Author's response to reviews

Title: Improved Sensitivity of an Interferon-Gamma Release Assay (T-SPOT.TB) in Combination with Tuberculin Skin Test for the Diagnosis of Latent Tuberculosis in the Presence of HIV Infection

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Author's response to reviews: see over
Improved Sensitivity of an Interferon-Gamma Release Assay (T-SPOT.TB) in Combination with Tuberculin Skin Test for the Diagnosis of Latent Tuberculosis in the Presence of HIV Co-Infection, by L. Elzi et al.

Dear Editor,

We thank very much for the very valuable comments and suggestions. Please find below our response to all raised concerns. All changes in the manuscript have been highlighted in yellow.

Reply to the Editor

- Editor: “Please add in the discussion, that we need better tools to detect latent tuberculosis. Among potential new strategies, the detection of IP-10 can be a useful tool.”
  
  - Reply: We appreciate this comment, and highlighted (page 14, discussion) the need of better tools such as IP-10 to detected latent tuberculosis. We also added the suggested references.

Reviewer 1

Major comments:

- Reviewer: “The purpose of this study in clinical terms needs to be more clearly defined.”
  
  - Reply: The purpose of this study was to identify HIV-infected individuals with latent tuberculosis, who qualify for preventive treatment in a country with low incidence rates of tuberculosis. As all patients included in our study developed active culture-confirmed tuberculosis within 6 months after enrolment in the Swiss HIV Cohort Study, we deduced that they had latent tuberculosis when they were enrolled in the Swiss HIV Cohort Study and were sampled. We specified the aim of our study in the introduction (page 5).

- Reviewer: “. the samples need to be tested fresh at the point of HIV infection.”
  
  - Reply: We agree with this comment that T-SPOT.TB is recommended to be performed with fresh blood. However, viability of frozen lymphocytes was checked meticulously, and the procedure of collection, freezing and thawing cells was
validated in several studies of the Swiss HIV Cohort Study (Khanna et al., J Virol 2009; 83:4404-11; Gasser et al., PLoS Med 2007; 4:e96; Rohrbach et al., Gut 2010, 59:1252-59). Moreover, T.SPOT.TB was always performed together with mitogens and nil controls, as described in the method section. We added these references in the methods section (page 7) and acknowledged this limitation in the discussion (page 14).

- Due to the very low incidence rate of tuberculosis in Switzerland (the incidence of tuberculosis was estimated to be 0.9% in a previous study of the Swiss HIV Cohort Study – Elzi et al, Clin Infect Dis 2007) we would have needed to test T-SPOT.TB in at least 6,400 HIV-infected patients enrolled in the Swiss HIV Cohort Study using fresh blood cells. Since half of patients with latent tuberculosis would have a positive skin test and therefore qualify for preventive chemotherapy, a sample size of about 14,000 patients would have led to approximately 60 patients developing active tuberculosis. Such a study would be very difficult to perform in Switzerland, where 600-700 HIV infected individuals are enrolled in the Swiss HIV Cohort Study each year. However, our results should be confirmed by other studies. This comment was added in the discussion (page 14).

- **Reviewer:** “…it would be helpful to define whether repeated testing is of value”; …

- **Reply:** We agree with the reviewer that repeating T-SPOT.TB sequentially in patients with initial negative results would be a very interesting research question. However, according to the protocol of the Swiss HIV Cohort Study, viable blood mononuclear cells are routinely stored at enrolment in the Swiss HIV Cohort Study and only once a year later. For this reason, further cells samples within 6 months after enrolment in the Swiss HIV Cohort Study were not available, and therefore we were not able to repeat the test at later time points before culture-confirmed active tuberculosis was diagnosed. We included a comment in the discussion (page 13).

