

ORGANIZATION OF LABORATORY FOR MONITORING SECURITY IN THE FOOD INDUSTRY IN ORDER TO DETECT THE PRESENCE OF ALLERGENS

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Abstract: Allergens are substances that cause allergic reactions. Allergic reactions differ from person to person in a sensitive and specific response to the presence of the same allergen. Groceries that often cause allergies are cow's milk, eggs, fish, crustaceans and shellfish, wheat, soy, peanuts, walnuts, almonds, hazelnuts and strawberries.

Organisation is the main factor for the success and the quality of a research in food industry laboratories, in order to detect the presence of allergens. All kinds of equipments are needed, as well as professional staff to perform the tests.

Allergen testing in the food industry is often performed using biochemical and separation methods. For analysis of deoxyribonucleic acid (DNA), the most suitable method is polymerase chain reaction (PCR) and electrophoresis. In our laboratory, we use immunological methods for qualitative and quantitative testing of allergens and we have two accredited methods: Enzyme-Linked Immunosorbent Assays (ELISA) and High Performance Liquid Chromatography (HPLC).

It is also necessary that staff have adequate competence in handling the specific equipment, performing tests, evaluating the results and signing test reports and calibration certificates, have adequate competences.

Laboratory have to prove that have been fulfilled all the requirements for validation. Validation includes: specification of requirements, characterization of method, verification that requirements can be fulfilled using the method.

The results of each test are presented in form of a report, which has to be correct, clear, unambiguous, objective and must include all the informations required by the client.

Key words: Organisation, Laboratory, Food industry, Allergens

Introduction (Organisation of laboratory)

Success and quality of a research in the food industry laboratories, in order to detect the presence of allergens, depends on its organization. The laboratory must be accredited in order to receive a license. Accreditation is done according to the ISO 17025 standard.

Our laboratory is organized as an independent organization and it is legally responsible. Management of the laboratory has taken all action to enable that analysis for the presence of food allergens in our laboratory run smoothly. In this sense, the following actions were taken:

- Organizational and staff structure is clearly defined to avoid the mixing of competences and responsibilities,
- Documentation is classified and marked to ensure traceability of documents and informations, from beginning of the research to the final results,
- Working process is documented and documentation is arranged in the workplaces,
- Competence and skills of staff are maintained and their further development is planned,
- Condition of the existing equipment is monitored,
- Equipment maintenance is planned,
- Plan for purchase of new and write off old equipment and
- Warehouse for the storage of samples is provided (JUS ISO 17025:2001).

Determination of the presence of allergens in food, consists of several stages:

- Receiving the total food sample and creating test samples,
- Recording samples,
- Preparation of the test samples,
- Analysis of the samples,
- Analysis of the test results,
- Control of the tests that are carried out,
- Verification of the obtained results and
- Reporting on the results obtained by testing (JUS ISO 17025:2001).

Scheme 1. Laboratory scheme

Reception, record and sample prepara- tion	Sensory analysis	Washing and sterilization of laboratory dishes and supplies	Hall	Physico-chem- ical analysis	Room for chromatog- raphy	Room for ELISA	Warehouse for chemicals, sup- plies and equip- ment
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Equipment

Laboratory has provided all kinds of equipment, for sampling, measuring and testing, as well as professional staff to perform tests in order to get accurate test results. Equipment and softwares used for testing, calibration and sampling, can bring appropriate accuracy. Before being placed into service, equipment must be calibrated and tested in order to establish compliance with the requirements of the laboratory specifications. The equipment is double checked immediately before use.

Equipment is operated by authorized staff. Competent staff can easily access to the instructions for use and maintenance of equipment. They have to keep records of each piece of equipment and its software. The records contain the following:

- Identification of parts of the equipment and its software,
- Name of the manufacturer, serial number,
- Compliance of equipment with the specification,
- The manufacturer's instructions or guidelines for their finding,
- Dates, results and copies of reports and certificates of all calibrations, settings and eligibility criteria,
- The maintenance plan and
- Any damage, failure or repair of the equipment (JUS ISO 17025:2001).

