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Research About the Efficency of Using Alternative Methods of Microbiological Food Expertise

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Abstract

In order to verify the efficiency of the rapid diagnosis alternative methods, in the Sanitary Veterinary and Food Safety Laboratory Brasov-Romania, we have examined a total of 9952 samples, collected from various processing units.

The examinations that were performed are: the quality indicators enumeration using TEMPO equipment; food pathogens detection using the VIDAS equipment and bacterial identification using the VITEK 2 COMPACT.

It was found that 4.3% of the examined samples showed positive results, most non-compliances parameters being recorded as Total Number of Germs (8.9%), Enterobacteriaceae (8.6%), and *Staphylococcus* spp. (8.3%) and the less at the parameters of *Salmonella* spp. (0.9%) *Listeria* spp. (1.8%) and *E. coli* O157 (0.0%).

Part of the serovarians identified by the Vitek 2 Compact method, have been additional confirmed to establish the degree of correlation, using the Kaufmann-White method.

Salmonella spp., serovars identified were: *Salmonella enterica* serovars: saintpaul, infantis, newport, enteritidis and taksony, and the species of *Listeria* spp., were: *L. monocytogenes*, *L. ivanovii* and *L. innocua*.

Pathogenic serovars, *Salmonella enterica* serovar enteritidis and *Listeria* monocytogenes, were confirmed as 100%. In the case of non-pathogenic *Salmonella* spp., the correlation between the two methods was 83.3% and in the case of *Listeria* spp, it was 100%. Infantis serovar identified by the Vitek 2 Compact method was confirmed by the Kaufmann-White method as taksony.

These values express the situation of the analysed samples, in terms of microbiological contamination and the results formed the basis for corrective measures implemented in the processing units and for the sanctions imposed on the origin lots in case, of the identification of bacterial species with toxigenic potential.

Keywords: Alternative method; Standardized method; Pathogens

Introduction

Decisions on food safety involve consideration of a wide range of concerns including the public health impact of foodborne illness, the economical importance of the agricultural sector, food industry, and the effectiveness and efficiency of interventions [1].

The presence of microorganisms is particularly important for the quality, wholesomeness and freshness of food status. Generally the microorganisms are those that reduce the nutritional value of the product, or can be edible by their pathogenic action, for the degradation and production of toxic metabolites [2-4].

Microbiological criterias are very important; they provide guidance in what concerns the acceptability of food and manufacturing processes, manipulation and distribution. For this reason they must be part of the procedures, based on HACCP principles and other measures for the hygiene control, by establishing a limit above which, a food product should be considered unacceptable and contaminated [5].

In food security, an important component of the field as a whole, is to achieve food security by the sector operators, in the self-prepared control programs elaborated in accordance with applicable laws, in wich they are obliged to survey all relevant parameters, having in view the specific activity of each unit.

In this respect, the European recent regulations, reunited in the so-called "hygiene package", aimed at preventing random food risks with the obligation to ensure the food safety circuit "from fork to plate", placing all responsibility to the producers, processors and suppliers of food resources, able to bring under qualified control the quality and food health [4,6,7].

Automatization in enumeration methods, can be very useful to reduce the time needed for the preparation of the average, serial dilution, counting colonies, etc. Many improvements in this field have been made, that allow laboratories to increase the efficiency and the

number of samples processed such as agar preparation machines, automated dilutors, automated counting devices and spiral plate [4,8].

An ideal detection system should include high specificity and sensitivity; fast response time; capability for mass production; elimination or simplification of sample preparation steps; minimal perturbation of sample; and providing continuous data analysis. Much progress has been made for the last decades, including automation and high throughput for sample processing and testing [9,10].

Lately, more and more rapid tests for microbiological food expertise are being used. In this regard, we can recall the tests based on the detection of antibodies and nucleic acid that revolutionized the methodology for the detection of microbial pathogens and their toxins [9].

