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Dried blood spots for aflatoxin B1 assessment in a field study with pregnant women

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BACKGROUND

- Aflatoxin B1 (AFB1), a highly carcinogenic fungal metabolite produced by the *Aspergillus flavus* and *Aspergillus parasiticus* fungi, has been linked to poor linear growth

- Assessing blood-based biomarkers requires a venipuncture and the maintenance of a cold chain: two aspects that limit research in community settings particularly in remote areas.

- Lab studies have suggested dried blood spots (DBS) as a viable, non invasive, low-cost alternative to venous blood draw for assessing AFB1 exposure (Xue et al. 2016).

METHODS

- Objective: Examine the agreement between AFB1-lysine adduct levels measured using a DBS versus a serum sample

- Random sub-sample of 296 pregnant women ages 16-49 enrolled in the USAID-supported Aflatoxin (AflaCohort) Birth Cohort Study in Banke, Nepal.

- Trained nurses collected blood samples once during pregnancy for AFB1 biomarker testing
  1. Capillary blood for DBS
  2. Venous blood

METHODS

Venous blood draw and DBS

- **DBS:** 3-5 large drops (c. 100 uL each) of whole capillary blood were collected from a finger stick using Whatman™ Qualitative Filter Paper.

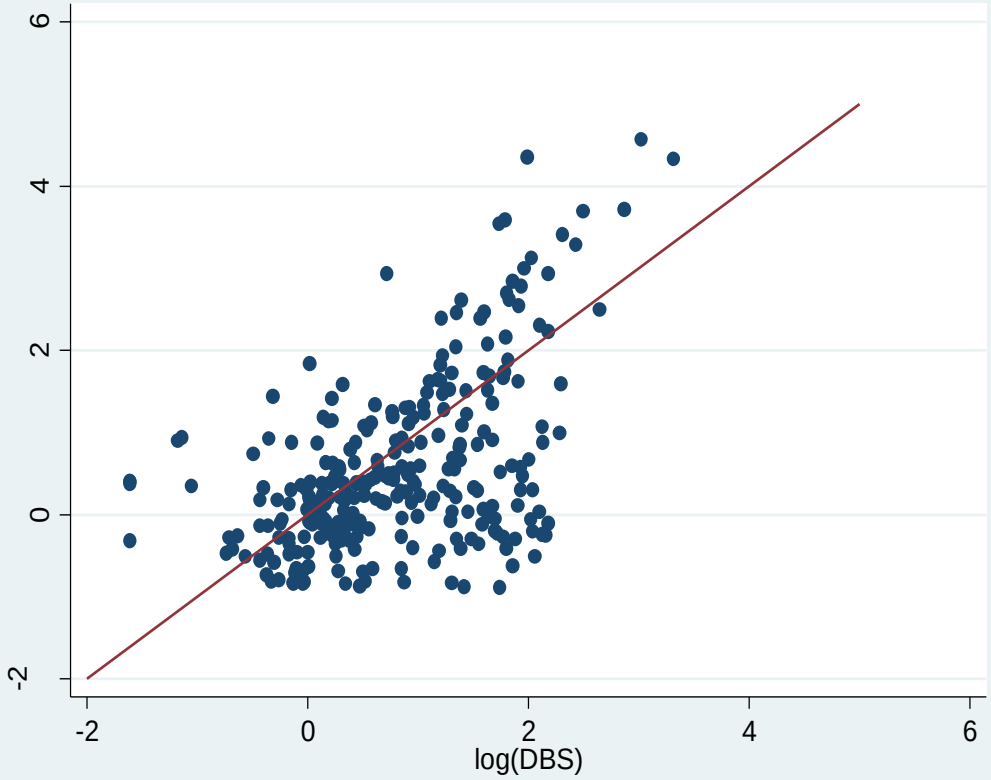
- **Venous blood:** A 3-5 ml venous blood sample was collected from the antecubital vein of each pregnant woman in using 5ml BD Vacutainer® blood collection red-top tubes.

