IDENTIFICACION DE ESPECIES COCIDAS POR ELISA EN ALIMENTOS BALANCEADOS
IDENTIFICATION OF COOK SPECIES BY ELISA IN FOOD BALANCED

INDEX

1. OBJECT:

Identification of specific proteins of animal origin for enzimoinmunoanálisis (ELISA) in cooked products.

2. IT REACHES:

Foods balanced for animal consumption.

3. AFFECTED AREAS:

Department of Evaluation and Development.

4. RESPONSIBILITY:

The Chief of Department: responsible for the execution of this document.
The Analyst: responsible for their execution.

5. RELATIONS:

ED-INS Nº001: "The microplate reader's control"
ED-INS Nº011: "General instructive for the analysis of Identification of Species"

6. DEVELOPMENT:

6.1. EQUIPMENT:

6.1.1. Scale granataria, sensibility: ± 0.01g.
6.1.2. Microplate reader with filter of 405 nm.
6.1.3. Vacuum Pump
6.1.4. Centrifugate
6.1.5. Grinder for mill of cereals.

6.2. Materials:
6.2.1. Automatic Micropipette 20-200 ml.
6.2.2. Automatic Micropipette of 8 channels of 20-200 ml.
6.2.3. Kit for identification of cooked species of the species to be analyzed.
Basic components: § Module of 96 wells covered with the specific antibody for each species. § Positive Control of each species § Antibody specific biotinilado of each species enzymatic Conjugated § Streptavidin-peroxidase Concentrated § ABTS (substrate) § Buffer peroxide-citrato (diluter of the substrate) § Solution stop § laundry Solution
The kit should have to be stabilized to room temperature before its use.
6.2.4. Filter paper.
6.2.5. funnels.
6.2.6. Rehearsal tubes.
6.2.7. Glasses of precipitate of 250 ml.
6.2.8. test tube of 100 ml.
6.2.9. Sieve of stainless steel of 2 mm. of opening.
6.3. drugs:
6.3.1. chloride of sodium, NaCl p.a., PM=58.44
6.4. Reference materials:
Not applicable
6.5. Reagents means and solutions:
6.5.1. Saline solution:
To dissolve 9 grams of chloride of sodium in distilled water and to take to volume in flask appraised of 1000 ml.
6.5.2. Preparation of the reagents and solutions of the kit:
6.5.2.1. Laundry solution:
To dilute 1:10 the concentrated laundry solution. To use distilled water as diluter.
6.5.2.2. Solution substrate:
To dilute 1:25 the concentrated ABTS, in the buffer citrato-peroxide.
6.6. Preparation of the sample:
6.6.1. Of being necessary, to use sieves for the separation of grasses, cereals, etc.
6.6.2. to mill the sample if it is in pellets shape. To homogenize for blended.
6.6.3. To weigh to the hundredth of gram, 35 g. of the sample selected in a glass of precipitation of 250 ml.
6.6.4. To add 100 ml. of solution 0.9% of NaCl.
6.6.5. To allow to rest 15 minutes at room temperature with eventual agitation. To filter.
Of being necessary to centrifuge to 3000 rpm for 10 minutes and to take the float.
6.6.6. The obtained extract is conserved until the seeding moment (in the refrigerator up to 36 hours. or in freezer during three months).
6.7. Calibrations and/or verifications:
Scale, thermometer, microplate reader and automatic pipettes.
6.8. Execution of the rehearsal:
6.8.1. Place 100 ml of the following controls for each species: · positive Control · negative Control in twelve wells (the control can be used positive of the other species). to Indicate the species used in the seeding protocol.

6.8.2. Seed 200 ml of the extracts of each sample obtained in 6.6.6.
6.8.3. Cover the wells and leave it at the working temperature during 1 hour.
6.8.4. Wash 3 times with laundry solution, filling and emptying the wells.

