

MONITORING OF RESIDUAL CONTENT OF ANTIBIOTICS IN FOOD

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ANTIMICROBIAL AGENTS IN VETERINARY

- Agricultural production includes some antimicrobial agents in veterinary medicine for prevention and treatment of livestock and poultry. However, due to their lack of efficiency, in some cases, manufacturers should apply antibiotic substances used for the treatment of humans (derivatives of tetracycline, penicilline, levomicetine, sulfonamides, furaxane, furazolidon and so on).

PROBLEMS OF ANTIMICROBIAL AGENTS

- The use of these drugs may lead to the subsequent development of a sustainable data substances of human microflora consuming foods that contain antibiotics. A person develops a goiter, and appointing him the antibiotic treatment is a high risk of treatment failure. Possibility of such meat products while importing, as well as the development of intensive livestock and poultry makes the problem of control highly relevant.

Chloramphenicol (levomycetine)

- Synthetic broad-spectrum antibiotic, which is prohibited for use in animal husbandry. The relative cheapness of product and high antibacterial efficacy leading to his unauthorized use of a fairly large scale, therefore in meats, liver, kidneys, eggs and other products frequently detected residues of levomycetine at concentrations ranging from 0.02 - 0.50 units/g of sample.
- 1 unit of activity corresponds to 1 mg of pure substance.

TETRACYCLINE

- A broad-spectrum antibiotic that is highly effective and used in medicine for the treatment of various diseases, as well as in veterinary medicine due to the high antimicrobial efficiency (chlortetracycline, oxytetracycline) that is unsafe in terms of sustainability of the microflora to the human antibiotic, consuming food products contaminated tetracyclines. MPC of tetracycline groups antibiotics residue in meat is 0.01 units/g. 1 unit corresponds to 1 μg . Russian Federation safety requirements of antibiotic residue content in meat products is not permitted and regulated at the level of 0,01 unit of the antibiotic activity (μg) in one gram of the sample.

SULFONAMIDES

- Sulfonamides is a group of chemicals derived from para-aminobenzolsulfamid. Para-aminobenzolsulfamid is the simplest connection class — also called white streptocide and is used in medicine so far. The result of sulfonamides action is a violation of the synthesis of nucleic acids in bacteria.
 - Many side effects of sulfonamides (sulfamethoxazole, sulfadimidine (synonyms sulfamethazine, sulfamezathine), sulfaquinoxaline) in human organism.

CIPROFLOXACIN

- Ciprofloxacin (according UPAC: 1-cyclopropyl-6-fluor-4-oxo-7-pyperasine-1-quinoline-3-carbonic acid) a synthetic antibacterial effective against many gram-positive and gram-negative bacteria.
- A synthetic broad-spectrum antibiotic of the fluoroquinolone class that inhibits enzyme deoxyribonucleic acid (DNA) gyrase needed for replication of DNA.
 - Many side effects of Ciprofloxacin in human organism.

RUSSIAN LEGISLATION

- In Russian Federation there is The State Standard (GOST R 55481-2013, introduced the 01.07.2014) “Meat and meat products. Qualitative method for detection of antibiotics residues and other antimicrobial chemotherapeutic agents”. This standard applies to all types of animal slaughter meat, poultry meat, offal and sets the microbiological method for qualitative determination of residues of antibiotics and other antimicrobial chemotherapy substances.
- There is The State Standard (GOST R 51447-99 (ISO 3100-1-91), introduced the 05.12.2009) “Meat and meat products. Methods of sampling” as well, where the quantification of residual antibiotics carried out by the internal standard peak area according to the amount of samples.

