## **Materials, reagents, and sample preparation**

## **Materials and reagents**

The individual mycotoxin liquid (1000 micrograms/milliliter) calibration standards of aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), M1 (AFM1), and 13C17-aflatoxin B1 (13C17-AFB1) (internal standard) were obtained from Sigma Aldrich (Bornem, Belgium). AFB1-lysine standards were supplied by Carleton University, Ottawa, Canada. The working solutions of AFB1, AFB2, AFG1, AFG2, AFM1, AFB1-lysine, and 13C17-AFB1 (10 micrograms/milliliter) were prepared in methanol, stored at -18 °C, and renewed monthly. Water was obtained from a Milli-Q® SP Reagent water system (Millipore Corp, Brussels, Belgium). Methanol (LC-MS grade) was purchased from BioSolve (Valkenswaard, the Netherlands), while acetonitrile (Analar Normapur) and ammonium acetate were obtained from VWR International (Zaventem, Belgium). Acetic acid (glacial, 100 %) was supplied by Merck (Darmstadt, Germany). Formic acid analytical grade (98–100%) and sodium chloride (> 99.5%) were from Merck (Darmstadt, Germany). Ultrafree®-MC centrifugal filter devices (0.22 Micrometer) were obtained from Millipore (Bedford, Massachusetts, USA).

## **Sample preparation for measurement of aflatoxins and fumonisins**

A protein precipitation method with few variations was used to prepare samples for analysis (31). Serum samples were thawed and measured for aflatoxins and fumonisins. Acetonitrile (ACN) was used for protein precipitation. One hundred microliter of ACN and 100 microliter of serum were quantitatively poured in eppendorf tubes. The centrifugation (15 minutes) of the mixture created two layers: a large aqueous layer on top and a circular flake of proteins at the bottom. An aliquot (160 microliter) of the supernatant was carefully transferred to glass tubes and evaporated using the TurboVap (40°C). The samples were reconstituted with 80 microliters of injection solvent and transferred to the centrifugal filter tubes. We spiked the injection vials with 5 microliters of an internal standard prior to analysis. Finally, the samples were transferred from tubes to the injection vials. Serum samples were analyzed in batches of five. For each batch, six standards and one blank calibrator were prepared for the calibration curve. The blank contained 80 microliters of internal standard (13C17-AFB1) and 120 microliters of injection solvent.