Many years ago, it had been predicted that traditional methods of microbiological examination will be replaced by automated, rapid methods [11]. Rapid early detection of food contamination is therefore relevant for the containment of food-borne pathogens. Conventional pathogen detection methods, such as microbiological and biochemical identification are time-consuming and laborious, while immunological or nucleic acid-based techniques require extensive sample preparation and are not amenable to miniaturization for on-site detection [12,13].

However, it should be noted that the results of the rapid diagnostic methods (which can be used in accordance with the provisions of Regulation 2073/2005) should be confirmed using standardized diagnostic methodologies [14,15].

Materials and Methods

In order to check the efficiency of fast alternative diagnosis methods in the Sanitary Veterinary and Food Safety Laboratory Brasov, Romania, 9952 samples were examined, taken from different processing units from the county of Brasov [16].

The microbiological examinations used were: the enumeration of quality indicators using TEMPO equipment; the detection of food pathogens using the VIDAS equipment. For the bacterial identification we used the VITEK 2 COMPACT (Table 1).

Analysed parameters	Samples		Rapid diagnostic methods used	
	No.	%	VIDAS	TEMPO
Salmonella spp.	4160	41.8	X	
Listeria spp.	1138	11.4	X	
Staphylococus spp.	624	6.3		X
Staphylococcal enterotoxin	52	0.5	X	
Campylobacter spp.	82	0.8	X	
E. coli	1056	10.6		X
E. Coli O157	8	0.08	X	
Enterobacteriaceae	864	8.7		X
Total Number of Germs	1440	14.5		X
Yeast and Molds	528	5.3		X
TOTAL	9952	100		

Table 1: The number of samples collected and examined by rapid alternative diagnostic techniques.

Tempo method

Fully automatic method, which allows the quantitative determination of bacterial germs, based on the traditional microbiology method, namely the multiple tube method. It shows sensitivity and ease of use, allows quick results to the classic method of working (3-7 d) and saving time in preparing culture media, glassware preparing for sterilization, packaging, inoculation, reading plates, autoclaving and washing glassware (Figures 1 A and B).

Figure 1: A. TEMPO-station preparation B. Reading station.

The TEMPO test is composed of a card with a transfer tube and a vial with a specific culture medium. The culture media is dehydrated, sterile, ready-to-use, disposable, selective TC (Total Coliforms), EC (*E. coli*), EB (Enterobacteriaceae), STA (*Staphylococcus* coagulase+) LAB (Lactic acid bacteria) or -TVC non-selective (Total Number of Germs), Y+M (Yeasts and Moulds), as identified by the bar code and the colour code (Figure 2) and TEMPO Stomacher bags (Figure 3).

Figure 2: Card with a transfer tube and a vial.

Figure 3: Type bags Stomacher.

The medium is inoculated with a dilution of the sample to be tested and it is transferred by filling tempo instrument in the tempo card. The medium is homogeneously dispersed in 48 well plates in three different volumes. The card is then hermetically sealed to avoid any risk of contamination during handling. Subsequently occurs the reading, interpretation and validation of results in a single transfer step.

Examples:

Tempo TVC tempo EC: During the incubation, the microorganisms present in the card reduces the culture medium substrate and causes the appearance of a fluorescent signal that is detected by the TEMPO reader. Depending on the number and size of positive wells, the tempo system inferred the Total Number of Germs (CCT test) or the *E. coli* (EC test) present in the original sample, according to the calculation, based on the most probable number method.

Tempo TC: The culture medium contains a fluorescent indicator which, when the pH is neutral, emits a signal detected by the tempo reader. During the incubation, the total coliforms present in the card, ferment lactose from the culture medium, resulting the decrease of pH and disappearance of the fluorescence signal. Depending on the number and size of positive wells, TEMPO system infers the total number of coliforms present in the original sample as calculated, based on the most probable number method.

Bacterial germs, matrices of which they can be identified and the time required for laboratory diagnosis are set out in Table 2.

