Evaluation of the Alere PIMA™ point-of-care CD4+ T-lymphocyte analyser compared to conventional laboratory testing, a literature review

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ABSTRACT

Human immunodeficiency virus (HIV) affects millions of people worldwide with the majority of those living in sub-Saharan Africa. The number of people receiving anti-retroviral therapy (ART) have gone up since ART has become more generic and cheaper but there are still a large number of patients eligible for ART that are not receiving it. The initiating of ART is based on the patient's absolute CD4+ T-lymphocyte count (CD4 count). This is usually measured on a flow cytometer, a method that is time consuming and requires laboratory infrastructure and trained technicians. In recent years several point-of-care tests for CD4 count have come on the market, one of those being the Alere PIMA™ CD4 analyser. It is the first point-of-care CD4 counter that does not utilise traditional flow cytometry methods. Ten articles were chosen to be in this literature review after a systematic search in the PubMed database, two other articles were chosen to be used for the background information. The objective of this literature review was to compile the results from these articles that evaluate the performance of the Alere PIMA™ point-of-care CD4 analyser compared to conventional laboratory based methods. The articles showed similar results with the Alere PIMA™ CD4 analyser and the average 95% limit of agreement for all articles was -211.3 to +228.4 CD4 cells/μl. Most articles agree that there needs to be more tests done on the Alere PIMA™ CD4 analyser but that it shows promising results for the future, and that the personnel collecting the samples need to have proper training in how to take capillary blood samples correctly.

Key words: HIV, CD4 count, PIMA, point-of-care
SAMMANFATTNING

Utvärdering av Alere PIMA™ patientnära CD4 T-lymfocyt räknare jämfört med konventionell laboratoriebaserad analys, en litteraturstudie

Humant immunbristvirus (HIV) drabbar miljoner människor världen över men majoriteten av de som drabblas bor i sub-Saharan Afrika. Antalet människor som lider av HIV och som står på anti-retroviral behandling (ART) har ökat sen medicinen blivit mer generisk och billigare men det är fortfarande ett stort antal människor som uppfyller kriterierna för att få ART men som ännu inte får det. När man påbörjar ART baseras på mängden CD4+ T-lymfocyter (CD4 antal) som patienten har i sitt blod och för att mäta detta så använder man vanligtvis flödescyometri, en metod som tar lång tid och kräver tillgång till fungerande laboratorier och utbildad personal. De senaste åren så har flera patientnära apparater för att analysera CD4 antalet kommit ut på marknaden och en av dessa är Alere PIMA™ CD4 analyser, det är det första patientnära CD4 cellräkningstestet som inte är baserat på traditionell flödescyometri. Tio artiklar valdes ut för att vara med i den här litteraturstudien efter en systematisk sökning i PubMed databasen och ytterligare två artiklar valdes för att användas i bakgrunden. Syftet med denna litteraturstudie är att sammanställa resultaten från dessa artiklar. Artiklarna har alla fått liknande resultat och medelvärdet för 95% limit of agreement var för alla artiklar -211.3 till +228.4 CD4 celler/μl. De flesta artiklar kommer överens om att det behövs göra mera tester på apparaten men att den visar lovande resultat för framtiden. Många artiklar anser även att personalen som samlar in kapillär proverna från patienterna behöver få ordentlig utbildning i hur man tar kapillärblodprov på korrekt sätt för att analyserna sedan ska kunna ge korrekta resultat.

Nyckelord: HIV, CD4 count, PIMA, point-of-care
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INTRODUCTION

Human immunodeficiency virus (HIV) is a deadly virus that can infect people through sexual intercourse, via blood or via mother-to-child transmission. Over 33 million people are living with HIV in the world today. Two thirds of those people live in the sub-Saharan Africa area. In the most recent years anti retro-viral therapy (ART), which is a treatment for HIV, has become cheaper and more generic and therefore available to more people. Especially poorer people living in rural parts of the world. The number of people receiving ART in low and middle income countries has increased substantially from 300 000 people in 2002 to 9.7 million in 2012. Although the number of people receiving ART has increased there are still millions of patients in the need of ART but aren't receiving it yet (1).