METHODS

- Dried blood spots were air dried and stored in Ziploc bags with a dessicant at -20 and then -80 C
- Venous samples were processed within 4 hours of collection and serum samples stored at -20 and then -80 C
- AFB1-lysine albumin adduct levels were assessed by high-performance liquid chromatography (HPLC) with fluorescence detection
- Pearson correlation analysis and Bland-Altman analysis was used to test the level of agreement between AFB1-lysine albumin adduct levels from the two different collection methods (DBS and serum)
- All analyses were conducted with Stata® SE version 14.

RESULTS

- DBS Geometric mean = 2.16 pg/mg of albumin (95% CI: 1.95, 2.39
- Serum geometric mean = 1.62 pg AFB1-lysine adducts/mg albumin (95% CI: 1.43, 1.84)
- A Pearson correlation coefficient = 0.5071 (p<0.001)



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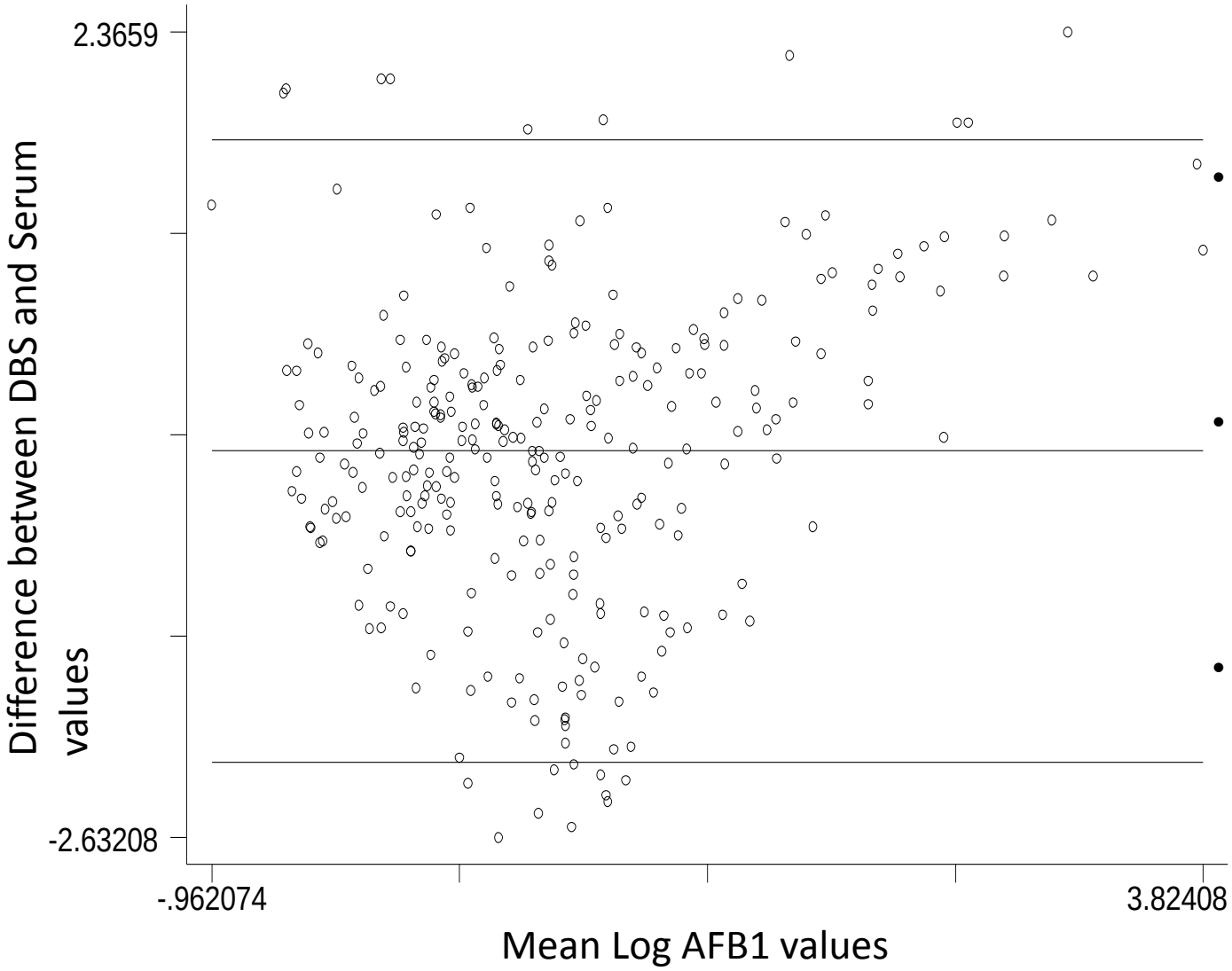
BLAND-ALTMAN



- Mean difference -0.284 (CI -0.401 to -0.168)
- Limits of agreement: -2.318 (CI -2.519, -2.116) to 1.749 (CI 1.548, 1.951)
- 60% of DBS samples showed higher AFB1 adduct levels than the serum samples
- DBS AFB1 adduct levels could be as small as 17.4% or as large as 1015% of serum AFB1.

