Author’s response to reviews

Title: Field evaluation of community usage of CD4 T lymphocytes counting by alternative single-platform flow cytometry in Chad

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Version: 2 Date: 24 April 2013

Author’s response to reviews: see over
To Editors of BMC Health Services Research
RE: Submission of revised manuscript.

N'Djamena, April the 25th 2013

Dear Editor,

Please find to your attention the revised version of the manuscript 1675375457824822 entitled: “Field evaluation of community usage of CD4 T lymphocytes counting by alternative single-platform flow cytometry in Chad”, by Koyalta and colleagues.

Thank you for willing to consider a revised version of our manuscript.

Thank you also for the comments from the Reviewers. We have responded to them on a point-to-point basis below. These comments mainly of technical nature have improved the paper, and likely helped us to better understand the scientific issues that the paper brings out. We hope that is reflected in the revised paper we have now submitted. Note that the full remarks of reviewer#1 were taken late into consideration, because of atypical presentation within the manuscript itself.

We trust that the revisions we have made, and our point-by-point responses, deal with any remaining concerns and that this manuscript is now suitable for publication in BMC Health Services Research.

Thank you very much for considering our manuscript.

Yours sincerely,

Dr Donato Koyalta
Answer to reviewers

Reviewer #1

Reviewer's report
Title: Field evaluation of the Chad national implementation of single-platform, volumetric, CD45-assisted pan-leucogating flow cytometry for community-based CD4 T lymphocytes monitoring in adults and children infected with HIV: Implications for decentralization of CD4 testing in resource-limited settings
Version: 2 Date: 11 February 2013
Reviewer: PAPA ALASSANE A DIAW
Reviewer's report:
No data regarding the evaluation as indicated in the title.
maybe you could change the title
Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests:
'I declare that I have no competing interests' below

We thank very much the above reviewer to consider our study “of importance in the field”. The main criticism of the reviewer concerns the title of the paper. To acknowledge the reviewer’s remark, we have changed the title as follows: "Field evaluation of community usage of CD4 T lymphocytes counting by alternative single-platform flow cytometry in Chad”.

Other comments presented within the manuscript itself

Abstract:

Methods: The methods does not match with the tilte. describ briefly How did you proceed to make the evaluation

To acknowledge the reviewer’s remark, we have added the in the methods section of the abstract, the following explanations: “For each Auto40 flow cytometer installed in Chad, the followings items were recorded : i) Location, environment, and level of each laboratory; ii) Provision of electricity and water; iii) Availability of human resources; iv) Reagents and consumables supplies; v) Medical activity and CD4 T cell enumeration cost in each site; vi) Quality assurance and control; vii) Instrument support. In addition, an open questionnaire was carried out in each study laboratory.”

Results: Give some results like correlation, difference plot, similarité or concordance etc...
The aim of our study is not to make comparison with a reference flow cytometry method, but to evaluate in the field the principal issues raised by the implementation of the single-platform, volumetric, Auto40 flow cytometer in 8 community HIV monitoring laboratories of different levels throughout Chad, a country with particularly difficult conditions, both the climate and the territorial extent, making the decentralization of the therapeutic management of HIV-infected patients challenging. We have largely quoted in the manuscript the previous evaluations of the Auto40, as follows in the introduction section:

“The Auto40 analyzer was initially intended for CD4 T cell enumeration based on primary CD4 gating, and has been validated in Senegal for measurement of absolute CD4 T cell count by reference to the FACSCount system [10].”

And

“The updated version of the Auto40 analyzer has been recently validated in Cameroon for CD4 T cell counting both in absolute number and in percentage [20, 21], and is therefore addressing the current WHO recommendations for CD4 T cell measurements in children less than 5 years [3].”

Introduction:

Page 4, first paragraph (highlighted in yellow): these sentences are not the part of objective

To acknowledge the reviewer’s remark, we have deleted these sentences.

Material and methods:

Page 5, 4th paragraph (Due to the stability…): no data about the stability

To acknowledge the reviewer’s remark, we have deleted the followings words: “Due to the stability of its optical bench,“.

Page 6, 2th paragraph (Therefore, 8 Auto40 flow cytometers (Apogee Flow Systems Ltd) were acquired at the price of 23,500 € per analyzer…): commercial issues without scientific interest

To acknowledge the reviewer’s remark, we have deleted price of the analyzer.

Page 6, 3th paragraph (the whole paragraph highlighted in yellow) : Commercial issues..
Page 6, 4th paragraph (at the end of this paragraph): What about the reference method used to evaluate the A40

The aim of our study is not to make comparison with a reference flow cytometry method, but to evaluate in the field the principal issues raised by the implementation of the single-platform, volumetric, Auto40 flow cytometer in 8 community HIV monitoring laboratories of different levels throughout Chad, a country with particularly difficult conditions, both the climate and the territorial extent, making the decentralization of the therapeutic management of HIV-infected patients challenging. As previously explained, we have largely quoted in the manuscript the previous evaluations of the Auto40, as follows in the introduction section.

Results:

Page 7, at the end of 1th paragraph (since the cassette fluid recycling drive costs 921 €...): Commercial issues...

To acknowledge the reviewer’s remark, we have deleted the commercial issues of these sentences.