Bacterial germs that can be identified	Matrix (examples)	The time taken (hrs)
E. coli	Meat, mechanically separated meat, cheese produced from milk treated in the heat, non-animal food products.	24
Staphylococus spp.	Cheese made from raw milk subjected to lower heat treatment then pasteurization and from heat-treated milk, milk powder, fish products.	24
Enterobacteriaceae	Cattle carcases, sheep, goats, horses, swine carcases, pasteurized milk and pasteurized milk products, milk powder, ice cream and dairy desserts, dried infant formulas and foods for medical purposes, egg products.	C
Total Number of Germs	Raw milk, cattle carcase, sheep, goats, swine, horses, poultry carcases, minced and mechanically separated meat.	48
Yeast and Molds	Bakery	72

Table 2: Bacterial germs, matrices of which they can be identified and the time required for laboratory diagnosis.

Method using the automatic analyser MiniVidas

It is a compact high performance system, for identifying highly pathogenic microorganisms (*Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni*, *E. coli* O 157, *Staphylococcal enterotoxin*), with the immunoassay principle.

Its use allows time saving for the preparation of culture media, glassware sterilization, packaging, labelling, inoculation, reading the plates, washing the autoclave and glassware, etc. using ready for use reagents.

MiniVidas automatic analyser offers the possibility of a large number of analyses, applications, safety and ease of use, being an automatic device, standardized, robust, allowing an objective reading and delivering a quick result compared to classical working methods (Figure 4).

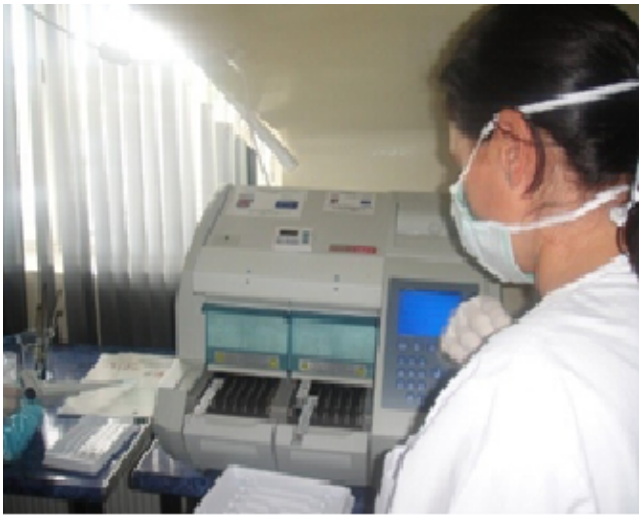

Figure 4: MiniVidas, automated analyzer.

Bacterial germs, matrices of which they can be identified and the time required for laboratory diagnosis is shown Table 3.

Bacterial germs that can be identified	Matrix	The time taken (hrs)
Salmonella spp.	All food during their shelf life	48
Lsteria monocytogenes	All food before leaving from the direct control of the processing units, food products available on the market during their shelf life (meat and meat products, raw material milk, cheese from unpasteurized milk,	72

	confectionery and pastry dishes, fish and fish products).	
Campylobacter jejuni	Bird carcases-in the warm season.	72
E. coli 157	Beef meat, minced meat and meat products containing beef.	72
Staphylococcal enterotoxin	Cheeses made from raw milk subjected to heat treatment than pasteurization and low heat-treated milk, milk powder, fish products.	72

Table 3: Bacterial germs, matrices of which they can be identified and the time required for laboratory diagnosis.

Vitek 2 Compact

The equipment Vitek 2 Compact, is an automatic system for the identification and biochemical confirmation and antibiotic test, able to select pathogenic and highly pathogenic micro-organisms isolated on solid media, their biochemical tests is performed extremely rapid while saving time in the results engendering.

Using it, saves materials and reagents. The used card saves all the necessary reagents for identifying and confirming and does not require any type of glass, heat sterilization, the pre-stage thermostat or other diagnostic operations.

