Reviewer's report

**Title:** Gut microbiota in children with type 1 diabetes differs from healthy children: a case-control study.

**Version:** 1  **Date:** 2 October 2012

**Reviewer:** Daniel Moore

**Reviewer's report:**

Murri and colleagues present a new analysis of the microbiome in T1D. The use of a strong control group and good analytical approaches. However, i think that some of the interpretations of their data are stronger than the data warrant and more attention should be paid to technical controls to allow the reader to better assess the data. Therefore, i suggest the following major compulsory revisions:

1. In the discussions, the authors propose that the microbial diversity is higher in the healthy children. It is not possible to conclude this from the presented data. Because of the sensitivity of the DGGE techniques, low frequency bacteria are not identified. Thus, it remains possible that those bacteria that were not detected by this analysis due to their low abundance could be much more diverse in children with T1D than controls (that is, children with T1D could have a large number of low abundance organisms whereas healthy children may have fewer).

2. The qPCR data should be presented with more detail. The use of a standard curve is laudable and a great way to do this analysis. However, for appropriate use of the standard curve, the authors need to report the efficiency of the qPCR with all primer pairs on their samples. If the efficiency is substantially greater than 110% or less than 90%, then the data may not be reliable. The sample may have PCR altering conditions that are not present in the standard curve.

3. Negative controls are not reported. The authors should determine and state whether any product is detected by PCR on a non-template control. Similarly, in the absence of template, is any product(s) detected by DGGE?

4. The authors also indicate a correlation between glucose control and the microbiome. The data would be made much stronger if they were able to show analysis on the same subject at a time when glucose control was different. Since glucose control is generally dynamic within an individual, this may be possible and would substantially strengthen the presentation and interpretation of the collected data.

I also hope the authors may consider the following discretionary revisions:

1. Did the authors control for other therapies that may alter the microbiome such as therapy with proton pump inhibitors or corticosteroid bursts?

2. The authors should consider including additional data relevant to the role of the microbiome in T1D from mouse studies such as the nature paper by Li Wen and colleagues or the several excellent recent papers on the role of SFB-Th17
interactions in mice. Do we have a grasp yet on the related mechanisms/interactions in humans? Does this study advance this field.

3. Could the authors discuss how these data relate to the concept and definition of enterotypes as forwarded in other studies?

**Quality of written English:** Needs some language corrections before being published

**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