6.8.5. Extract the liquid in the last laundry for extraction with the vacuum pump. Taking care of not touching the bottom of the wells. To dry off with absorbent paper.
6.8.6. Add to each well 25 ml of the conjugated biotinilado anti-species corresponding to the species to identify. To cover the wells and to leave to the working temperature during 1 hour.
6.8.7.Wash 3 times with laundry solution, filling and emptying the wells.
6.8.8.Extract the liquid in the last laundry for extraction with the vacuum pump, taking care of not touching the bottom of the wells. To dry off with absorbent paper.
6.8.9.Add to each well 25 ml of the one conjugated enzymatic. To cover the wells and to leave at the working temperature during 30 minutes.
6.8.10.Wash 6 times with laundry solution, filling and emptying the cells.
6.8.11. Extract the liquid in the last laundry for extraction with the vacuum pump, taking care of not touching the bottom of the wells. To dry off with absorbent paper.
6.8.12.Add to each well 50 ml. of the solution substrate prepared in 6.5.2.2. Cover the wells and leave it at the working temperature during 30 minutes.
6.8.13.Add to each well 50 ml. of the solution stop.
6.8.14. Read the absorbance's at 405nm. in the microplate reader. The reading of data has to be registers in the chart "I Rehear: Species" emitted by the microplate reader.
6.9. Critical points of control:

Point critical Value acceptable Registration
1 - Temperature of work 20-25ºC Notebook of the analyst
2 - Times of incubation 1hour or 30 minutes as it corresponds Notebook of the analyst
6.10. Results (See Annex III):
6.10.1. It is establish a cut off value above which the samples are positive for each analyzed species.
NOTE : A positive result is confirmed by reiteration of the analysis in another shift of samples.
6.10.2. Source of uncertainty:
a) To consider : heavy of the sample, solution volume for the extraction, temperature of kits storage and extracts, temperature of the rehearsal, preparation of solutions of the kit, times of incubation, seeding volumes and repetitively of the same ones, readings of optic density and repetitively of the same ones.
b) Not to consider: Taking of sub sample for the analysis, temperature of Prosecution of the sample, laundries, extraction process.
Court value = half value of the negative controls + 3 deviations standard

7. I REGISTER AND FILE:
The seeding protocol, Nº used kit, times of incubation, ambient temperature to the one beginning and finalization of the rehearsal and thermometer with which the temperature is measured and registered in the analyst's notebook.
The chart "Rehearse: Species" emitted by Elisa's Reader it is filed next to the "Schedule of calculation for the identification of species in balanced foods" in the portfolio "Identification of species - cooked Products - Data and results."

For samples of the program of prevention of BSE the result registers in the one corresponding notebook of revenues of samples (AP-BSE-0014).
8. ANNEXES:
Annexed Nº Titles Quantity of Pages
Annexed I (F000) Listing of Distribution of the Procedure 1
I annex II (F001) Leaf of Modifications 1
I annex III calculation Schedule for the identification of species in balanced foods
1
9. HISTORICAL: § Procedure: "Identification of species in products cooked for enzimoinmunoanálisis" (version 30/09/03). § Version 001(01/03/04) § Version 002(21/07/04)
10. REFERENCES: - ELISA-TEK Microwells kit, Cooked meat speciation kit, Instructions for uses. (5106*1
rev.ds31020) - Ronald G. Berger, Identification of animal species in cooked and canned meat and poultry products, USDA/FSIS, Microbiology Laboratory Guidebook, 3rd ed., 1998, 17:1 -
11). - BIOKITS, Cooked species identification test kit, Instructions for uses. Version 03/03 - Ansfield et to the, Production of to sensitive immunoassay for detection of ruminant and porcine, heated to> 130ºC at 2.7 bar, in Animal compound Feedstuffs, Food and Agricultural Immunology (2000), 12, 273-284. - Pallaroni, Bjorklund, von Holst, Unglaub; Determination of rendering plant sterilization conditions using to commercially available ELISA test kit developed for detection of cooked beef, Journal of AOAC. - Norma ISO-IEC 17025 (1999), Point 5.4

ANNEX III: Calculation chart for the identification of species in balanced foods
Date:
ANALYST: Species:
ABSORBANCE
Control negative species 1
Control negative species 2
Control negative species 3
Statistical parameters of the controls negative values
Half V.
desvest (n=12)
cv%
3DS
v.middle + 3DS (cut off value)
Absorbancia half Result observations shows it controlled: