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Faculty of Food Science

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COMPARISON OF RAPID METHODS FOR DETECTING FOOD-BORNE PATHOGENS

Theses

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Budapest

2014

PhD School/Program

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Field: Food Science

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The applicant met the requirement of the PhD regulations of the Corvinus University of Budapest and the thesis is accepted for the defence process.

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1. INTRODUCTION

Food scandals of the last few years have drawn attention to the issue of food safety. Detection period for pathogenic microorganisms by using time-consuming conventional culturing procedures may last for 5 or more days. To shorten the standardised, long and expensive working processes used for the detection of pathogens a great variety of procedures have been developed lately.

For the detection of pathogens a great variety of procedures have been tried out during the latest periods of time in order to shorten the standardised, long and expensive working process. To introduce rapid microbiological methods applied in laboratories comparative studies should be carried out. Methods which are the most suitable for the optimal operation of the laboratory can be selected on the basis of such measurement results.

Alternative testing methods, independently self-developed methods, standardised methods falling beyond the field of operation, additions and amendments made to the standardised methods applied in the accredited laboratory should be validated internally by the laboratory itself or externally by international organisations of validation (AOAC, AFNOR, MICROVAL). Studies targeting matrices not investigated by bodies of validation are to be classified into the category falling beyond the field of operation; therefore in cases of such samples in-house validations are performed according to the MSZ EN ISO 16140 standard.

2. AIMS OF STUDY

Comparative analysis and assessment according to the ISO 16140 standard of modern methods used for the detection from food products of the most frequently occurring pathogenic bacteria/species considered to be important from the point of view of food safety have been established as the aims of my work in order to verify the safety of the routinely performed diagnostic laboratory applications.

When selecting the alternative microbiological methods the aspects of the appropriate relative values of sensitivity, specificity and accuracy, the occurrence of matrix effect, the daily sample amount, the time period available for the tests, the level of automation, the possibilities of data storage and analyses, the promptness of servicing, and finally the cost of the tests are intended to be used as subjects of my analytic work. As a matter of course the availability of culture media, equipment and reagents in Hungary at the time when the herein paper has been prepared were also taken into account as important criteria.

Main steps of accomplishment:

1. Comparison of results obtained by modern rapid methods and standardised methods being at our disposal in the cases of *Salmonella* spp., *Listeria monocytogenes*, *E. coli* O157 and thermotrophic *Campylobacter* causing diseases of food origin.

   Applied alternative microbiological diagnostic methods:

   - Culture media containing chromogenic substrate
   - Immunological assays
   - Molecular microbiological assays (real-time PCR)
2. Comparison of performance characteristics of alternative diagnostic methods with the data measured by the international organisations, and in cases of divergent matrices establishment of such on the basis of the MSZ EN ISO 16140 standard.
3. Analysis of the effect of the food matrix exerted on the different testing methods.
4. Optimisation of the enrichment conduction.
5. Selection of testing method with especial regard to the duration of the testing.

3. MATERIALS AND METHODS

Experimental materials:

Artificially infected and naturally occurring (selected from routine samples) samples were used in my work. Micro-organisms used for the tests are obtained from the Microbiological Laboratory of FoodMicro Kft. [FoodMicro Ltd.] and from the strain collection of the University of Turin, Italy.

Within the frame of the *Salmonella* tests altogether 85 naturally infected and 36 artificially infected samples were used to carry out the experiments in the cases of the modern differential culture media and immunological methods. Test strains were used for the artificially infected samples. As food matrices minced meat, cold cuts, dairy products and salads were employed.

In case of tests carried out by using the real-time PCR method the matrices of raw meat, cold cuts, chocolate, seasoning, environmental sample, and feeding stuff were investigated. In case of the real-time PCR tests I examined altogether 1445 routine laboratory samples; and in these cases raw meat, cold cuts, chocolate, seasoning, environmental sample, feeding stuff (for pets) were used as matrices.

Within the frame of the *Listeria monocytogenes* tests altogether 147 naturally infected samples, as well as 4 artificially infected samples together with 1 control sample per each product group were used to carry out the experiments in the cases of the modern differentiating culture media and immunological methods. As experimental matrices minced meat, salad, milk, frozen vegetables, frozen ham, and frozen noodles were employed.
Within the frame of the *E. coli* O157 tests altogether 47 hygiene samples collected in plant areas and 3 artificially infected minced meat samples were used to carry out the experiments in the cases of the immunological methods and the real-time PCR method.
Within the frame of the *Campylobacter* tests altogether 40 commercial samples of cold stored and cut poultry meat samples were used to carry out the experiments in the cases of the conventional and the qPCR method. Samples were analysed at $t = 0$, $t = 6$, $t = 24$, and $t = 48$ hours. Calibration curves were established in the cases of the four enrichment broths and of the Ringer solution.

Experimental methods:

To carry out my experimental work available modern rapid methods and standardised methods were used to detect food-borne *Salmonella* spp., *Listeria monocytogenes*, *E. coli* O157 and thermotrophic *Campylobacter* bacteria. The applied alternative microbiological diagnostic methods were the followings:

- **New modern differential agars** (culture media containing chromogenic substrate: Harlequin *Salmonella* ABC, SM2 ID, Compass *Salmonella*, Harlequin *Listeria* Agar, RAPID'*L.mono*, Compass *Listeria* Agar, Harlequin™ SMAC-BCIG, Brilliance Campy Count Agar).
- **Immunological assays** (VIDAS ICS2-SLM, VIDAS LMO2, VIDAS UP *E. coli* O157:H7).
- **Molecular microbiological assays** (real-time PCR TaqMan *Salmonella enterica* Detection Kit (ABI), BAX® System PCR Assay (DuPont Qualicon) and a qPCR method developed for the detection and determination of the *Campylobacter* count).