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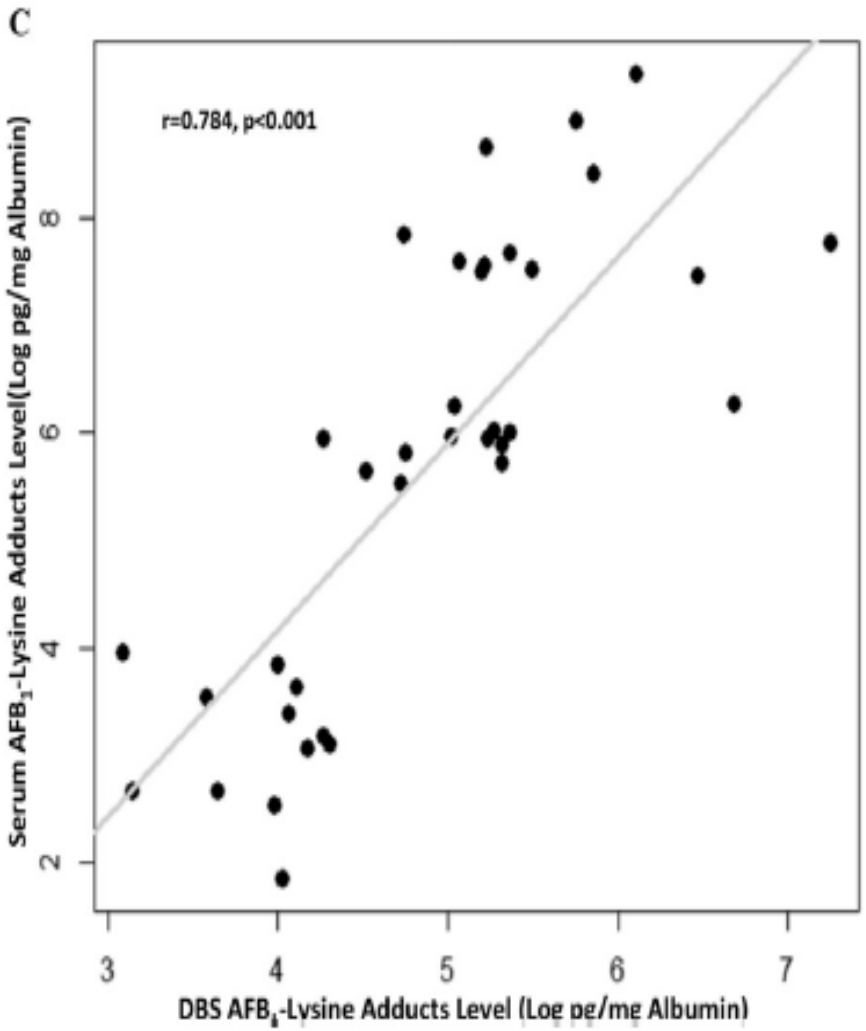
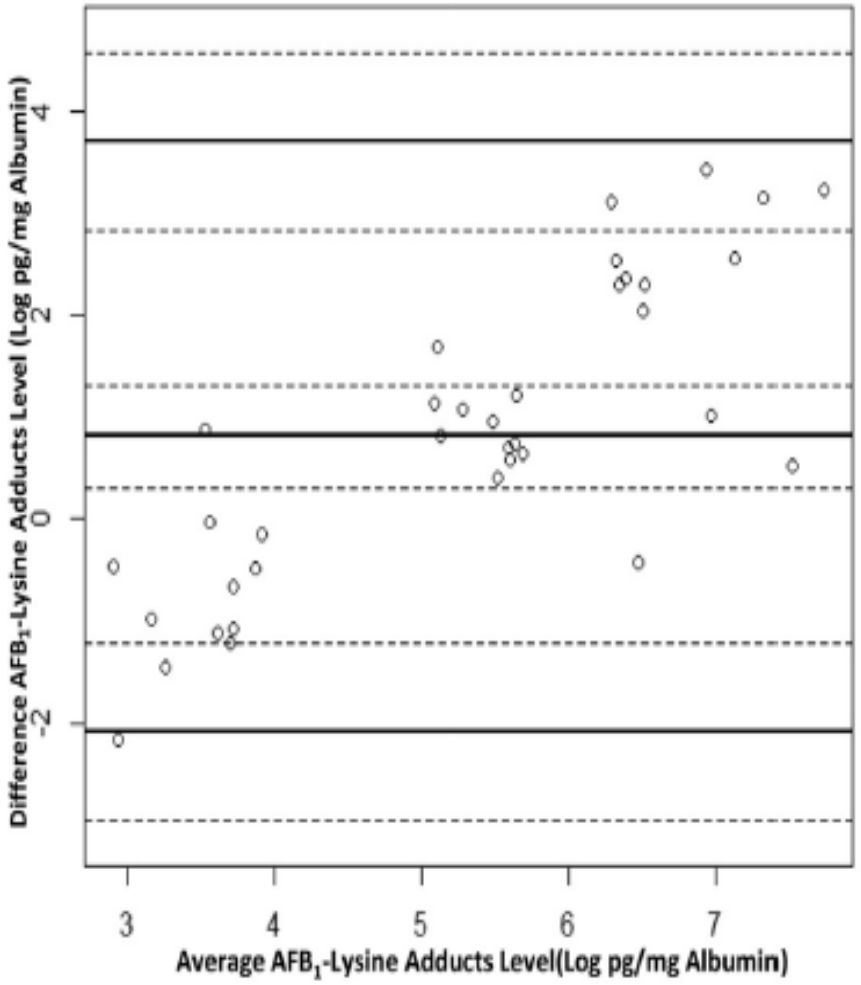
DATA FROM UGANDA- DBS VERSUS SERUM





