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Qualitative detection of food parthogens, micotoxins and allergens using alternative methods

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Abstract

This paper presents the eight methods we validated within ICA- Assay Laboratory , concerning detection of three pathogens (*Salmonella, Listeria*, and *Staphylococcus*), micotoxins (aflatoxin, ochatoxin, DON) and allergens from milk and egg. Pathogens were detected using ELFA technique (enzyme linked fluorescent assay) and micotoxins and allergens were detected using ELISA technique.

Keywords: pathogens, micotoxins, allergens, ELFA, ELISA

1. Introduction

Introducing HACCP sistem in food industry of our country and legal regulations, compulsory after UE adheration are only two reasons that led to validation idea of some methods within ICA - Assay Laboratory. In this respect we compared alternative methods for pathogen detection with the reference methods. Micotoxins and allergens alternative methods were validated using standards.

2. Material and methods

Validation for alternative qualitative analysis method VIDAS Salmonella (VIDAS SLM).

The validation technique, for this alternative method, was comparison of performance of alternative method and the reference method (SR EN ISO 6579:2003).The Vidas SLM kit is an automated system using an enzyme immunoassay which detects *Salmonella* antigens using ELFA method (Enzyme Linked Fluorescent Assay).

The Vidas SLM methods consists of pre-enrichment in buffer peptone water followed by enrichment in selective broths for 6 to 8 hours followed by post-enrichment in M broth for 16 to 20 hours, after which the Vidas test is coonducted.

Each test is composed of two parts:

- the disposable SPR serves as the solid phase as well as the pipetting device for assay. The SPR is coated with anti-*Salmonella* antibodies, absorbed on its surface;
- the strip which contains all the ready to use reagents required for the assay.

There are tested 50 samples belonging to the following product families: milk, biscuit, vegetal fat, spawn and spawn salad fish, meat and meat products.

From this, 33 samples where natural samples and 17 samples where artificialy contaminated with a reference strain (*Salmonella anatum* ATCC 9720). The results obtained were interpreted according to the SR EN ISO 16140:2005 standard regarding to practicability, relative sensitivity and relative accuracy .

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Validation for alternative qualitative analysis method VIDAS Listeria monocytogenes 2 (VIDAS LMO2).

The validation technique, for this alternative method (Vidas LMO2), was comparison of performance of alternative method and the reference method (SR EN ISO 11290-1:2000).

The Vidas LMO2 test is an enzyme immunoassay test which detects *Listeria monocytogenes* antigenes using the ELFA method on the Vidas analyzers.

Each test is composed of two parts:

- the disposable SPR, which serves both as the solid phase and the pipetting device for the test. The SPR is coated with anti-*Listeria monocytogenes* antibodies absorbed on its surface;
- the strip, which contains all ready-to-use reagents necessary for the test: washing solution, alkaline phosphatase-labeled anti-*Listeria monocytogenes* antibodies and substrate.

The Vidas LMO2 method consists of an enrichment of half-Fraser incubated 24h to 26 h at 30°C±1°C, after which 1 ml of the suspension is transferred into 10 ml of undiluted Fraser broth, incubated 24h to 26h at 30°C±1°C. The Vidas LMO2 test is performed with an aliquot of undiluted Fraser broth.

In this study were tested 85 samples belonging to the following product families: milk, fish, meat and meat products, of which 44 samples where naturally contamined and 41 artificially contaminated with *Listeria moonocytogenes* ATCC 7644.

The results obtained were interpreted according to the SR EN ISO 16140:2005 standard, regarding to practicability, relative sensitivity and relative accuracy.

Validation for alternative qualitative analysis method VIDAS Staph enterotoxin II (VIDAS SET2).

The validation technique, for this alternative method, was comparison of performances of alternative method and the reference method (SR EN ISO 6888:2002)

The detection of staphylococcal enterotoxins mainly consists of two steps:

1. Extraction/Concentration: The sample is mixed and homogenised with distilled water. The toxins diffuse in water and are recovered, after two centrifugations, in the supernatant.This aqueous phase is concentrated overnight by dialysis;
2. Immuno-enzymatic detection

In this study where tested 56 samples belonging to the following product families: milk (40 samples), meat and meat products (16 samples), of which 27 samples where naturally contamined and 29 artificially contaminated. The results obtained were interpreted according to the SR EN ISO 16140:2005 standard, regarding to practicability, relative sensitivity and relative accuracy.

Validation of ELISA method for total aflatoxins determination.

The method proposed for intralaboratory validation within ICA-Assays Laboratory is an alternative analysis for quantitative determination of total aflatoxins in matrixes like: corn, cornmeal, corn germ meal, corn gluten meal, raw and roasted peanuts, peanut butter, popcorn, rice, tree nuts and wheat.