The human immunodeficiency virus attacks the immune system, specifically the CD4+ t-lymphocytes (T-helper cells) of the host. As time goes by it makes the immune system so weak that it can't defend itself against common diseases that healthy people would have little to no problem with, so called opportunistic diseases. A common way of tracking the HIV infection and to see how far it has gotten in destroying the host immune system is to measure the absolute number of CD4+ T-lymphocytes (also called CD4 count) in the blood of the infected individual. The absolute CD4 count is also used to determine whether or not a person shall receive anti retro-viral therapy (ART) and to monitor the progression of treatment. According to the World Health Organisation (WHO), the guideline threshold set in 2010 was to initiate ART if the patient had less than 350 CD4 cells/µl. The 2013 guidelines however, suggest that ART should be initiated when the patient has a CD4 count of less than 500 CD4 cells/µl (1,2).

The standard method for CD4 counting today is by using fluorescence-activated cell sorting flow cytometry such as the BD FACSCalibur or BD FACSCount (BD biosciences, San Jose, United States of America). A method that requires a lot of resources, time and access to well established laboratory infrastructure. This means that patients that live in rural areas where there is no access to any laboratory that can provide CD4 counting either have to have their samples transported to a laboratory, which can be very difficult due to hot temperatures and bad roads, or the patient has to
travel to a laboratory, which isn't an option for many patients that live in rural parts of the world. This means that these patients often don't get started on ART even though they are eligible for it (3).

An option to laboratory based flow cytometry is point-of-care CD4 counting instruments. That would be instruments that can be used outside of laboratory settings and by people that aren't trained laboratory technicians. These instruments could increase the number of people that get started on ART and help prevent mother-to-child transmission of HIV (3).

One point-of-care CD4 counting instrument that came on the market in late 2009 is the Alere PIMA™ CD4 T-lymphocyte analyser (Alere™, Los Angeles, United States). It is the first instrument available for CD4 counting that isn't based on traditional flow cytometry, instead it uses dual-fluorescence image analysis. This is a method based on static cytometry (image 1). In this method it uses labelled anti-hCD3 and anti-hCD4 antibodies (4,5).

It is a portable, lightweight, rechargeable instrument that uses cartridges with all the necessary reagents. It can analyse both venous and finger-prick blood and can be used in temperatures up to 40°C. When the analysis is complete the result shows up on the display of the machine, both as absolute CD4 count and as CD3+/CD4+ ratio. Quality
control cartridges comes with the machine which are intended for daily use, to assure the accuracy of the instrument (5).

![Diagram of FACSCalibur flow cytometer](http://wwwbdbiosciences.com/instruments/facscalibur/features/index.jsp)

Abbreviations: FSC forward scatter; SSC side scatter

Figure 2: An overview of the methodology used in the FACSCalibur flow cytometer (Available from http://wwwbdbiosciences.com/instruments/facscalibur/features/index.jsp).

The PIMA analyser is based on image cytometry which differs from flow cytometry in the way that it uses a static image instead of a flow of cells (figure 1). The PIMA utilises fluorescence marked anti-hCD3 and anti-hCD4 antibodies to count the cells and to calculate the absolute number of CD4+ cells (4, 6). In traditional flow cytometry the cells pass a laser in a flow as can be seen in figure 1, the laser hits the cells and the instrument measures side scatter and forward scatter. This gives the size and granularity of the cell. In fluorescence-activated cell sorting the cells are also marked with fluorescent dyed antibodies directed at different surface proteins on the cells which give the option of sorting the cells based on the different surface proteins expressed, this is illustrated in figure 2. This methodology requires larger amounts of the sample and the machines used are big and require constant electricity and skilled, trained laboratory technicians to operate them. The technology that the PIMA analyser is based on allows
it to be compact and to be handled by pretty much anyone. The PIMA is based on static imaging particle analysis which was originally performed manually in a microscope (figure 1). The method counts the number of CD3 and CD4 cells using anti-hCD3 and anti-hCD4 antibodies that has been marked with a fluorochrome. The static part means that it counts the cells by taking a photo of one visual field at a time and the machine then counts the number of cells and calculates the absolute CD4 count (7-9). The PIMA analyser comes with special cartridges that contain all the necessary reagents. The person operating the machine simply has to add capillary or venous blood to the cartridge, and insert it in to the machine which delivers an answer of the absolute CD4 count in approximately 20 minutes. This means that the patient can get their result quickly, and does not have to return later for their result as they would have to do if they got their CD4 count by regular flow cytometry (6).