Xue et al 2016

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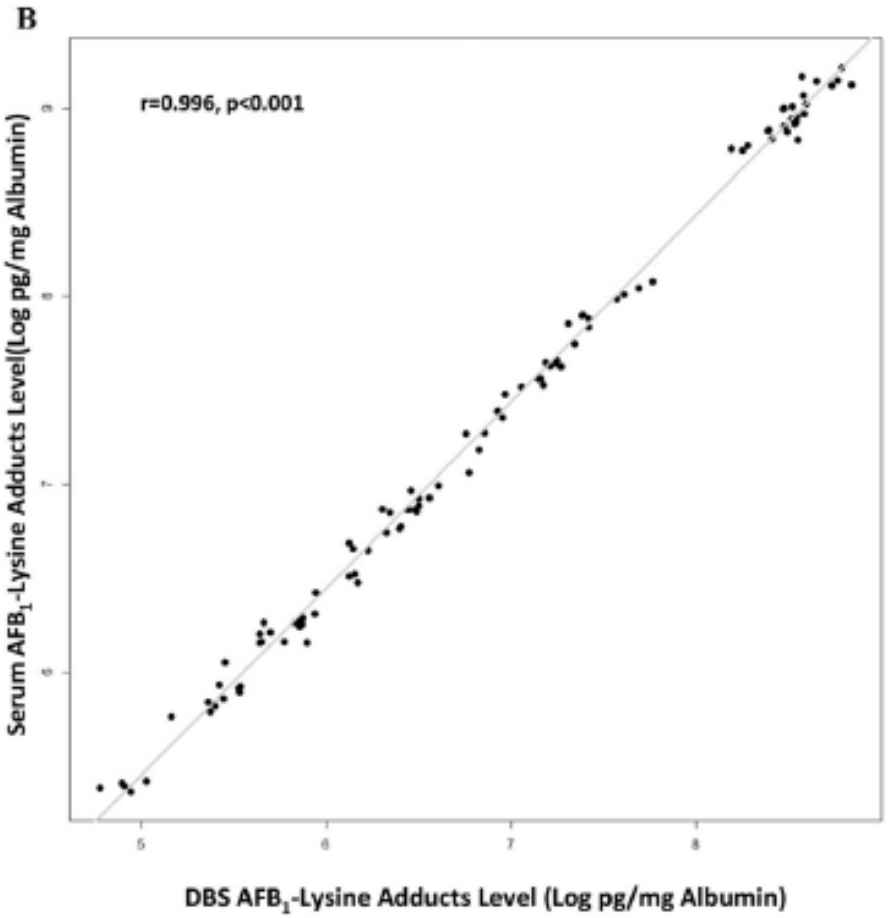
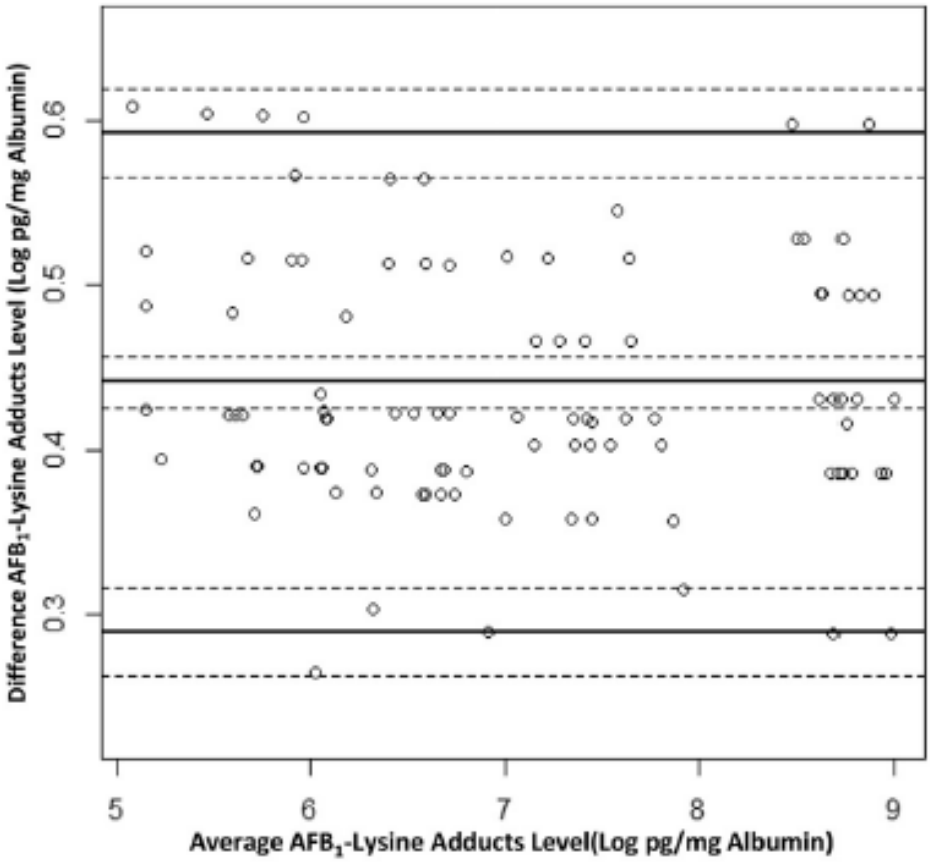
ANIMAL DATA





Xue et al 2016

LIMITATIONS AND CONSIDERATIONS

- Higher levels in DBS

  DBS samples from whole blood include RBCs, WBCs and other plasma components: Complex matrix for binding aflatoxins than in serum samples

  AFB1 exposure in whole blood samples is significantly different from serum

- Low levels of aflatoxin in the sample
- Type of filter paper
- Method and technique including spot size and spot overlap

CONCLUSIONS

- The current study is the first to evaluate AFB1 biomarkers in adults using DBS samples in a region with relatively low levels of aflatoxin exposure.
- Contrary to previous studies showing strong correlations in samples with higher aflatoxin levels, our study showed a modest correlation between AFB1-lysine adduct levels in DBS and serum samples.
- Limits of agreement are similar to other human data but much wider than from animal data. Need to understand the clinical significance of the limits of agreement
- While DBS might allow research in more limited settings, specialized and well trained clinical personnel are key for data quality

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