diagnostic accuracy

This CD4 counting method could improve clinical decision making in patients with HIV disease in settings with limited resources

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Body mass index cut offs to define thinness in children and adolescents: international survey

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ABSTRACT

Objective To determine cut offs to define thinness in children and adolescents, based on body mass index at age 18 years.

Design International survey of six large nationally representative cross sectional studies on growth.

Setting Brazil, Great Britain, Hong Kong, the Netherlands, Singapore, and the United States.

Subjects 97 876 males and 94 851 females from birth to 25 years.

Main outcome measure Body mass index (BMI, weight/height^2).

Results The World Health Organization defines grade 2 thinness in adults as BMI <17. This same cut off, applied to the six datasets at age 18 years, gave mean BMI close to a z score of −2 and 80% of the median. Thus it matches existing criteria for wasting in children based on weight for height. For each dataset, centile curves were drawn to pass through the cut off of BMI 17 at 18 years. The resulting curves were averaged to provide age and sex specific cut-off points from 2-18 years. Similar cut offs were derived based on BMI 16 and 18.5 at 18 years, together providing definitions of thinness grades 1, 2, and 3 in children and adolescents consistent with the WHO adult definitions.

Conclusions The proposed cut-off points should help to provide internationally comparable prevalence rates of thinness in children and adolescents.

INTRODUCTION

Much has been written about the epidemic of child obesity but malnutrition in infants, children, and adolescents poses a considerably larger public health problem internationally, and in the developed world anorexia nervosa is the third most common chronic condition of adolescence.[1] Obesity and malnutrition represent opposite extremes on the spectrum of adiposity, and both are routinely quantified in terms of weight and height relative to the child's age. Yet the classification of malnutrition in later childhood and adolescence is currently unsatisfactory because of the lack of suitable cut offs for international use.[2]

BMI has been used since the 1960s to assess obesity in adults and more recently in children. International BMI cut offs for child overweight and obesity, based on data from six countries, have been developed.[3] The