RUSSIAN PRACTICE

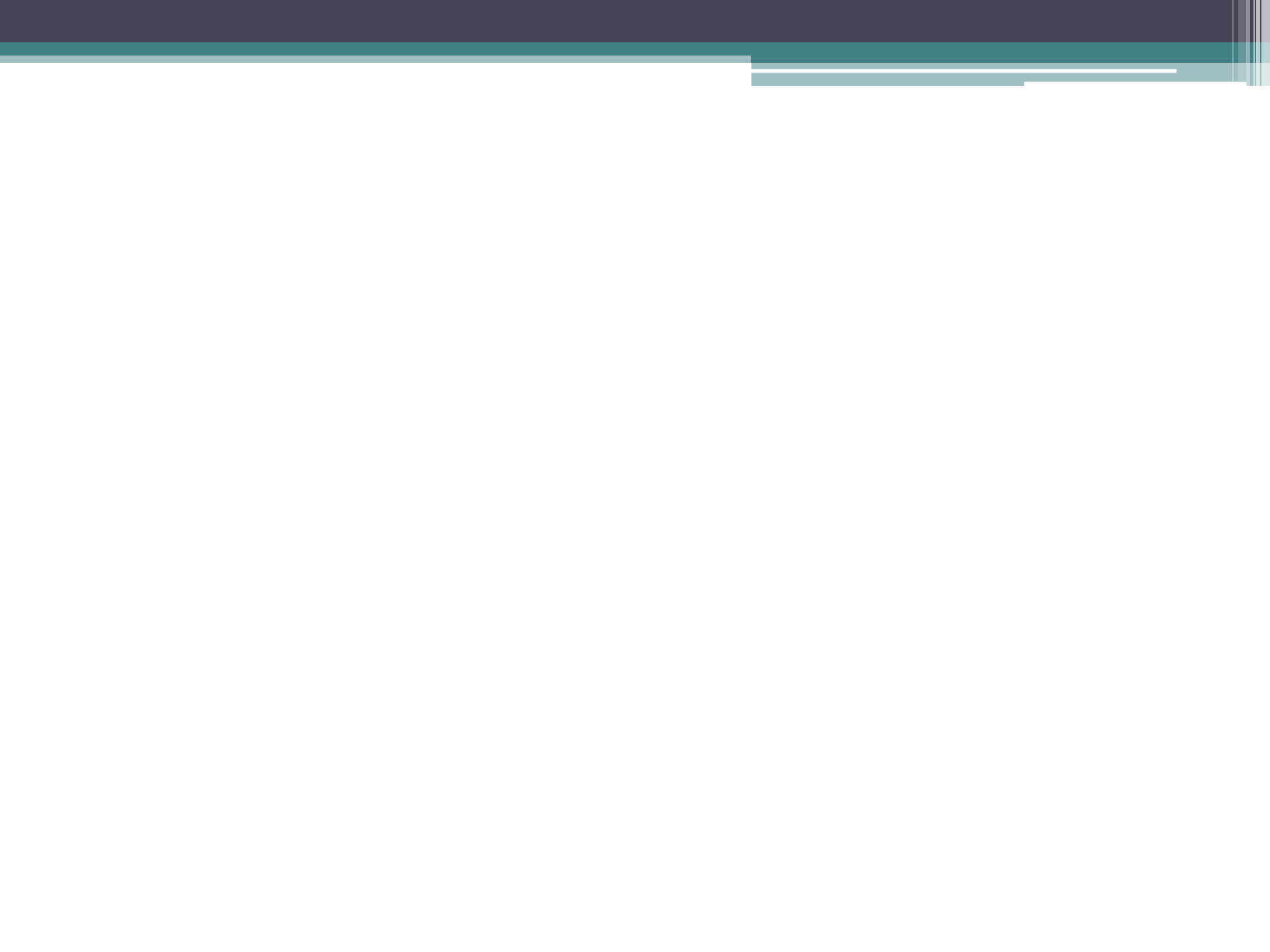
- Currently for analytical determination of residues of antibiotic drugs they use microbiological methods based on registration growth test cultures of microorganisms in the presence of the standard quantities of antibiotics or extracts; high performance liquid chromatography (HPLC); liquid chromatography with mass spectrometry (LCMS); thin-layer chromatography (TLC), which allows to register the appearance of individual spots of the test material; fluorescent analysis based on the formation of fluorescent complex antibiotic with special organic chromophore.
 - Gas chromatographic method was not used because of the complexity of the transfer of antibiotics in the volatile state.

