

Limit of detection (LOD) calculated from experiment on an Infinite<sup>®</sup> 200 instrument was equal to 0.3 HA unit/ $\mu$ l, while LOD calculated from the experiment on a Portable Analyzer was equal to 0.06 HA unit/ $\mu$ l. LOD was calculated according to the equation [2]:

$$LOD = P(C_{antigen} = 0) - 3 \cdot St. deviation$$

**Conclusions.** Fluorescence polarization immunoassay of the Avian Influenza virus was successfully carried out. Antibodies H5 have been shown to have a high ability to bind with positive serum Avian Influenza virus. Portable Fluorescence Polarization Analyzer was tested for Avian Influenza virus system. It was demonstrated that the instrument works properly for it. High-throughput screening of Avian Influenza virus could be performed on both instruments.

## REFERENCES

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## FLUORESCENCE POLARIZATION IMMUNOASSAYS FOR DEOXYNIVALENOL DETECTION

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Mycotoxins are secondary metabolites produced mainly by molds under certain conditions. Deoxynivalenol (DON), a major trichothecenes mycotoxin that is mainly produced by *Fusarium graminearum*, can induce acute and chronic toxic effects on both human and animals through contaminated foods and feeds. Therefore, surveillance and control of DON and its derivatives in foods and feeds are of importance for producers to protect consumer health [1].

Fluorescence polarization immunoassay (FPIA) is a homogeneous competitive immunoassay method based on the increase in fluorescence

polarization of fluorescent-labelled small antigens (tracers) bounding with specific antibodies. Due to its simplicity, it is a readily automated method which is suitable for high-throughput screening and widely used for studying molecular interactions such as protein–protein or protein–DNA interactions [2].

**Aim.** (i) Studying molecular interactions of the deoxynivalenol with antibodies and (ii) evaluation the scope of the FPIA method for DON analysis.

**Materials and methods.** Fluorescence polarization was measured on the Infinite<sup>®</sup> 200 instrument (Tecan Group Ltd., Switzerland). Binding DON with the antibody was assessed by the measurement of the fluorescence polarization value after competitive binding of the tracer and DON with the antibodies. Immunoassay was performed using “Aokin mycontrol DON” analytical Kit.

**Results and its discussion.** A typical sigmoidal FPIA curve (Fig. 1), which is in accordance with the theory, was obtained. The curve was obtained by mixing a DON solution in several dilutions with constant amounts of antibodies and tracer solutions followed by fluorescence polarization measurement. At a fixed temperature, the value of the fluorescence polarization will be directly dependent on the effective molecular size of the fluorophore [2]. For small-sized molecules, such as an unbound tracer, the value of fluorescent polarization is low, while for large-sized, such as a tracer bound to antibody, high. In the absence of the analyte in the sample, the antibodies are more associated with the tracer, which leads to a high signal.

Limit of detection (LOD) was equal to 0.03 µg/ml while the limits of DON in food varies from 0.5 to 2.0 µg/g [3]. LOD was calculated according to the formula [2]:

$$LOD = P(C_{antigen} = 0) - 3 \cdot St. deviation$$

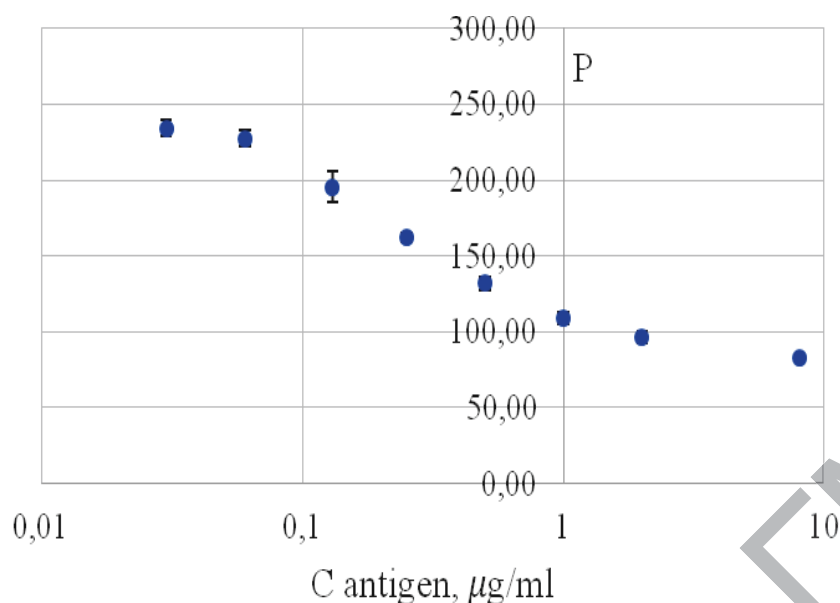


Fig. 1 Typical FPIA standard curve: analytical signal (fluorescence polarization value) vs. analyte concentration (in logarithmic coordinates)

**Conclusions.** Fluorescence polarization immunoassay of the DON was successfully carried out. Antibodies have been shown to have a high ability to bind with DON. LOD was equal to 0.03  $\mu\text{g/ml}$ . FPIA method could be considered as a fast high-throughput screening method, but not as a method of monitoring the content of toxin in food.

## REFERENCES

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