Page 8, at the end of the last paragraph: What about the results of the evaluation: concordance, accuracy, sensibility, similarity etc... as indicated the first objective is to assess the performance of the A40 method

The aim of our study is not to make comparison with a reference flow cytometry method, but to evaluate in the field the principal issues raised by the implementation of the single-platform, volumetric, Auto40 flow cytometer in 8 community HIV monitoring laboratories of different levels throughout Chad.

Discussion:

Page 11, at the end of 2th paragraph: more commercial comments than scientific discussion regarding the evaluation as indicated in the title. Maintenance issues were not well discussed.

The title has been changed, as recommended. We have discussed the maintenance issues in the Discussion section, as follows: “Routine maintenance recommended by the manufacturer was performed regularly, as well as the contractual annual maintenance operated by the technician of the distributor coming from France. Although expensive, the required maintenance has been properly carried out by the national
AIDS program allowing an excellent operation of the entire park of Auto40 flow cytometers."

**Conclusion:**

*Page 11, 3th paragraph (our observations point that a CD4 T cell analyzer should be chosen carefully...) : give results in CHAD. What about the response of your first objective.*

We have yet answered to this reviewer’s concern.

*Page 12, line 2: No significant responses regarding the first objective of the study. No external quality control was performed. It would be very important to confirm the reliability of the method by an EQA.*

The reviewer is right. International nor national EEQ is not yet available in Chad.
Reviewer #2

Reviewer's report

Title: Field evaluation of the Chad national implementation of single-platform, volumetric, CD45-assisted pan-leucogating flow cytometry for community-based CD4 T lymphocytes monitoring in adults and children infected with HIV: Implications for decentralization of CD4 testing in resource-limited settings

Version: 2 Date: 11 February 2013
Reviewer: David Barnett

Reviewer's report:

Firstly, many thanks to the authors for submitting this manuscript. The manuscript describes a field and community evaluation of the Auto40 mini flow cytometer. Whilst this is an interesting report it does not have any scientific cross comparison with other techniques and therefore cannot be assessed on the basis of science alone. I cannot decipher if this is just a “report” on the reliability and robustness of the instrument or a true scientific evaluation of the instrument, that should involve instrument to instrument comparison. If it is the former, which I think it is, then this (in my view) limits it as a frank scientific publication. Yes there is a lot of valuable information within the manuscript that describes the cost and laboratory characteristics but this should also be underpinned by the science. The reader will need to know how the CD4 counts compare to other techniques available. Thus, their statements at the end of the abstract may require rewording because they cannot state it is a trustable CD4 measurement method unless comparison of CD4 counts to a predicate instrument has been undertaken. One other aspect that the authors may wish to consider is the use of the term Pan-leucogating. To my knowledge Dr Debbie Glencross patented this approach (prior to the original publication in Cytometry 2002) and the approach they describe is NOT the pan-leucogating approach as originally described and therefore this may raise some legal issues. The authors should check this out with the manufacturer as I would not wish them to fall foul of any patenting issues (note I am not on the patent and have no commercial interest in pan-leucogating).

Level of interest: An article of limited interest

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests

We thank very much the above reviewer for its encouragement to submit our study, to consider that our study contains “a lot of valuable informations”. We feel our study in a fully scientific study, because our approach to collect information is based on an objective questionnaire, and the interpretation of the results is rational, impartial and unbiased. We do agree that our study is not based on the pathophysiological approach developed by Claude Bernard, and that there is basically no “control group”. This field evaluation however is original by its design (a study carried out at the scale of a whole
country), and by the scope of its conclusions interesting not only Chad and its 10 millions of inhabitants, but also all sub-saharan African countries where the HIV epidemic is highly prevalent and which are concerned by the immunological monitoring of their HIV-infected patients and the hot problem of decentralization in favor of resource-constrained populations.

**Point 1.** The reader will need to know how the CD4 counts compare to other techniques available. Thus, their statements at the end of the abstract may require rewording because they cannot state it is a trustable CD4 measurement method unless comparison of CD4 counts to a predicate instrument has been undertaken.

The CD4 T cells counting by Auto40 flow cytometer has been yet evaluated in prior studies, such as Dakar, Senegal [Dieye et al., 2011], and Yaoundé, Cameroon [Mbopi-Keou et al., 2012], by comparison to reference flow cytometers (such as FACSCalibur). These previous validations of the Auto40 flow cytometer are quoted in the text.

To acknowledge the reviewer’s concern, we have re-written the conclusions mainly in the abstract and discussion, as follows: “The Chad experience on the Auto40 flow cytometer indicates that its usage of in resource-limited settings should be mainly reserved to reference (level 1) or district (level 2) laboratories, rather than to laboratories of health care centres (level 3).”

**Point 2.** One other aspect that the authors may wish to consider is the use of the term Pan-leucogating. To my knowledge Dr Debbie Glencross patented this approach (prior to the original publication in Cytometry 2002) and the approach they describe is NOT the pan-leucogating approach as originally described and therefore this may raise some legal issues. The authors should check this out with the manufacturer as I would not wish them to fall foul of any patenting issues (note I am not on the patent and have no commercial interest in pan-leucogating).

To acknowledge the reviewer’s remark, we have deleted the term “pan-leucogating” thorough the manuscript, and kept the adequate references.

**Point 3.** Quality of written English: Not suitable for publication unless extensively edited

The manuscript has been corrected by a native English-speaking researcher of our lab.