The Alere PIMA™ analyser is designed to work in field settings using capillary blood. It is easy to use which means it can be handled by personnel that do not have extensive laboratory training. This gives the PIMA™ analyser all the abilities it needs to be a good option for CD4 counting in rural areas where traditional flow cytometry isn't available (5).

AIM
To compile the results from different scientific articles that evaluate the performance of the Alere PIMA™ point-of-care CD4 analyser in HIV-positive patients compared to conventional laboratory based methods. And determine if the Alere PIMA™ point-of-care CD4 analyser is a good substitute for conventional laboratory testing in rural areas where laboratory infrastructure is unavailable.
METHODS

Criteria of the systematic literature search
All articles were found in the NCBI - PubMed database by using the criteria that are described in Table 1. The articles were then chosen based on abstract and after that selection, all articles were read and the relevant ones where included in this literature review. Ten articles were chosen to be a part of the review while two others were chosen to be used for background facts about point-of-care testing and CD4 counts.

Table 1: Summary of results by systematic literature search

<table>
<thead>
<tr>
<th>Database</th>
<th>Search words</th>
<th>Search Criteria</th>
<th>Results</th>
<th>Selection criteria</th>
<th>Selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCBI - PubMed</td>
<td>HIV CD4 PIMA</td>
<td>Human Free full text available No more than 5 years old</td>
<td>6</td>
<td>Selected based on abstract</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>PIMA point of care HIV</td>
<td>Human Full text available No more than 10 years old</td>
<td>13</td>
<td>Selected based on abstract</td>
<td>6</td>
</tr>
</tbody>
</table>

The articles that were chosen matched the criteria for this literature review. They were all articles that evaluated the performance of the Alere PIMA™ point-of-care CD4+ analyser. The articles that weren't chosen did not focus on the subject that this literature review covers.

Ethics considerations
All articles that were used in this literature review were ethically approved by authorities in their respective countries and all studies were performed on voluntary patients.

RESULTS
Ten articles about the reliability and performance of the Alere PIMA™ CD4 analyser have been read and reviewed and the results will be presented here. Eight out of the ten studies were performed in sub-Saharan Africa while the other two were performed in Thailand and the United Kingdom as can be seen in table 2. The study by Jani et al. (10)
was performed in Mozambique.

All ten articles used either capillary blood or both capillary and venous blood in their studies and in this review the focus is going to be on the capillary finger-prick analysis and not the venous blood.

The reference method used in all articles was laboratory based standardised flow cytometry of venous blood samples performed by trained laboratory technicians at a hospital where such technology was available.

Table 2: The articles used in this review and some basic facts

<table>
<thead>
<tr>
<th>Article</th>
<th>Number of participants</th>
<th>Place where the study was performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jani et al., 2011</td>
<td>n=697</td>
<td>Mozambique</td>
</tr>
<tr>
<td>Mtapuri-Zinyowere et al., 2010</td>
<td>n=165</td>
<td>Zimbabwe</td>
</tr>
<tr>
<td>Alassane Diaw et al., 2011</td>
<td>n=300</td>
<td>Senegal</td>
</tr>
<tr>
<td>Mwau et al., 2013</td>
<td>n=1,549</td>
<td>Kenya</td>
</tr>
<tr>
<td>Sukapirom et al., 2011</td>
<td>n=203</td>
<td>Thailand</td>
</tr>
<tr>
<td>Manabe et al., 2012</td>
<td>n=206</td>
<td>Uganda</td>
</tr>
<tr>
<td>Glencross et al., 2012</td>
<td>n=96</td>
<td>South Africa</td>
</tr>
<tr>
<td>Mnyani et al., 2012</td>
<td>n=296</td>
<td>South Africa¹</td>
</tr>
<tr>
<td>Myer et al., 2013</td>
<td>n=521</td>
<td>South Africa¹</td>
</tr>
<tr>
<td>Herbert et al., 2012</td>
<td>n=269</td>
<td>United Kingdom</td>
</tr>
</tbody>
</table>