In the laboratory, there are procedures for safe handling, transportation, storage, use and planned maintenance of equipment to prevent contamination or damage. Equipment that was mishandled and that is damaged, must be withdrawn from use. In order to prevent its further use, this equipment must be separated and clearly indicated that it is out of use until it is repaired.

LIST OF EQUIPMENT NEEDED TO DETERMINE ALLERGENS

Allergens are antigens which cause allergic reactions. Most food allergens belong to a group of proteins that are resistant to heat, proteolytic enzymes, and pH change. However, the immune system of a person can react in a very small amount of allergen (quantity in ppm). Different persons have a different sensitive and specific response to the presence of the same allergen. Avoiding contact with the allergen is

the only way that people can help prevent allergic reactions (Grujić, 2015).

Groceries that often cause allergies are cow's milk, eggs, fish, crustaceans and shellfish, wheat, soy, peanuts, walnuts, almonds, hazelnuts and strawberries. In adults, approximately 90% of allergic reactions to food are caused by peanuts, nuts, fish and shellfish, and for children, eggs, milk, soy and flour (www.tehnologijahrane.com).



Figure 1. Food that most often cause allergies (Anonymous, 2015)

In our laboratory quantitative and qualitative determination of food allergens is carried out (eggs, milk, soy, peanuts). Two accredited methods are used:

- Biochemical method - ELISA and
- Separation method - HPLC chromatography.

As for equipment, these methods require two instruments: an instrument for ELISA and HPLC instrument. In addition to the basic, for a successful measurement an additional equipment and materials are necessary: allergen kit for extraction, filter paper, graduated cylinder for measurements (125 ml), microwell reader, bottles for preparation and washing of a solution, high-speed mixer, paper towels, waterproof marker, water bath or hot plate to maintain the temperature, analytical scale, membranes for filtration, automatic titrator, fume hood (Anonymous, 2010; Noack group, 2011).



Figure 2. Microwell reader (Anonymous, 2010)

Microwell reader is easy to use. Interactive LCD screen is a touch screen with USB mouse option. It enables automatic calculation of results and permanent data storage. It has possibility of entering more than 120 tests (Anonymous, 2010).

HOUSING CONDITIONS AND ENVIRONMENT

Laboratory equipment for testing or calibration, including energy sources, lighting and environmental conditions, allow the proper carrying out tests. Environmental conditions must not endanger the results or adversely affect the required quality of the measurement. Special attention must be paid to sampling, testing and calibration, especially if it is done outdoors. It is necessary to document technical requests, related to the housing conditions and the environment (JUS ISO 17025:2001).

In the laboratory, it is necessary to monitor, control and write environmental conditions. Special attention is focused on biological sterility, dust, electromagnetic disturbances, radiation, humidity, electrical supply, temperature and sound level. These are factors that can affect the measurement of allergens in food.

If the environmental conditions endanger the results, the test must be canceled. Environment, in which are organized activities that are incompatible with the examination, must be effectively separated. It is necessary to control access and use of space, which affect the quality of testing (JUS ISO 17025:2001).

Analysis of Required Staff

The management of the laboratory has provided the competence of all personnel handling the specific equipment, performing tests, evaluating the results and signing test reports and calibration certificates. The personnel that is trained must be under the supervision of a competent person and be habilitated on the basis of appropriate education, experience and skills for performing certain tasks.

The staff responsible for providing opinions and interpretations of test reports, in addition to appropriate qualifications, training, experience and sufficient knowledge of the tests that are performed, should also:

- Possess adequate knowledge about the technology of production of the product which are tested or the manner of their use, as well as any defects or degradation during the use,
- Know the general regulations and standards and
- Understand significance of discrepancies, relating to the use of the products (JUS ISO 17025:2001).

Laboratory has a permanent staff or contract staff. If it is hired additional technical staff and support staff, it is necessary to ensure the supervision of such staff and their competence. Laboratory shall maintain records of work descriptions for managerial, technical and support staff involved in the study. Work descriptions include:

- Responsibilities for planning and evaluation of test results,
- Responsibilities for providing opinions and interpretations,
- Responsibilities for modifying methods, development and validation of new methods,
- Necessary knowledge and experience,
- Training programs and
- Manager responsibilities.

Staff working in the laboratory has undergone allergy tests before the start of the analysis. People who are not sensitive to the presence of allergens are selected to perform the analysis.

