JAGIELLONIAN
CENTER OF INNOVATION

GLUTEN DETECTION – SELECTED TECHNIQUES

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Immunoanalytical methods are currently preferred in gluten detection. They include the **enzyme immunosorbent assay** (ELISA), which is most popular and most commonly used for routine analysis of food allergens. It is so due to its sensitivity, precision and extensive standardization possibilities. ELISA is also the most commonly used technique in commercially available sets for detection of allergen levels in both foodstuffs, and in meals and drinks [1].

Two forms of ELISA test – **sandwich** ELISA and **competitive** ELISA – are recommended for the testing of gluten level in food..

The sandwich technique is based on the use of two antibodies. The coating antibody (green in the figure) is attached to the surface of the plate, where the assay is carried out. The plate is washed with a solution containing the analyzed material. If protein to be detected (antigen, red triangles in the figure) is present in the material, it will become bound to antibodies set on the plate. After adding the analyzed material, the plate is washed. At this point the antigen is already separated from the analyzed mixture and bound to the plate surface. Then, another antibody is added. This one is marked with an enzyme. This antibody is also specific for the detected protein. After another washing a sandwich structure is formed – the antigen is trapped between two layers of antibodies. Finally, a substrate for the enzyme bound to the second antibody is added. Compounds yielding a color product as a result of the enzymatic reaction are usually used, as they may be easily measured using the spectrophotometric method. Signal strength is proportional to the amount of trapped antigen..

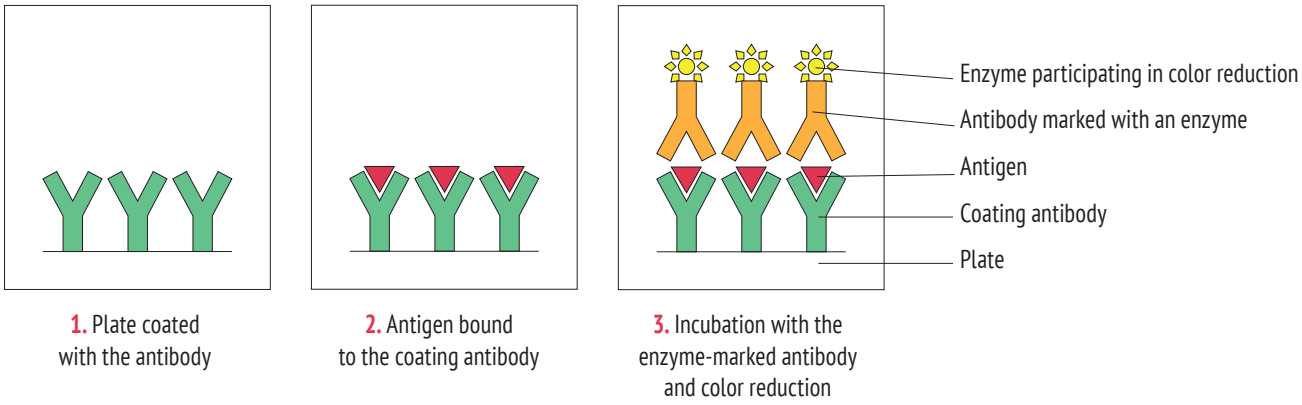

Fig. 1 Simplified outline of the sandwich ELISA assay

The currently recommended method for determination of gluten level is the R5 (Mendez) ELISA sandwich test, using two R5 antibodies binding to gliadin – a protein contained in gluten. It is a highly sensitive and specific test, particularly useful if the level of the analyzed protein is low and if assayed protein is accompanied by high concentrations of other proteins. The detection limit of the test is 1.56 ppm (parts per million), and it is recommended by the Codex Alimentarius Commission as a preferred method for determination of gluten level in food [2]. However, the method is not sufficiently precise to determine gluten content in hydrolyzed products. R5 ELISA competitive test, based on a single monoclonal antibody, allows for precise determination of the level of both gluten as a whole, and its fragments. According to the Codex Alimentarius Commission, this modification of the R5 test is recommended for the analysis of the level of hydrolyzed gluten. The detection limit of the method is 0.3 ppm in liquid samples.

An ideal test for the presence of gluten should recognize those regions of the protein that are responsible for its immunotoxic effect. Despite the fact that not all those regions have been identified yet, tests based on the monoclonal G12 antibody seem to meet that criterion, and their use for determination of gluten in food is recommended by, for example, AGES – the official control office of Austria [3]. The G12 antibody recognizes a α-2 peptide fragment of gliadin. This region is mostly responsible for immunotoxicity of gluten. G12 antibody-based sandwich ELISA gave very promising results in tests of various samples, with the detection limit of 0.6 ng/ml. Moreover, the obtained results were well correlated with immunotoxicity of cereals from which the compound had been extracted. Also, a competitive variant of the ELISA assay with the G12 antibody has been developed. The method is highly sensitive, with the detection limit of 0.44 ppm for gliadin. It is also highly reproducible [4].

Another group of methods is based on detection of genes responsible for production of gluten components using the polymerase chain reaction (PCR). The most rapid and convenient techniques are based on quantitative real-time PCR. Contrary to classic PCT, with application of fluorescent techniques quantitative PCR allows for the monitoring of the amount of a product in real time. Due to that, the whole analysis procedure is relatively fast and allows for elimination of the stage of product estimation after the end of reaction. The method enables detection of DNA already at the level of 20 pg DNA/mg of the analyzed material [5]. Comparison of this test with the R5 ELISA assay demonstrated that it was able to detect DNA in products in which gluten level was below the R5 ELISA detection limit.

The last group of methods is based on the use of biosensors providing a measurable signal proportional to concentration of the assayed analyte. Biosensors based on absorption, fluorescence or surface plasmon resonance (SPR) are most commonly used. The SPR technique is highly effective, provides results in real time, may be automated, and is highly sensitive. The technique offers a reduced time of analysis and automation [6].

All the discussed techniques are used by the Jagiellonian Center of Innovation for the rendered services.

Literature:

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