GENERAL INFORMATION
Ochratoxin-A is a nephrotoxic and hepatocarcinogenic mycotoxin produced by Penicillium verrucosum and Penicillium viridicatum in temperate and cold climates and by a number of Aspergillus species such as A. ochraceus in warmer and tropical areas of the world. Ochratoxin-A has been shown to occur in various cereals and other plant products, coffee beans and coffee products, wine, animal feeds. Due to contaminated feed or food the ochratoxin-A also can occur in pig kidneys, liver and muscle, blood, urine and faeces from pigs and in human blood.

For ochratoxin-A maximum levels (MLs) are established legally in Europe. Depending on the fact whether products are used directly for human consumption or the products still have to be processed. The MLs vary from 2 to 10 μg/kg (ppb)

PRINCIPLE OF THE METHOD
The Ochratoxin-A Rapid Test is a competitive enzyme immunoassay on nitrocellulose for the screening of ochratoxin-A in food samples (corn, rice, wheat, sorghum, barley, oats, rye, coffee, rice, dry beans and wine and spices).

Rabbit antibodies to mouse IgG are immobilised in the test line (T) of the nitrocellulose membrane. A mouse anti-ochratoxin-A, sample, and enzyme labelled ochratoxin-A are added sequentially. The unbound conjugate is removed by a washing step. A chromogen substrate (tetramethylbenzidine) is then added. Bound enzyme transforms the chromogen substrate into a blue coloured product appearing as a color band.

CONTENTS AND COMPOSITION
Devices: 2 x 5 cassettes.
Reagent A: 3 bottles. Extraction solution.
Reagent B: 1 vial. Dilution buffer.
Reagent C: 1 vial. Antibody solution, yellow cap.
Reagent D: 1 vial. Enzyme conjugate, green cap.
Reagent E: 1 vial. Washing buffer, white cap.
Reagent F: 1 vial. Tetramethylbenzidine substrate, blue cap.
Filters and Syringes: 10 units each.

STORAGE AND STABILITY
Store at 2-8 °C. Each component is stable until the expiry date marked in the label. Liquid components are stable once opened until the expiry date marked in the label if they are stored at the recommended temperature, well closed and care is taken to prevent contamination during their use.

Indications of deterioration:
- Reagents: Blue color of the substrate (Reagent F).
- Devices (cassettes): rips on the sealing bag, presence of lines or spots in the membrane before performing the assay.

REAGENT PREPARATION
All the reagents are provided ready to use.

PRECAUTIONS
- Ochratoxin-A is a carcinogenic and toxic compound. Avoid contact with mouth and skin. Be aware the ochratoxin-A are not inhaled. Any material contaminated with ochratoxin should be destroyed or decontaminated by addition of sodium hypochlorite solution (10% v/v).
- Avoid contact of all biological materials with skin and mucous membranes. Do not pipette by mouth.
- Do not eat, drink, smoke, store or prepare foods, or apply cosmetics within the designated work area.
- TMB is toxic by inhalation, in contact with skin and if swallowed; observe care when handling the substrate.
- Do not use components past expiration date and do not intermix components from different serial lots.

SAMPLE TREATMENT
A homogenous sample has to be obtained from a representative part of the compound.

1. Sample (50-100 g) is ground and pulv erised into a fine homogeneous compound.
2. Sample extraction: For the detection of 4 ppb: 5 g of ground sample is extracted with 15 mL of Reagent A.
3. Shake by hand at room temperature for 3 minutes and leave the sample to settle and to obtain clean supernatant.
4. For cereals and green coffee: Draw 1.4 mL of dilution buffer (Reagent B) with a syringe and draw 1 mL of supernatant to the 2.4 mL mark. Mix gently. Fix the filter to the syringe.

For other samples: Draw approximately 1.5 mL of the supernatant with a syringe. Fix the filter to the syringe.

PROCEDURE
Allow all the reagents and devices warm up to room temperature.
1. Open the Device package and take out the required amount of cassettes. Place the device on a flat surface. Store the unused cassettes into the sealing bag and reseal it, keeping the desiccant inside.
2. Place 2 drops of Reagent C onto the middle of the well. Allow liquid to flow-through completely.
3. Add 20 drops of sample extract with the syringe. Allow liquid to flow-through completely.
4. Add 2 drops of Reagent D. Allow liquid to flow-through completely.
5. Wash membrane with 1 drop of Reagent E. Allow liquid to flow-through completely.
6. Rinse membrane with 3 drops of Reagent E. Allow liquid to flow-through completely.
7. Add 5 drops of Reagent F and observe color development. An optimal interpretation of results is achieved 5 to 6 minutes after application.

READING
Examine the presence of color bands inside the well of the cassette.

Negative result. Two color bands appear: one in the side “T” and another in the side “C” of the well.

Positive result. A color band appears only in the side “C” of the well.

Invalid result. Absence of color bands. Retest the sample using a new cassette.

ASSAY CHARACTERISTICS
- Cut-off: 4 ppb.
- Specificity: The antiserum used cross-reacts with ochratoxin-A (100 %) and with ochratoxin-B (9 %).

BIBLIOGRAPHY