Equipment is easy to use, with reduced labor time, intuitive software and connection to ATLAS Vet LIMS System. The method is fully automated, for diagnosis using different cards, which enables the identification of bacteria: Gram positive (GP), Gram negative (GN), anaerobic bacteria (ANC), *Campylobacter* spp. (NH), *Corynebacterium* spp. (CBC), Yeasts and Molds (YST) and *Bacillus* spp. (BCL), eg. *Salmonella* spp. (Figure 5).

Figure 5: Steps of identification of *Salmonella* spp.

Bacterial germs that can be identified and the required time for laboratory diagnosis are mentioned in Table 4.

Bacterial germs that can be identified	Time needed to perform laboratory tests (hrs)
E. coli	4-6
Staphylococcus spp.	4-6
Salmonella spp.	5-6
Listeria monocytogenes	7-8
Campylobacter jejuni	8-12
E. coli A 157	5-6
Corynebacterium spp.	4-6
Bacillus spp.	4-6
Yeasts and molds	4-6

Table 4: Bacterial germs that can be identified and the required time for laboratory diagnosis, using Vitek 2 Compact.

Results

After analyzing the 9952 samples by rapid alternative methods, the following results is shown Table 5.

Parameters analysed	Samples		D. c. results			
	No.	%	Inconsistent		Compliant	
			No.	%	No.	%
Salmonella spp.	4160	41.8	36	0.9	4124	99.1
Listeria spp.	1138	11.4	21	1.8	1117	98.2
Staphyloccocal spp.	624	6.3	52	8.3	572	91.7
Staphylococcal enterotoxin	52	0.5	4	7.7	48	92.3
Campylobacter spp.	82	0.8	3	3.7	79	96.3
E. coli	1056	10.6	64	6.1	992	93.9
E. coli O157	8	0.08	-	-	8	100
Enterobacteriaceae	864	8.7	74	8.6	790	91.4
Total Number of Germs	1440	14.5	128	8.9	1312	91.1
Yeast and Molds	528	5.3	38	7.2	490	92.8
TOTAL	9952	100	420	4.3	9532	96.7

Table 5: The results of the examination samples using fast alternative techniques of diagnosis.

In all cases, the interpretation was done in accordance with regulation 2073/2005 in which the microbiological safety criterias are provided, which defines the acceptable character of the products, and also safety microbiological criteria of food products that should establish a line above which a food product must be considered unacceptably contaminated.

It was found that 4.3% of the examined samples showed positive results, most of the non-concordances recorded for Total Number of Germs (8.9%), Enterobacteriaceae (8.6%), and *Staphylococcus* spp. (8.3%) and the less in the case of *E. coli* O157, *Salmonella* spp. (0.9%) and *Listeria* spp. (1.8%).

These values express the situation of the analysed samples, under the aspect of microbiological contamination, and the results has constituted the basis for corrective measures implemented in the processing units and for the sanctions imposed on the origin lots in case of bacterial species with toxigenic potential were identified. The ultimate were put under distraint until confirmation or refutation of the results obtained by reference tests.

The reference methods used to confirm the positivity of cases obtained after use fast alternative techniques of diagnosis were as follows (Table 6).

Parameters analysed	Reference test used
Salmonella spp.	ISO 6579/2003 AC/2006
Listeria spp.	ISO 11,290 - 1,2
Staphylococcal spp.	ISO 6888 - 1.2
Staphylococcal enterotoxin	European screening method of the EU-RL
Campylobacter spp.	ISO 16649
E. coli	ISO 16649 -1.2
E. coli O157	ISO 16649 -1.2
Enterobacteriaceae	ISO 21,528 - 1.2
Total Number of Germs	ISO 4833/2003
Yeast and Molds	ISO 21527-1

Table 6: The reference methods used to confirm positivity of cases.

Using only the standardized methods in the food microbiological expertise, has some disadvantages: they are laborious; requires a longer working time (3-6 days delay of finished product delivery and providing a delayed data response from the monitoring program of hygiene); a greater amount of consumables and many suppliers; the results can be subjective (many false negative and false positive), they depend on the experience and expertise of persons involved in analytical process and high uncertainty of measurement.