THERE ARE A NUMBER OF PROBLEMS

- First, the chromatographic identification involves the review of specific substances on the residence time of chromatographic peak. We know a significant number of reasons why the retention times may vary and even match for certain substances. In the case of very low concentrations of substances, this feature is often insoluble problem, even with the use of internal standard defined substances. Leading manufacturers of chromatographic equipment offer to secure the double identity of a substance in accordance with peak use, for example, the UV-spectrum or mass spectrum of substance followed by comparison with a standard database of computer data. **This approach to the determination of the residue of dangerous impurities in the raw food is reliable enough, but requires very expensive analytical equipment and could not be recommended for mass analysis.**

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

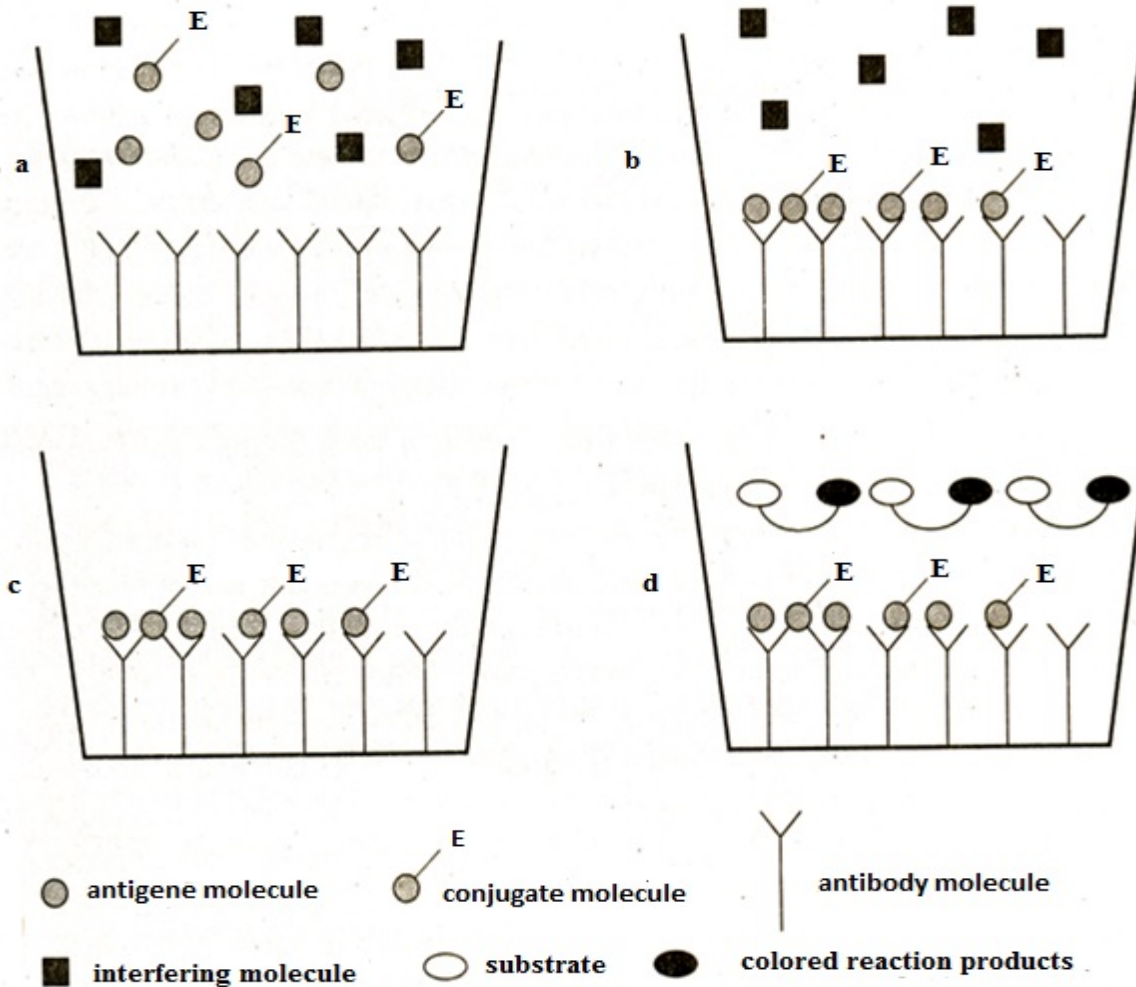
- Taking into account the requirements of rapid methods for monitoring food (sensitivity, selectivity of the method, results, speed of execution, cost of analyses), the most preferred method is immune-enzyme analysis ELISA - method that meets all the requirements for methods of routine control.
- For the analysis of chemical compounds in food and biological objects they use mainly a variant of the "competing" immune-enzyme analysis, therefore, it is only on this version of the ELISA we stay.