Intralaboratory validation technique used was by comparison of results of Veratox for aflatoxin HS (8031) method with an aflatoxins containing standard and determining of results uncertainty based on 9 repeated trials on corn flour.

There were tested 58 samples of 10 matrixes: cereals, grist, brewing malt and malt rootlets, beer, peanuts, sunflwer and pumpkin seeds, spice, mushrooms, molasses.
Equipments: a high speed blender, an orbital agitator, a grinder, a laboratory scale, a microwell reader with a 650 nm filter.

We read optical densities in a microwell reader, aflatoxins concentrations are obtained by plotting on the standard curve of optical densities of standards the optical densities of samples; the exact concentration is obtained with a log/logit formula.

Validation of ELISA method for ochratoxins determinations

The method proposed for intralaboratory validation within ICA-Assays Laboratory is an alternative analysis for quantitative determination of ochratoxins in matrixes like: corn, barley, green coffee, different dried fruits (apricots, dates, raisins, figs).

Intralaboratory validation technique used was by comparison of results of Veratox for ochratoxin (8610) method with an ochratoxins containing standard and determining of results uncertainty, based on 9 repeated trials on corn flour.

There were tested 33 samples of 9 matrixes: cereals, grist, brewing malt and malt rootlets, beer, sunflwer and mustard seeds, mushrooms, gluten.

Equipments: a high speed blender, an orbital shaker, a grinder, a laboratory scale, a microwell reader with a 650 nm filter.

We read optical densities in a microwell reader, ochratoxins concentrations are obtained by plotting on the standard curve of optical densities of standards the optical densities of samples; the exact concentration is obtained with a log/logit formula.

Validation of ELISA method for DON determination.

The method proposed for intralaboratory validation within ICA-Assays Laboratory is an alternative analysis for quantitative determination of DON in matrixes like: wheat, barley, other raw and processed cereals, etc.

Intralaboratory validation technique used was by comparison of results of Veratox for DON HS (8332 code) method with a DON containing standard and determining of results uncertainty, based on 9 repeated trials on corn flour. There were tested 37 samples of 5 matrixes: cereals, corn flour, grist (sun flower and soy), brewing malt and malt rootlets, beer, mustard seeds.

Equipments: a high speed blender, an orbital shaker, a grinder, a laboratory scale, a microwell reader with a 650 nm filter. We read optical densities in a microwell reader, DON concentrations are obtained by plotting on the standard curve of optical densities of standards the optical densities of samples; the exact concentration is obtained with a log/logit formula.

Validation of ELISA method for egg allergen determination.

The method proposed for intralaboratory validation within ICA-Assays Laboratory is an alternative analysis for quantitative determination of unprocessed or thermal processed egg proteins in food matrixes like: pasta, salad dressings, cake mixes or ice creams. Intralaboratory validation technique used was by comparison of results of Veratox for egg allergen (8450 code) method with an egg allergen containing standard and determining type A uncertainty, based on 9 repeated trials on mayonnaise sauce.

Equipments utilized were: a high speed blender, an orbital shaker, a grinder, a watwr bath capable to maintain 60°±1°C, a Vortex shaker, a laboratory scale, a microwell reader with a 650 nm filter.

We read optical densities in a microwell reader, egg allergen concentrations are obtained by plotting on the standard curve of optical densities of standards the optical densities of samples; the exact concentration is obtained with a log/logit formula.

Validation of ELISA method for milk allergen determination

The method proposed for intralaboratory validation within ICA-Assays Laboratory is an alternative analysis for quantitative determination of casein- a milk and whey protein in food matrixes like: juices, sauces, sorbets, etc .

Intralaboratory validation technique used was by comparing results of Veratox method for milk allergen (8460 code) with a milk allergen-containing standard, and determining of type A uncertainty, based on 9 repeated trials on vinaigrette sauce.

Equipments utilized were: a high speed blender, an orbital shaker, a grinder, a water bath capable to maintain 60°±1°C, a Vortex shaker, a laboratory scale, a microwell reader with a 650 nm filter.We read optical densities in a microwell reader, total milk allergen concentrations are obtained by plotting on the standard curve of optical densities of standards the optical densities of samples; the exact concentration is obtained with a log/logit formula

3. Results and Discussions

Qualitative detection of Salmonella in food.
Practicability: Negative results are obtained in 2 days using alternative method against 3-6 days using the reference method. Positive results are obtained in 5-6 days using alternative method (including classical tests described in the standard reference including purification step), as with the reference method.

Sensitivity = 100% (positive confirmate samples x 100 / positive confirmate samples + fals negative samples).

Accuracy: false negatives: 0; additional positives, alternative method: 0; concordant results: 50.

The concordance rate between the reference method and Vidas SLM test is 100%.

Qualitative detection of Listeria monocytogenes in food.

