Validation of a rapid "QuickTest" for aflatoxins in peanuts and maize in Timor-Leste

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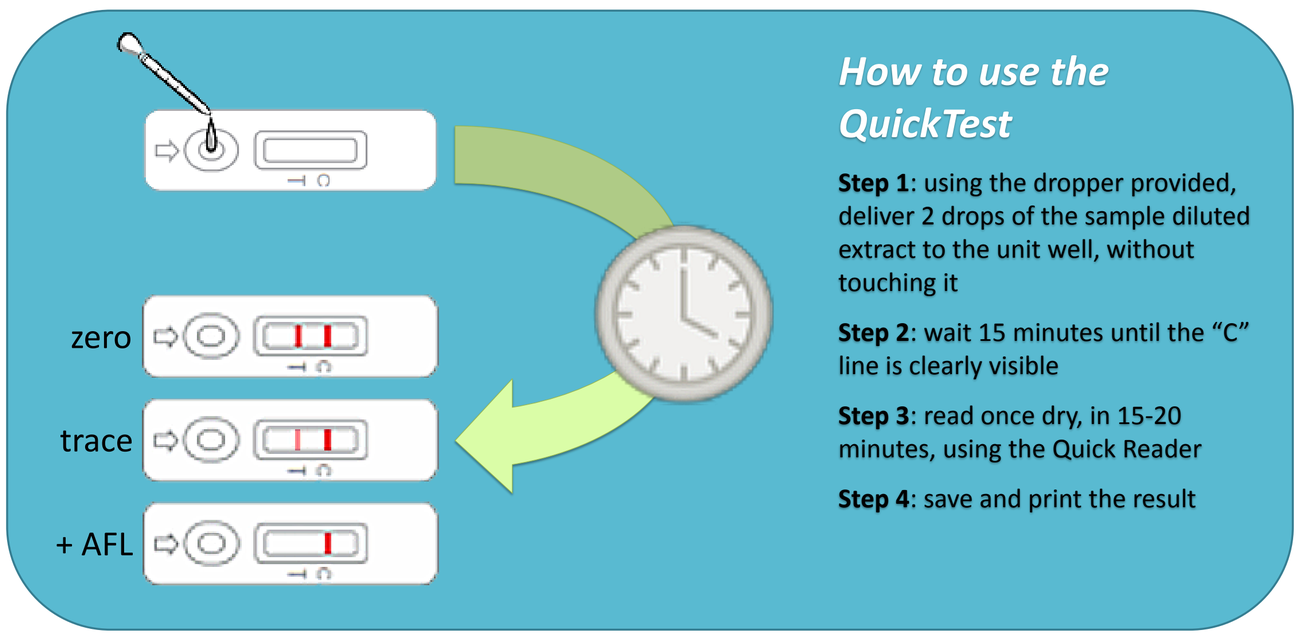
Introduction

The occurrence of carcinogenic aflatoxins in agricultural produce is a major concern worldwide but particularly in the humid tropics. Aflatoxin has been identified as a threat to human health in Timor Leste (Ministry of Health, 2015) and prevented export of some agricultural produce in recent times. The fungal species, *Aspergillus flavus* and *A. parasiticus*, can infect various crops – frequently peanuts and maize – either prior to harvest or under moist conditions in produce stored after harvest leading to excessively high concentrations of aflatoxins B1 and G1; this contamination can sometimes exceed thousands of parts per billion (ppb) in individual kernels of peanuts or other grains, whereas maximum residues limits are in the range of about 2-20 ppb, depending on the country.

Screening to detect aflatoxin contamination often relies on fluorescence and has been achieved by reference standards using thin layer chromatography, adsorption on minicolumns or HPLC. Specific antibodies to aflatoxins have provided an alternative means to conduct immunoassays as ELISA (Lee et al., 2004). More recently, lateral flow display has been employed (Masinde et al., 2013) to develop rapid QuickTests™ employing immunogold nanoparticles for quantification using a suitable reader.

Fig 1. Workshop for training SoL personnel in the use of Aflatoxin QuickTest



Conclusions

This validation study was successful: it proved the accuracy of the AQT, which renders results comparable to those obtained by the standard analytical methods.

Advantages of the new technology are the ease of use, rapid development time (15 min), no need for dangerous chemicals, straightforward reading of test results and low cost of the AQT units. Moreover, very little and inexpensive equipment is required.

Based on the demonstrated ease of use of the Aflatoxin QuickTest to evaluate aflatoxin contamination in Timor-Leste, the grain procurer and processor 'Timor Global' has decided to implement the AQT technology in its processing plant with proposed training of its staff in April 2016. It is anticipated that application of this technology may allow rapid, accurate and low cost screening of Timorese agricultural products to meet strict food safety standards for the export market in the near future.

References

Lee, Nanju A., Wang, Shuo, Allan, Robin D. and Kennedy, Ivan R. (2004). A rapid aflatoxin B1 ELISA: Development and validation with reduced matrix effects for peanuts, corn, pistachio, and soybeans. *Journal of Agricultural and Food Chemistry* 52, 2746 – 2755.

Masinde, Lily A., Sheng Wei Xu, Xin Zhang, Yan Yuan M., Kennedy, Ivan R. and Wang, Shuo (2013). Colloidal gold based immunochromatographic strip for the simple and sensitive determination of aflatoxin B1 and B2 in corn and rice. *Microchimica Acta* 180, 921-928.

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Objective

In mid-2015, the Seeds of Life project commissioned this new technology to be applied to peanuts and maize in Timor-Leste. The aim was to test whether the Aflatoxin QuickTest could be a reliable and affordable means of screening Timorese agricultural produce for aflatoxin.

To meet this objective, in November 2015 a workshop was held in Dili with more than 10 participants for demonstration and training in the application of QuickTests (Fig. 1). Seed samples collected from a comprehensive survey around the country were analysed using the Aflatoxin QuickTest (AQT).

Methodology

Sample collection: A survey was conducted in 2013, 2014 and 2015 to collect maize and peanut kernels from markets, seed producers and households in 42 districts of Timor-Leste. A subsample of 50 peanut and 30 maize samples were analysed using the AQT. In addition, 3 samples of cassava and 3 of locally made peanut butter were also analysed during the workshop.

Sample extraction: 100 g of kernels were ground and then extracted with 200 ml of 80% methanol containing 4% NaCl. The extract was filtered and 4 ml of supernatant were collected for subsequent analysis by both AQT and HPLC.

Aflatoxin QuickTest (AQT): An aliquot of the extract was diluted in phosphate buffer solution (ratio 1:10) to make it ready for AQT analysis. Two drops of this solution were added to an AQT strip (see box). After 15 minutes the strip was read using a Quick Reader, and the results printed and recorded.

High-performance liquid chromatography (HPLC): Analysis of a set of extracts (33 peanut samples) by this standard analytical method were done in the NATA-certified laboratory of the Peanut Company of Australia (PCA) at Kingaroy (Queensland, Australia).

Results

The results by both analytical methods were compared in order to validate the AQT procedure. The set of 33 peanut samples showed an r^2 value of 0.989 on regression (Fig. 2). Data for the maize samples are now being acquired in Sydney and will be analysed the same way.

The AQT used can detect aflatoxins B1, G1 and M1, and some aflatoxins B2 and G2. The sensitivity of the test is from 1 to 40 ppb in the sample. For readings above 40 ppb, the sample extracts can be diluted further and re-analysed again, increasing the range up to 400 pbb in the sample.

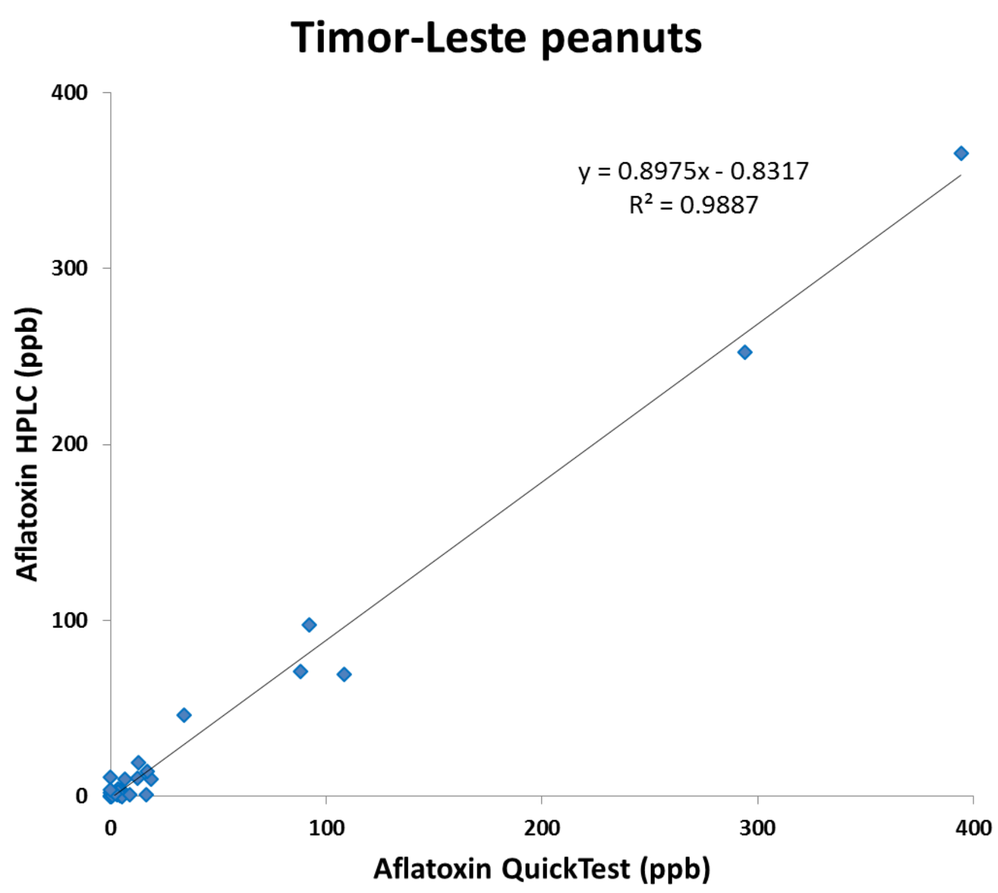

Fig 2. Validation of Aflatoxin QuickTest using peanut samples from Timor-Leste (n = 33)