Management has appointed specific staff to do particular types of sampling, testing or calibration, as well as for the issuance of test reports, giving opinions and interpretations and working with certain types of equipment. The laboratory shall keep records of the relevant authority, competence, education and professional qualifications, training and experience of the entire technical staff as well as staff under contract. In electing the staff for testing in laboratories, preference is given to people with experience and those who have already worked the same or similar jobs. This information must be easily accessible and shall contain data about the confirmation of the authorization or competence (JUS ISO 17025:2001; Grujić, 2015).

Laboratory staff in the food industry, that are doing tests for the presence of allergens, is: employee on administrative duties, records and preparation of test reports, employees on the performance of physical and chemical analysis, employee on the preparation and storage of samples and additional tasks in the laboratory (JUS ISO 17025:2001).

Sampling

Laboratory owns a plan and procedures for sampling in determining food allergens. The plan consists in the fact that materials, semi-finished or finished products are mixed, milled and separated. Working surfaces and equipment should be cleaned prior to production. It is necessary to prepare the extraction solu-

tion or to use one that has already been prepared. A method for determining the presence of food allergens consists in preparation of the reagent, after which is held for 30 minutes at room temperature. After that, the sample is taken, a buffer is added, mixed and the test is carried out. Reading the result follows at the end (Noack group, 2011).

Plan and sampling procedures must be available at the place where samples are taken. Whenever it's possible, sampling plans must be based on the appropriate statistical methods. Sampling process indicates the factors that need to be controlled to ensure the validity of the results and calibration.

Sampling is process of taking a portion of the substance as a whole, for the purposes of testing. Sampling procedures should describe the sampling plan and preparation of the sample or samples, in order to obtain the necessary information. There have to be procedures for writing relevant data about the activities related to the sampling. Records should include a procedure that was used for sampling, identification of the person who has carried it out, environmental conditions and diagrams or other ways to identify the place of sampling (JUS ISO 17025:2001; Anonymous, 2010).

HANDLING THE SAMPLES FOR TESTING

Accredited laboratory has procedures for the transportation, receipt, handling, protection, storage and preservation of samples for testing. It is necessary that the samples, which get into the laboratory for testing, have the specification of their identity. Specification contains both physical and chemical characteristics, labeling, bacteriological requirements, criteria of purity, *ie.* the absence or permitted amount of foreign bodies (www.tehnologijahrane.com).

After receiving the sample for testing or calibration, it is necessary to write down any irregularities or deviations from normal or specified conditions. It is necessary to prevent any damage, loss or contamination of the sample for testing, during its storage, handling and preparation. It is essential that there is adequate guidance for the sample to be tested. If the samples need to be stored under specific environmental conditions, these conditions shall be maintained, monitored and recorded. Sampling procedure and information on storage and transport of samples, including information affecting the result of the test, should be available to those responsible for receiving and transport (JUS ISO 17025:2001).

Testing Methods

Laboratories in the food industry use appropriate methods for all tests, within the boundaries of their jurisdiction. They use a validated test methods, including methods for sampling, which meet the needs of the client and are suitable for testing in laboratories. In our laboratory immunological methods for qualitative and quantitative testing of allergens are used. For qualitative detection of food allergens we use immunochromatography, immunoblotting, immunoelectrophoresis; and for quantitative detection, we use ELISA. When it comes to DNA methods, qPCR is used for the qualitative and quantitative analysis (Noack group, 2011).

Immunochromatography method is used to determine allergens in almonds, gluten, hazelnut, shell, casein, eggs, peanuts, soybeans; and ELISA test is used for anlysis of peanuts, eggs, milk, hazelnut, almonds, gluten, histamine, soy, mustard.

If the client does not require a test method, laboratories may select appropriate methods that have been published as an international, national or regional standard, or have been published by some technical institution or published in relevant scientific papers or magazines or specific manufacturers of the equipment. Nowadays, the most commonly used method is ELISA, because it is an internationally recognized, fast method and the results are relevant to HPLC method (JUS ISO 17025:2001).

