Reviewer's report

Title: Fragile X protein in newborn dried blood spots: implications for newborn screening

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Reviewer: Flora Tassone

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This is a follow up study of the development of a simple and inexpensive immunoassay capable of measuring FMRP levels in dried blood spots. A larger set of samples from the general population was used to establish the variability in FMRP expression detected by the assay.

The feasibility of the assay was tested in fresh and archived spots showing that a potential FMRP degradation may have occurred in archived spots and suggesting that its utility would be most effective if applied to fresh samples. Overall it is a good and reliable technique and the feasibility of the analysis is appropriate. The study is important as a quantitative methodology able to reliably measuring FMRP expression levels is lacking.

This reviewer has the following comments:

In the abstract:

Methods:

From the abstract it is not possible to distinguish whether the only difference between the “2000 fresh random DBS” and the “set of 76 newborn DBS that had been stored…” is the age of the spots. May want to also explain that the 2000 samples were anonymous and that you retrospectively analyzed the 76 samples from controls and 1 FXS case.

Results:

It is somewhat confusing when the author refers to the first and second set, I may suggest to use a descriptive adjective to distinguish the samples, ie “fresh DBS” and “aged DBS” instead or being more specific.

The author reports a mean distribution about 7 fold higher than that of normal adults in the first set. Which normal adult samples were used for comparison? If the author refers to FMRP levels obtained from fresh DBS versus FMRP levels from older DBS, this should be stated instead. Or was form a previous study?

From the sentence: “Despite the degraded signal from these samples, it was possible to recognize all the affected males”…. There were affected males out of the 76 from the general population? It is clear only later on in the Result section maybe just mention in the abstract that the 76 blood spots included samples from full mutation males as well as normal individuals.
In the Results section
line 136-138: Please describe further: is the Covance anti FMRP against a different species than rabbit? Are the two antibodies selecting different epitopes?

line 165: The author state that “FMRP concentration (pM) in each sample (3-mm-diameter disk eluate) was calculated by comparison to dilutions of an abbreviated FMRP standard (GST-SR7).” The quantification of FMRP is very important for the conclusions in this paper and there is no detailed description of the GST-SR7 FMRP standard used and how exactly were the dilutions compared in order to quantify FMRP concentration for the DBS. Some specifics should be added or reference the authors’ previous study.

line 188: n=? for full mutation and normal

…….”Despite the loss of detectable FMRP with DBS storage time, the qFMRP assay identified all of the full mutation males”...

I believe that the problem could be that the loss of FMRP the author shown to be due to prolonged storage could lead to false positives.

Based on their observations, how would the authors propose the finding of a low FMRP sample with this test could be handled? Follow up with CGG sizing test? Also based on the presented results with aged blood spots, what are the authors’ recommendations for the age range of the spots that should be used to obtain reliable data?

Level of interest: An article of importance in its field

Quality of written English: Acceptable

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