They remain, however, very important, being the ones on which we base to report the results of other diagnosis methods used in microbiology.

The concordance of the results was 100%, which demonstrates that the use of fast alternative methods can be successfully used, generating results equivalent to those obtained by using the reference method (Table 7).

Parameters analysed	Non-concordant samples at rapid alternative methods	Samples not in concordance with the reference tests	
		No.	%
Salmonella spp.	36	36	100
Listeria spp.	21	21	100
Staphylococcus spp.	52	52	100
Staphylococcal enterotoxin	4	4	100
Campylobacter spp.	3	3	100
E. coli	64	64	100
E. Coli O157	-	-	100
Enterobacteriaceae	74	74	100
Total Number of Germs	128	128	100
Yeast and Molds	38	38	100
TOTAL	420	420	100

Table 7: Concordance results obtained from the use of the reference methods.

Part of the serotypes identified by the Vitek 2 Compact method were analyzed at the Institute of Hygiene and Veterinary Public Health Bucharest Romania, using the Kaufmann-White method. Thus, in the case of *Salmonella* spp, a number of 12 serovars were analyzed, representing 33%. The isolated serovars were: *Salmonella enterica* serovars: saintpaul, infantis, newport, enteritidis and taksony (Table 8).

Method used	Serovars identified									
	Salmonella enterica from which serovar									
	enteritidis		taksony		infantis		newport		saintpaul	
	No.	%	No.	%	No.	%	No.	%	No.	%
Kaufmann-White method	2	16.7	2	16.7	3	25.0	3	25.0	2	16.7
Vitek 2 Compact method	2	16.7	1	8.3	4	33.3	3	25.0	2	16.7
Correspondence		100		50		75		100		100

Table 8: *Salmonella* spp. serovars, identified.

A correlation of 83.3% was found and it was being noted that the infant serotype identified by the Vitek 2 Compact method was confirmed by the Kaufmann-White method as taksony. It was observed that seovar *enteritidis* is the only one with pathogenic potential of the isolates, being confirmed in 100%. The other identified serovars have a low degree of pathogenicity, assisting thermal processing of food which does not constitute a risk of generating food poisoning to consumers.

In the case of *Listeria* spp., 7 species were analyzed, representing 33%. The isolated species were: *L. monocytogenes*, *L. ivanovii* and *L. innocua* (Table 9).

The method used	L. monocytogenes		L. inanovi		L. innocua	
	No.	%	No.	%	No.	%
Kaufmann-White method	2	28.6	4	57.1	1	14.3
Vitek 2 Compact method	2	28.6	4	57.1	1	14.3
Correspondence		**100**		**100**		**100**

Table 9: Species of *Listeria* spp., isolated and confirmed.

There is a 100% correlation between the two used methods.

Conclusions

During food microbiological expertise using alternative methods, non-concorcondance have been identified in 4.3% of the examined samples. Most non-concordances were recorded for total number of germs (8.9%), Enterobacteriaceae (8.6%) and *Staphylococcus* spp. (8.3%) and the lowest in the case of *E. coli* O157 (0%), *Salmonella* spp. (0.9%) and *Listeria* spp. (1.8%).

The results formed the basis for corrective measures implemented in processing units and sanctions imposed on the lots of origin for the identification of bacterial species with toxigenic potential. They were sequestered until results confirmation or refutations were obtained by the reference tests.

Part of the serotypes identified by the Vitek 2 Compact method (*Salmonella* spp. and *Listeria* spp.), were confirmed by the Kaufmann-White method in 83.3%, in the case of *Salmonella* spp., and 100% in the case of *Listeria* spp. Pathogenic serovars of *Salmonella* spp. and *Listeria* spp. with a risk of generating food poisoning have been confirmed in 100%.

We recommend that rapid diagnosis methods can be used in particular, to carry out the self-monitoring program of the units, taking into account the short time necessary to generate analysis report.

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