¹ Performed on pregnant women

The number of participants in the studies varied from 96 to 1,549 patients with the majority of the studies having somewhere in between 200 and 300 patients. As seen in table 2, two of the studies were performed on pregnant women, in this review they are compared to all the other results equally when evaluation the results. The two studies performed on pregnant women got varied result. The study performed by Mnyani et al., 2012 (17) showed that there was no difference in the mean bias of the PIMA at different gestational ages while the one by Myer et al., 2013 (18) showed a tendency to underestimate the number of CD4 cells more in women that had gone longer in their pregnancy.
### Table 3: Summary of statistical data of the articles used in this review

<table>
<thead>
<tr>
<th>Article</th>
<th>Median absolute CD4 count (reference)</th>
<th>Median absolute CD4 count (PIMA)</th>
<th>Mean Bias(^2)</th>
<th>95% Limits of agreement (LOA)(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jani et al., 2011</td>
<td>376 cells/μl</td>
<td>318.5 cells/μl</td>
<td>-57.6 cells/μl</td>
<td>-250.9 to +145.2</td>
</tr>
<tr>
<td>Mtapuri-Zinyowere et al., 2010</td>
<td>266.5 cells/μl</td>
<td>234.1 cells/μl</td>
<td>+7.6 cells/μl</td>
<td>-173.8 to +189.0</td>
</tr>
<tr>
<td>Alassane Diaw et al., 2011</td>
<td>364.0 cells/μl</td>
<td>313.0 cells/μl</td>
<td>-39.0 cells/μl</td>
<td>-258.0 to +179.0</td>
</tr>
<tr>
<td>Mwau et al., 2013</td>
<td>-</td>
<td>-</td>
<td>+8.6 cells/μl</td>
<td>-235.4 to +252.7</td>
</tr>
<tr>
<td>Sukapirom et al., 2011</td>
<td>500.0 cells/μl</td>
<td>446.0 cells/μl</td>
<td>+4.5 cells/μl</td>
<td>-173.4 to +164.3</td>
</tr>
<tr>
<td>Manabe et al., 2012</td>
<td>396.0 cells/μl</td>
<td>329.7 cells/μl</td>
<td>-66.3 cells/μl</td>
<td>Not available</td>
</tr>
<tr>
<td>Glencross et al., 2012</td>
<td>617.5 cells/μl</td>
<td>719.4 cells/μl</td>
<td>+105.7 cells/μl</td>
<td>-336.0 to +547.0</td>
</tr>
<tr>
<td>Mnyani et al., 2012</td>
<td>367.0 cells/μl</td>
<td>352.0 cells/μl</td>
<td>+20.5 cells/μl</td>
<td>-133.9 to +175.0</td>
</tr>
<tr>
<td>Myer et al., 2013</td>
<td>402.0 cells/μl</td>
<td>388.0 cells/μl</td>
<td>+22.7 cells/μl</td>
<td>-129.2 to +174.6</td>
</tr>
<tr>
<td>Herbert et al., 2012</td>
<td>450.0 cells /μl</td>
<td>391.5 cells/μl</td>
<td>-58.5 cells/μl</td>
<td>Not available</td>
</tr>
</tbody>
</table>

\(^1\)The LOA means that 95% of all patients in the study got results that were inside of the given parameters compared to their personal reference flow cytometry blood sample.

\(^2\)The mean bias is the average number of CD4 cells that the PIMA analyser results varied from the reference flow cytometry results.