SCHEMATIC PROCESS OF ENZYME IMMUNOASSAY (ELISA)

- Ready made kits (sets) for ELISA in the complete supply manufacturer typically includes all the necessary materials to perform the analysis, buffer and standard solutions. Antibodies to a particular chemical compound (antigen) blood serum obtained from living organisms are adsorbed on a solid surface of the Tablet (media), usually at 96 holes.

SCHEMATIC PROCESS OF ENZYME IMMUNOASSAY (ELISA)



SCHEMATIC PROCESS OF ENZYME IMMUNOASSAY (ELISA)

- a-the start of immunosorbption process; b- the completion of the immunosorbption; c- the tablet washing; d- the chain reaction development.
- During the contact of media activated surface with a solution containing antigens (e.g., tetracycline), part of the antibodies specifically interacts with molecules of controlled compounds, i.e. deactivated. In a competitive ELISA version controlled solution is mixed directly into the hole of the tablet with a solution of the so-called conjugate (antigen molecules), which is chemically linked (labeled) with molecules of the enzyme. Over a period of incubation of the tablet (from 30 minutes to 2 hours) at a certain temperature antibodies on the surface of the media are deactivated as a result of the immunosorbption of labeled and no labeled antigens (fig.c).

SCHEMATIC PROCESS OF ENZYME IMMUNOASSAY (ELISA)

- After the procedure of the tablet washing following the incubation, on the surface of supporter there are only adsorbed antigens, and the ratio of labeled and no labeled antigens depends on the concentration of antigens in the controlled solution (fig. c).
- At the stage of developing a solution of the so-called substrate is added into tablet holes.
- The enzyme molecule fragment adsorbed from solution conjugate together with the labeled antigen on the surface of the holes, catalyzes a chemical reaction making the substrate in the colored compound (fig. d).

SCHEMATIC PROCESS OF ENZYME IMMUNOASSAY (ELISA)

- After a certain time the color reaction development a fixative reagent is added into tablet holes and the optical density of the contents of each hole is measured . After the optical density measuring in holes it's easy to build a calibration curve automatically or manually and then you can calculate the concentration of the controlled test compound in the controlled trial.
- Applied analytical methods for determination of antibiotics distinguish by the minimum detectable level of different substances depending on the antibiotic properties (table 1). For analytical determination of chemical toxicants complex in food using methods differ in complexity and sensitivity.

THE MINIMUM LEVELS OF ANTIBIOTICS IN MEAT PRODUCTS DETERMINED BY DIFFERENT METHODS

Method	Determined substance	Detection limit	MPC, no more	Analysis time, h
TLC	Tetracycline	0,1 µg/g	0,01 units/g	5
fluorescence spectroscopy	Tetracycline	1 µg/g	0,01 units/g	4
ELISA	Tetracycline	6 pg/g	0,01 units/g	3
ELISA	Levomicetine	1 ng/g (µg/kg)	0,01 units/g	1

CONCLUSION

- The most successful combination of sensitivity, speed and cost is ELISA method that allows fast enough to hold screening of food products by the maximum number of indicators. The standard methods loose to the method of ELISA for simplicity and minimal detectable level of the test material. This method enables you to determine the content of harmful impurities at a level of 0.1 ng/ml.
- The most justified is the application of the method of ELISA to determine the complex organic toxicants, including hormones and antibiotics.



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