To determine the allergens in our laboratory, we use the following methods: *in vitro* determination of specific antibodies in food products using conventional physico-chemical and biochemical methods, *in vitro* determining the content of general and specific IgE antibodies; testing reaction to allergens in animals and clinical trial patient response, using different tests on the skin, respiratory or digestive organs.

For the analysis of the allergens can be used various methods. Classical physico-chemical and biochemical methods, which are used to test protein, are as follows: determination of nitrogen according to Kjeldahl method, nephelometry, colorimetry, chromatography (SEC, ion-exchange chromatography, affinity chromatography, HPLC, FPLC), electrophoresis (SDS-PAGE, capillary electrophoresis, 2D-electrophoresis), spectrophotometry, mass spectrometry, and PCR (DNA specific for the allergens). Immunological methods that are in use: counter electrophoresis, immunoblotting, immunodiffusion test for identification of enzymes related to immunosorbent assay (ELISA), enzyme-linked immunospot assay (ELISPOT), radiological tests (Grujić, 2015)

BIOCHEMICAL METHODS

Testing of allergens in the food industry is often performed using biochemical methods, which detect immunoenzyme reaction. The most commonly used are ELISA and Western blot analysis (Butorac et al., 2013).

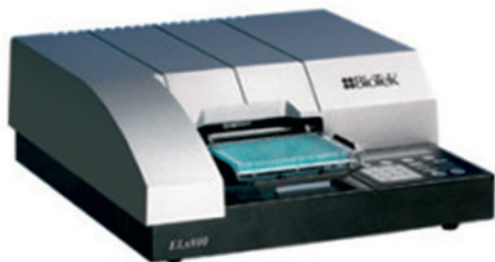


Figure 3. Enzyme-Linked Immunosorbent Assays (ELISA) (Anonymous, 2015a)

Elisa

Enzyme-Linked Immunosorbent Assays (ELISA) have important applications in a clinical diagnostics, while their use in detection of food allergens is intensively applied in the past decade. Immunochemical methods are based on the use of specific detectors for allergen in food, ie. an antibody recognizes an antigen. The enzyme converts the colorless substrate to a colored product (Butorac et al., 2013).

These methods are fast, semi-quantitative, selective and sensitive methods which are suitable for a large number of samples and does not require long-term preparation of samples. These are some of the advantages of these methods:

- In the food industry, these methods are used to identify food allergens (milk, peanuts, hazelnuts and eggs), pesticides, mycotoxins and pathogens in a sample and
- Half an hour after the reaction is complete, it is possible to read out the results (Grujić, 2015; Butorac et al., 2013).

The disadvantages are:

- Long development time,
- Difficulties in detecting problems during the test and
- Possibility of cross-reactions.

Kits, commercially offered, differ over the threshold of detection of allergens. The obtained results are compatible with the results obtained by HPLC.

Western blot analysis

Western blot analysis is used to detect specific proteins from the sample. Sample proteins are separated with sodium-dodecylsulfate polyacrylamide gel electrophoresis on the basis of their molecular weight.

After that, they are transferred to a nitrocellulose membrane, a nylon or synthetic, and exposed to the specific primary antibody. Specifically labeled secondary antibody binds on the (primary) antibody, which is detected as light emission at the site of the specific binding, *ie.* dark line.

Western blot analysis is obligatory method for detecting food allergens by binding immunoglobulin E (IgE) to allergens (Butorac et al., 2013).

MOLECULAR-GENETIC METHODS

Molecular genetic methods are based on the analysis and amplification of deoxyribonucleic acid (DNA). For the analysis of DNA is mainly used polymerase chain reaction (PCR) and electrophoresis on agarose or polyacrylamide gels.

Methods and modifications of PCR

PCR is fast, simple and specific method, which uses a variety of thermostable DNA polymerase enzymes, for reproduction of a specific part of the DNA molecule. For the PCR reaction is necessary to prepare a mixture, consisting of: DNA to be tested, thermostable polymerase, primers and the same concentration of all four deoxynucleoside triphosphates.

Primers are short, specific oligonucleotides with sequences complementary to the part of DNA that needs to be amplified. Each PCR cycle consists of three stages: denaturation, elongation and termination. By using this method allergens present in almond were detected (Butorac et al., 2013; Nielsen, 2010).