As can be seen in table 3, most articles reported their 95% Limits of agreements. The mean 95% limits of agreement for the articles where this was available was -211.3 to +228.4 cells/μl.

The articles got varied results (table 3) and the mean bias for all articles varied from -66.3 cells/μl to +105.7 cells/μl with a mean for all articles of -60.8 cells/μl.
Table 4: Percentage of patients misclassified below and above the threshold

<table>
<thead>
<tr>
<th>Article</th>
<th>Misclassification in favour of treatment, misclassified as below the threshold of 350 cells/μl</th>
<th>Misclassified not in favour of treatment, misclassified as above the threshold of 350 cells/μl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jani et al., 2011</td>
<td>14.8%</td>
<td>2.2%</td>
</tr>
<tr>
<td>Mtapuri-Zinyowere et al., 2010</td>
<td>2.4%</td>
<td>4.2%</td>
</tr>
<tr>
<td>Alassane Diaw et al., 2011</td>
<td>20.0%</td>
<td>9.0%</td>
</tr>
<tr>
<td>Mwau et al., 2013</td>
<td>16.6%</td>
<td>20.6%</td>
</tr>
<tr>
<td>Sukapirom et al., 2011</td>
<td>Not available</td>
<td>Not available</td>
</tr>
<tr>
<td>Manabe et al., 2012</td>
<td>20.5%</td>
<td>6.8%</td>
</tr>
<tr>
<td>Glencross et al., 2012</td>
<td>0.0%</td>
<td>31.0%</td>
</tr>
<tr>
<td>Mnyani et al., 2012</td>
<td>7.0%</td>
<td>7.0%</td>
</tr>
<tr>
<td>Myer et al., 2013</td>
<td>11.0%</td>
<td>8.0%</td>
</tr>
<tr>
<td>Herbert et al., 2012</td>
<td>12.0%</td>
<td>5.2%</td>
</tr>
</tbody>
</table>

¹ Used a limit of <300 cells/μl

The misclassification of patients can be seen in table 4. The patients misclassified below the limit were patients who had more than 350 CD4 cells/μl in their reference sample but less than 350 CD4 cells/μl on the PIMA™ analyser. The patients misclassified above the limit were patients who had less than 350 CD4 cells/μl in their reference sample but got a result on the PIMA™ analyser that was higher than 350 CD4 cells/μl.

For all articles an average of 11.6% of patients were misclassified below the limit and in favour of treatment and 10.4% of all patients were misclassified above the limit and wouldn't receive treatment if based on the PIMA™ result. Most articles agree on the misclassification of patients as acceptable apart from the articles by Mwau et al., 2013 (13) and Glencross et al., 2012 (16) that got significantly different results compared to the other articles and therefore did not agree on the misclassification being acceptable.
All studies were performed in field settings. Some articles had laboratory technicians perform the tests on the PIMA™ analyser and some had nurses or other personnel perform them. One article, the one by Jani et al., 2011 (10) had nurses and laboratory technicians perform the tests to compare if there were any differences between their results. The difference in mean bias between the nurses and the laboratory technicians was 9.5 CD4 cells/μl. The nurses mean bias was -52.8 CD4 cells/μl while the technicians was -62.3 CD4 cells/μl.

In this review, the focus is on how the PIMA performs when using capillary finger-prick blood, although several articles have evaluated it using both capillary and venous blood. In the article by Jani et al., 2011 (10) they found that the mean bias between capillary and venous blood was -9.0 CD4 cells/μl, while in the article by Alassane Diaw et al., 2011 (12) they got results that weren't normal when comparing the two and could therefore not calculate an accurate mean bias. The study performed by Mwau et al., 2013 (13) got a mean bias between capillary and venous blood of -7.7 CD4 cells/μl, which is similar to the one that Jani et al., 2011 (10) got.