Practicability: Negative results are obtained in two days using the alternative method against 5-12 days using the reference method. Positive results are obtained in 3-4 days using alternative method (including confirmation according to classical tests of the reference method, with purification step included), against 7 – 11 days using the reference method.

Sensitivity = 100% (positive confirmate samples x 100 / positive confirmate samples + fals negative samples).

Accuracy: false negatives:0; aditional positives, alternative method:0; concordant results:50.

The concordance rate between the reference method and Vidas LMO2 is 100%

Qualitative detection of staphilococcus entherotoxin in food
Practicability: Negative results are obtained in 1 day using the alternative method against 3-7 days using the reference method. Positive results are obtained in 1 day using alternative method, against 3-7 days using the reference method.

Sensitivity = 96,55% (positive confirmate samples x 100 / positive confirmate samples + fals negative samples).

Accuracy: false negatives:1; aditional positives, alternative method:28; concordant results:55.

The concordance rate between the reference method and Vidas SET2 is 98,2%

Determination of total aflatoxins content by ELISA method.

For determining of the uncertainty of measuring we made 9 determinations in repeatability conditions on maize flour. x(the mean value of 9 parallel determinations)= 0,7

STD (standard deviation)= 0,1; STDrel,%= (STD x 100)/ x_{man} = 14.3. $U_A = \pm k\ s / \sqrt{n} = \pm (2 \times 0.1)/3 = \pm 0.1$; (type A standard uncertainty).

Determination of ochratoxins content by ELISA method

For determining of the uncertainty of measuring we made 9 determinations in repeatability conditions on maize flour.

x (the mean value of 9 parallel determinations) = 0,8 STD (standard deviation)= 0.1 ; STDrel,% = (STD x 100)/ x_{mean} = 12,5; $U_A = \pm k\ s / \sqrt{n} = \pm (2 \times 0.1)/3 = \pm 0.1$.

Determination of DON content by ELISA method

For determining of the uncertainty of measuring we made 9 determinations in repeatability conditions on maize flour.

x(the mean value of 9 parallel determinations) = 95,4; STD (standard deviation) = 1,1; STDrel,% = (STD x 100)/ x_{mean} = 1,2; $U_A = \pm k\ s / \sqrt{n} = \pm (2 \times 0.1)/3 = \pm 0.8$.

Results obtained concerning determination of egg allergen content by ELISA method

For determining the uncertainty of measuring we made 9 determinations in repeatability conditions on mayonnaise sauce. x the mean value of 9 parallel determinations) = 0,3 STD (standard deviation)=0,2; STDrel,% = (STD x 100)/ x_{mean} = 66,7; $U_A = \pm k\ s / \sqrt{n} = \pm (2 \times 0.1)/3 = \pm 0.3$.

Results obtained concerning determination of total milk allergen content by ELISA method

For determining the uncertainty of measuring we made 9 determinations in repeatability conditions on vinaigrette sauce.

x (the mean value of 9 parallel determinations) = 1,2; STD (standard determinations)=0,7; STD (standard deviation)= 0,1; STDrel,% deviation) = 0,1; STDrel,% = (STD x 100)/ x_{mean} = 8,3; $U_A = \pm k\ s / \sqrt{n} = \pm (2 \times 0.1)/3 = \pm 0.1$.

4.Conclusions

All results obtained are concordant with expected results. The concordance between reference methods and Vidas tests are 100% for qualitative detection of *Salmonella* and *Listeria* and 98,2% for staphilococcus enterotoxin detection. The Vidas methods are reliable. Type A uncertainty for a confidence interval of minimum 95%, are: ±0,1 (total aflatoxins, ochratoxins and milk allergens); ± 0,8 (DON); ± 0,3 (egg allergens).

References

SR EN ISO 16140:2005. Microbiology of food and animal feeding stuffs - Protocol for the validation of alternative methods (ISO 16140:2003);

SR EN ISO 11290-1:2000. Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of *Listeria monocytogenes*. Part 1: Detection method;

SR EN ISO 6579:2003. Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp (ISO 6579:2002);

SR EN ISO 6888:2002. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird-Parker agar medium (ISO 6888-1:1999);

AOAC Official Methods 993.16 – Total aflatoxins in Corn. ELISA Method, AOAC-IUPAC Method

Metoda ELISA Veratox pentru determinarea aflatoxinelor totale (cod 8031)

Metoda ELISA Veratox pentru determinarea ochratoxinelor (cod 8610)

Metoda ELISA Veratox pentru determinarea DON (cod 8332)

Metoda ELISA Veratox pentru detrminarea alergenilor totali din lapte (cod 8460)

Metoda ELISA Veratox pentru determinarea alergenilor din oua (cod 8450)

Ghid EURACHEM/CITAC-Cuantificarea Incertitudinii in Masurarea Analitica, Editia a II-a, 2000.