Author's response to reviews

Title: Human T-lymphotropic virus type 1 (HTLV-1) prevalence and quantitative detection of DNA proviral load in individuals with indeterminate/positive serological results.

Authors:

Francesca Vitone (vitonfra@yahoo.it)
Davide Gibellini (davide.gibellini@unibo.it)
Pasqua Schiavone (pasqualinaschiavone@libero.it)
Antonietta D'antuomo (dantuomo@med.unibo.it)
Lorenzo Gianni (blumimma@libero.it)
Isabella Bon (isabellabon@virgilio.it)
Maria Carla Re (remc@med.unibo.it)

Version: 2 Date: 16 December 2005

Author's response to reviews:

Dear Editor,
Enclosed herewith please find the revised manuscript entitled: Human T-lymphotropic virus type 1 (HTLV-1) prevalence and quantitative detection of DNA proviral load in individuals with indeterminate/positive serological results" (former title: Human T-lymphotropic virus type 1 (HTLV-1) prevalence and quantitative detection of DNA proviral load in a group of individuals originating from endemic areas and living in Italy), which has been modified according to the referees' suggestions for publication in BMC Infectious Diseases.

In particular,
1. Referee Nb (Olvero Varnier)
2. Page 1: The title has been modified, as suggested.
3. Page 2: Abstract: we now better specify our aims
4. We changed the last paragraph of the abstract, making our conclusion concordant with the aim of the study
5. Since we have modified the title and the abstract (as suggested by this referee) we did not shorten the paragraph: SYBR Green-based real time PCR analysis of HTLV-I indeterminate and reactive Western-blot patients.
6. We added the references requested
7. We delete the word "novel"
8. The sentence "Our method may also be useful for screening blood donors" has been changed to "Our method may also be useful to analyze PBMCs from blood donors with serological indeterminate results", even though infection diagnosis is currently undergoing radical changes (implementation of nucleic acid amplification testing for blood and tissue donors)
9. The sentence "SYBR Green real time PCR suggests a possible application and screening in the diagnosis of HTLV-1" has been changed to "SYBR Green real time PCR suggests a possible application to cut short doubtful diagnosis of HTLV-1 infection"

Discretionary revision
1. 1 page 1: we changed the sentence in the abstract: all the sera were firstly analysed by serological methods (ELISA and/or Western Blotting)
2. 2 page 3 We deleted the word "Re"
3. page 9 We did not separate this paragraph since, as clearly expressed, we have clinical information regarding only one patient.

Referee Ndegree2 (Graham Taylor)
- Background. According to referee's suggestion we modified the text and added a couple of references [Taylor, 1998 and 1999] concerning the first and the second points addressed by this referee.
- We have better specified the percentage (up to 2.5%) of indeterminate results that need to be confirmed as underlined in several published article (25, 21, 17, 8, 14, 26).
- Methods. MT2 cell lines were used to create a reference curve to quantify the presence of DNA and we believe that deleted genomes might not have an impact on the accuracy, since the same sensitivity level (up to 10 copies of viral DNA) has been found with scalar dilutions of HP1 plasmid where the HTLV-I 117bp
pol fragment was cloned (please see the chapter: SYBR Green-based real time PCR analysis of HTLV-I indeterminate and reactive Western-blot patients). Moreover Lee et al (30) and Albrecht et al (29) focused on quantitative PCR and used MT2 cell line to built a reference curve considering 2.1 HTLV-I genome/cell. Hence we believe that the indicated number of copies represents the correct value of amplifiable target.


Results. As requested we now show the complete data concerning the sensitivity and specificity of our assay (pages 9 n 10). The sensitivity (up to 10 plasmid copies) of real time PCR was assessed by scalar dilutions of HP1 plasmid where the HTLV-I 117bp pol fragment was cloned. The specificity of SYBR Green real time PCR was determined by parallel scalar dilution not only of Jurkat DNA (as in the former version of our manuscript) but also in 8E5LAV cell lines analysis (see materials and methods and results).

Discussion We modified the sentence emphasising that the determination of proviral load could be an important step in the pathogenesis of HTLV-associated disease such as in HAM/TSP patients where a significant correlation has been found between the proviral load and neopterin concentration (related to inflammatory process in the spinal cord lesion).

Fig.1 (now Figure 2) Electrophoresis agarose gel and Southern blot analysis of amplicons indicated the presence of a specific band at 117 bp (Figure 2). The presence of a larger additional band observed in three negative samples represents an HTLV-I unrelated fragment. In fact this fragment did not hybridize with the HTLV-I specific probe and displayed a higher melting temperature (about 84 degreesC) than HTLV-I specific amplicon Tm.

Referee Ndegrees3 (Steven Jacobson)

A figure representing the Western Blot profile (previously shown only in the table) has been added.

We added a more complete figure (Figure 2, previously Figure 1) that shows Electrophoresis agarose gel and Southern blot hybridization of all 11 samples with indeterminate or positive Western blot assays. We apologize for having sent a figure that did not include all our results and hence led to misinterpretation.

We strongly agree with the referee's comment concerning only the specificity of Real time PCR and we modified the text. In particular we underlined the specificity of our assay as assessed by the lack of positivity by control cell lines (Jurkat and 8E5LAV) and in blood donors in the study.

In addition we have discussed the possibility that seroindeterminate samples might be found PCR positive (please see discussion and correlated references)

Quality of written English: A mother-tongue professional English editor has revised the paper.

Hoping you will now find the paper acceptable for publication, I look forward to hearing from you at your earliest convenience.

Yours sincerely
Dr. Maria Carla Re
Department of Clinical and Experimental Medicine Section of Microbiology
University of Bologna
St. Orsola Hospital
Massarenti, 9
40138 Bologna Italy