SEPARATION METHODS

Liquid Chromatography

Liquid chromatography is separation method used to separate the products between two phases: stationary and mobile phase. For the determination of certain food ingredients, it is commonly used high performance liquid chromatography (HPLC) (Butorac et al., 2013; Nielsen, 2010a).

The most commonly used chromatographic separation technique is chromatography of the reverse phases. It uses a polar solvent as a mobile phase and the stationary phase is non-polar, silica gel covered with a long chain hydrocarbons. HPLC is used for the determination of protein, organic acids, vitamins, amino acids, sugars, additives, mycotoxins, pesticides, antibiotics, lipids and pigments (Butorac et al., 2013; Nielsen, 2010a).

Capillary electrophoresis

Capillary electrophoresis is separation technique where the products are separated based on differences in their electrophoretic mobility. The separation can be carried out based on differences in charge and mass, isoelectric point or molecular weight. It is used for the analysis of proteins, peptides, carbohydrates, nucleic acids, inorganic ions, viruses and micro-organisms which are found in food (Butorac et al., 2013).

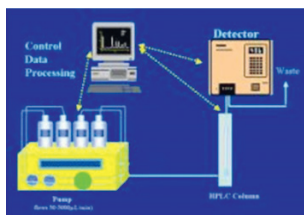


Figure 4. Liquid chromatography (Anonymous, 2015a)



Figure 5. HPLC chromatography (Anonymous, 2015a)

Validation of Methods

Validation is verification that have been fulfilled individual requirements for a specific use, by testing and presenting objective evidences. To confirm the suitability of the methods for the intended use, laboratory validated non-standard methods, which were developed in the laboratory, as well as standard methods used outside the predicted area of use or modified standard methods. Laboratory documents the obtained results, procedures used for validation, and statement that the method is suitable for the intended purpose.

Validation may include procedures for sampling, handling and transportation. In case of introducing some changes in the validated non-standard methods, the impact of these changes is necessary to be documented. After the estimation of the purpose, scope and accuracy of the values that can be obtained by the validation methods, they must be in accordance with the client's needs (JUS ISO 17025:2001).

Validation includes: specification of requirements, characterization of method, verification that requirements can be fulfilled using the method.

EVALUATION OF THE MEASUREMENT UNCERTAINTY

Laboratories in the food industry that are performing tests for the presence of allergens have to apply the instructions for estimation of measurement uncertainty. In certain cases, the nature of the test methods can prevent severe and statistically significant calculation of the measurement uncertainty. In that case, the laboratory should try to identify all the components of uncertainty, make the estimation acceptable and ensure that the way of reporting the results does not provide a false impression about uncertainty.

Estimation of the measurement uncertainty depends on: demands of the test methods, customer requirements, existence of narrow limits on which are based decisions about the compliance with the specification. When assessing the measurement uncertainty, there should be taken into account all the components of uncertainty that were significant at a time (JUS ISO 17025:2001).

Sources contributing to the uncertainty include: reference materials, methods, equipment, environmental conditions, properties and condition of the sample during the analysis, as well as the perpetrator.

Reporting

The results of each test or series of tests made by the laboratory, must be presented accurately, clearly, unambiguously and objectively. The results are presented in the form of a report and must include all information required by the client, which are necessary for the interpretation of test results, as well as information required by the method.

Each test report includes:

- Title (test report),
- Name and address of the laboratory, a place of testing, if it differs from the address of the laboratory,
- Unique identification of test report (serial number) and identification of each page that allows its recognition as a part of the test report,
- Name and address of the client,
- Identification of used methods,
- Date of receipt of the test sample, if it is crucial to the validity and application of the results, and dates of performing tests,
- Plan with sampling procedures,

- Results of the tests with measurement units, and
- Names and surnames, functions and signatures of the persons who have approved the test report (JUS ISO 17025:2001)

The test report as a permanent record should include and total number of pages. If the interpretation of test results is required, test reports include:

- Deviations, additions or exclusions, compared to the test method and information about the special conditions,
- Statement about the estimated measurement uncertainty, and
- Additional information that may be required by specific methods, clients or groups of clients.

Report should be adapted to each type of test, as well as to reduce the possibility of misunderstanding or misuse. After the issuance of the document, its amendments must be made in the form of another document.

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