A large number of articles saw that the PIMA consistently underestimates the CD4 count compared to laboratory testing. Several of the articles including the ones by Jani et al., 2011 (10), Alassane Diaw et al., 2011 (12), Sukapirom et al., 2011 (14) and Manabe et al., 2012 (15), also mention getting results where the PIMA underestimates the absolute number of CD4 cells more in patients with higher absolute CD4 counts than in patients with lower counts.

DISCUSSION
This literature reviews purpose is to evaluate the performance of the PIMA™ analyser in regards to the initiation and monitoring of ART in HIV positive patients. For this purpose I believe that the misclassification of patients is the most important aspect to consider since this is what decides whether or not a patient will receive ART. As one can see in table 4, the number of patients misclassified varies between the articles and this could be because of a number of factors. Two of the factors that play a role are the number of patients participating in the study and the mean absolute CD4 count of those
patients. As many of the studies have come to the conclusion that on patients with higher absolute CD4 counts the PIMA™ analyser tends to underestimate the number of CD4 cells more. The variation in results could also be because of variation in the quality of the capillary sample collection.

In the study performed by Glencross et al., 2012 (16) they got a much larger 95% LOA span than any of the other articles. This could have many different explanations one being the relatively small number of participants in the study. Another being that they performed it under poorer circumstances than any of the other studies and that the results somehow suffered because of it. Even taking in Glencross et al., 2012 (16) the mean 95% LOA for all articles is about +/−200 CD4 cells/μl. This is more than one would have hoped for, but the results are still informative for evaluating the Alere PIMA™ CD4 analyser.

Many of the articles have also got the result that the PIMA tends to underestimate the number of CD4 cells more in patients with higher CD4 counts than in patients with lower counts. When the threshold for initiating ART is 350 CD4 cells/μl it does not play as big a role as it would do if the higher threshold of 500 CD4 cells/μl, which was suggested by the WHO in 2013, was used. And underestimating the CD4 count will give more people ART than need it which I consider better than the opposite. But of course more people receiving ART costs more money, and in some places in the world that would be a huge waste of resources.

All articles, apart from the one by Glencross et al., 2012 (16), have come to the conclusion that the results of the PIMA compared to laboratory based methods show promise and that the PIMA is a good point-of-care option for CD4 counting. Most articles agree that the PIMA tends to underestimate the CD4 count and that this might be a thing that the machine always does, which in that case would make it reproducible. I believe that the PIMA is an excellent option to use in rural areas as it is small, easy to use and relatively cheap. This would benefit a lot of HIV-positive patients as they would have access to CD4 counting close to where they live and could obtain the results quickly and conveniently. I know the PIMA isn't completely in line with the reference
method but I believe that it should be taken to use in rural areas as it might be the only option for a large number of patients. The performance of the PIMA is relatively steady yet a few variables are seen. The skill and knowledge of the person collecting the capillary blood sample is one thing that could affect the results. As is the temperature at which the cartridges are stored. To be able to assure the best quality, it would be of great help if personnel operating the PIMA received basic training in how to properly collect a capillary blood sample.

In 2013, the WHO released new guidelines that suggest ART should be started when the patients have less than 500 CD4 cells/μl. Following these guidelines would increase the number of patients receiving ART and therefore also increase the number of patients in need of treatment monitoring and regular CD4 counting. This would most likely also mean that an instrument like the PIMA analyser would be of even greater help as it could relieve some of the workload otherwise put solely on the laboratories. And increase access to CD4 counting in rural areas. It is therefore of utmost importance to do more testing on the PIMA so that it can be used in routine analysis around the world as soon as possible.

**CONCLUSION**

The Alere PIMA™ CD4 analyser shows promising results and is an interesting option for CD4 counting in the future. It could potentially decrease the number of patients that are lost to follow up after their HIV diagnosis substantially. And give HIV-positive patients living in rural areas access to CD4 cell counting and therefore also give them access to ART. But before one can say that the Alere PIMA™ CD4 analyser is an adequate substitute to conventional laboratory based flow cytometry there needs to be more testing done. In those cases that the machine is to be used the person operating it needs to have had proper training in how to collect a capillary blood sample correctly.
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