- **Reviewer:** “…the reason for negative tests needs further assessment, e.g. by measurement of T regulatory cells …”

- **Reply:** As reported in the discussion (page 12), in a country with low incidence rates of tuberculosis, it is unlikely that our patients with initial negative T-SPOT.TB or tuberculin skin test have been infected after enrolment in the Swiss HIV Cohort Study, developing active tuberculosis in a short period of time, i.e. 6 months after study entry. However, it might be possible that false negative T-SPOT.TB results rely on increasing immunodeficiency resulting from acute tuberculosis itself. Due to the small amount of viable peripheral blood mononuclear cells routinely stored (3 aliquots
of 1.5 million cells) we were not able to perform additional analysis such as the suggested measurement of T regulatory cells. This was added in the limitation (page 14).

- **Reviewer:** “Were samples initially positive and then became negative as the time point of active tuberculosis approached?”
- **Reply:** As discussed above, we were not able to repeat T-SPOT.TB at later time points, since frozen cells samples at later time points before diagnosis of active tuberculosis were not available.

- **Reviewer:** “A comparison with the demographics of those who did not develop tuberculosis might be helpful to elucidate whether a combined negative elispot and tuberculin skin test were of pathogenic significance”
- **Reply:** We agree with the reviewer. As subjects who did not develop active tuberculosis were not included in our study, we could not estimate specificity of T-SPOT.TB and tuberculin skin test. However, the focus of this study was on sensitivity, because improvement of sensitivity is the main diagnostic need in latent tuberculosis in HIV-infected individuals. We have already acknowledged this limitation in the discussion (page 14).

- **Reviewer:** “…the first step is to validate the method…”
- **Reply:** As suggested by the reviewer, we added the issue of time between collection and freezing of sample “according to a specific protocol” in the methods section (page 7).

- **Reviewer:** “The time from collection of the sample to freezing appears to be especially important…”
- **Reply:** As discussed above, viability of frozen lymphocytes was checked regularly and this method was validated in several studies within the framework of the Swiss HIV Cohort Study (Khanna et al., J Virol 2009; 83:4404-11; Gasser et al., PLoS Med 2007; 4:e96; Rohrbach et al., Gut 2010, 59:1252-59). These references were added in the methods section (page 7).

- **Reviewer:** “The most important correlation between a positive elispot test is with duration of exposure to tuberculosis which needs to be included in the variables listed in Table 2”.

- **Reply**: Unfortunately, data on duration of exposure to tuberculosis were not recorded in the database of the Swiss HIV Cohort Study. However, information on whether patients had travelled to regions endemic for tuberculosis in the 2 years previous to diagnosis of active tuberculosis was available. We did not find any significant differences concerning T-SPOT.TB results and travel history.

- **Reviewer**: “..the grey zone of values recommended by the US FDA should be included”
- **Reply**: As suggested, we included the interpretation criteria used in the USA in the methods section (page 7).

- **Reviewer**: “The term “fair mitogen” needs to be defined…”
- **Reply**: We agree with the reviewer that the term “fair mitogen control” may be misleading. We specified in the results section (page 9) that patients with a fair mitogen control had less than 20 spots positive.

- **Reviewer**: “As the number of spots varies among individuals, correlation with CD4 count by Spearman’s test is not sensible – the figure speaks for itself and is sufficient”
- **Reply**: As suggested, we omitted the p-value of Spearman test.

**Other comments**

- **Reviewer**: “Is an elispot performed at entry in the cohort?”
- **Reply**: It is recommended to perform a tuberculin skin test and/or an interferon-gamma release assay in all HIV-infected individuals at enrolment into the Swiss HIV Cohort Study.

- **Reviewer**: “Is chemoprevention recommended on the basis of the tuberculin skin test?”
- **Reply**: Yes, in the Swiss HIV Cohort Study preventive treatment of latent tuberculosis is recommended in case of a positive tuberculin skin test, after exclusion of active tuberculosis.

- **Reviewer**: Did any member of the cohort receive chemoprevention?
- **Reply**: In the present study, none of the patients developing active tuberculosis had previously received chemoprevention.
Reviewer 2
We thank the reviewer for the appreciation and positive comments.

References:


