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Analysis of gamma-delta T cells in black South African patients with active tuberculosis

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LIST OF CORRECTIONS EXAMINER 2:

General:

The report contains very interesting data and yet it is poorly presented with many oversights. It is a pity that the candidate did not take the time to polish the report off before submitting because it detracts from its true value.

The introduction is very well written and contains relevant information to set the scene.

It is such a pity that the report is so full of mistakes: the data set appears unique for South Africa and is therefore worthy of publishing.

Thank you for the valuable comments and opportunity to correct and improve this manuscript. Each comment from the examiner has been noted and corrected in the manuscript as outlined below:

1. The candidate reference authors as XXX *et al* in italics. Similarly, *in vitro* or *in vivo* should also be italized throughout the text.

The referencing has been altered using Endnote, Vancouver style and is thus all numbered in the text. *In vitro* and *in vivo* has been italicized throughout the text.

2. Page VII: These are lists of abbreviations and not nomenclature as currently indicated. Also, the abbreviation should be listed first followed by the full description.

'Nomenclature' has been changed to 'list of abbreviations' and the abbreviation was listed first followed by the full description - pages V11 and V111.

3. The figures simply appear within the text but the candidate does not refer to these figures (except figure 12)

Figures 1 up to and including figure 7 have been removed from the text. The list of figures is now as follows:

FIGURE NUMBER	TITLE OF FIGURE	PAGE NUMBER
Figure 1	Spill over in AmCyan channel	21
Figure 2	Spill over in PerCP CY5.5 channel	22
Figure 3	Spill over of fluorochromes into CD8 Beta APC	22
Figure 4	Box plots of gamma delta T cells in all patient groups	29
Figure 5	Flow cytometric plots of gamma delta T cells	30
Figure 6	Box plots of cytotoxic T cells	31
Figure 7	Flow cytometric plots of helper and cytotoxic T cells	32
Figure 8	Correlation graphs	33

These figures have been referred to in the text as appropriate.

4. The legend of figure 3 should be reworded since it appears that the CD4 helper cells or the CD8 cytotoxic cells are the antigen presenting cells as currently worded.

This figure 3 has been entirely removed as suggested by examiner 1.

5. Page 9: the VDJ gene re-arrangements of the TCR relate only to the T cells and not B cells.

B cells have been removed from this sentence in the text on page 6.

7. Page 14: the stain is the Zeihl-Neehlsen stain: spelling and caps!

This figure and legend has been removed.

8. Page 22: the description of patients included in the study (hence study samples) poses the biggest problems. On page 22 the candidate refers to patients who were HIV-positive but had no current symptoms or signs of tuberculosis. However all of these patients had previously been treated for tuberculosis: how can the candidate be certain that these immune parameters measured returned to "normality" post tuberculosis therapy? This is not even discussed in the context of her results. Furthermore, the so-called "controls": the appendix clearly indicates that 4 subjects never consented to HIV testing. And to make matters worse, one such control was HIV positive and yet included as a control subject. There are major discrepancies between the text and appendix as provided.

The original data sheets from patient and control and spreadsheets have been reviewed. The shorthand that was used in the demographic spreadsheet has been corrected for clarification. There were no changes required to the data analysis.

Please refer to the list of corrections of examiner 1 in which these errors have been explained. The finding that Group B patients had previous Tuberculosis has been discussed in the report under Section 4 (see page 35).

9. Page 23: the staining procedure of the PBMCs should be reviewed. It seems as if the cells were aspirated post staining.

The paragraph now reads as follows in Section 2.3:

'Peripheral blood mononuclear cells (PBMCs) were isolated by density centrifugation over Ficoll Hypaque gradients. The PBMC's were spun at a speed of 3000rcf (relative centrifugal force) and washed three times in phosphate buffered saline (PBS). An antibody cocktail was prepared which consisted of each appropriate volume of the nine antibodies multiplied by the number of samples for testing. The staining volume of each antibody was obtained by titration. Each sample was then stained using the antibody cocktail for each day, to minimize pipetting inaccuracies.'