I have carried out comparative studies on the alternative rapid methods (agar containing, chromogenic substrate, VIDAS, real-time PCR) and the conventional methods used for the detection of the four most frequently occurring food-borne pathogens (*Salmonella* spp., *Listeria monocytogenes*, *E. coli* O157 and *Campylobacter jejuni*). On the basis of the MSZ EN

ISO 16140 standard I have determined the performance characteristics (relative accuracy, relative specificity, and relative sensitivity) of the methods being suitable for the detection of each pathogen. During the comparison with the results obtained by the international organisations of validation I have established that food matrices used for the validation procedures do not cover all food types that may occur in a routine laboratory. I also determined the performance characteristics of given methods in cases of divergent matrices.

4. RESULTS AND CONCLUSIONS

4.1 Results of the tests carried out by using chromogenic culture media

4.1.1 Investigation of *Salmonella* on chromogenic media

Relative accuracy, relative specificity and relative sensitivity measured on the COMPASS *Salmonella* and on the SM2 culture media show 100% match with the results of the reference method.

Results obtained on the HARLEQUIN *Salmonella* culture medium showed relative sensitivity with 100%, relative accuracy with 99.2% and relative specificity with 98.6% in comparison with the results of the reference method. I observed neither false positive, nor false negative results in the cases of the COMPASS *Salmonella* and the SM2 culture media. Concerning the COMPASS *Salmonella* culture medium the AFNOR international validation data show lover values of relative accuracy (97%), relative specificity (96.9%) and relative sensitivity (97.1%). In the tests carried out with the HARLEQUIN *Salmonella* culture medium I obtained false positive result in case of one minced meat sample, which result was not verified by the biochemical and serological tests.

4.1.2 Investigation of *Listeria monocytogenes* on chromogenic culture media

I observed neither false positive, nor false negative results in the tests using Harlequin™ *Listeria*, COMPASS *Listeria* and RAPID'*L.mono*™ culture media.

Based on my experiments it can be established that the results obtained with the Harlequin™ *Listeria*, COMPASS *Listeria* and RAPID'*L.Mono*™ show 100% match with the results of the reference method. Concerning the COMPASS Listeria Agar the AFNOR international validation data show lover values of relative accuracy (97.3%), relative specificity (98.9%) and relative sensitivity (95.6%). Concerning the RAPID'*L.Mono*™ Agar the AFNOR international validation data again show lover values of relative accuracy (98.6%), relative specificity (97.9%) and relative sensitivity (99.2%).

4.2 Results of the tests carried out by using immunological assays

4.2.1 Investigation of *Salmonella* with the VIDAS method

It can be established that results measured by using the VIDAS SLM, and the VIDAS ICS2+SLM methods show 100% match with the results of the reference method.

By using the VIDAS ICS2+SLM method I obtained values of relative accuracy to be 99.2%, relative specificity to be 98.6% and relative sensitivity to be 100%. In case of VIDAS SLM, I observed neither false positive, nor false negative results. In the tests carried out with the VIDAS ICS2+SLM method I obtained false positive result on the chromogenic medium in one case of minced meat testing, which result was not verified by the biochemical and serological tests. Concerning the VIDAS ICS2+SLM method the AFNOR international validation data show lover values of relative accuracy (97.4%), and of relative sensitivity (95.5%), and higher value concerning relative specificity (99.4%).

4.2.2 Investigation of *Listeria monocytogenes* with the VIDAS method

Results measured by using the VIDAS LMO2 method show 100% match with the results of the reference method. In the case of VIDAS LMO2, I observed neither false positive, nor false negative results. Concerning the VIDAS LMO2 method the AFNOR international validation data show lover values of relative accuracy (97.6%), relative specificity (98.3%) and relative sensitivity (96.7%).

4.2.3 Investigation of *E. coli* O157 with the VIDAS method

It can be established that results measured by using the VIDAS UP method very good match level is shown with the results of the reference method. In the case of VIDAS UP method, I observed neither false positive, nor false negative results. Concerning the VIDAS UP method the AFNOR international validation data show lover values of relative accuracy (98.4%), relative specificity (96.9%) and relative sensitivity (97.4%).

4.3 Molecular assays

4.3.1 Investigation of *Salmonella* with the real-time PCR method (ABI)

Based on my experiments it can be established that the results of the real-time PCR measurement, with the exception of the seasoning samples, show deviation from the results of the reference method. In cases of all tested sample groups the presumptive positive samples were further examined by a standardised method. I observed false positive results in 10 samples of raw meat, in 4 samples of seasoned meats, in 2 samples of cold cuts, in 12 samples of feeding

stuffs, in 11 environmental samples, and in 2 samples of vegetables and fruits. I observed no false negative result in any of the samples. Concerning the real-time PCR method, the AFNOR international validation data show higher values of relative accuracy, relative specificity and relative sensitivity, except the cases of feeding stuff samples, where data of relative sensitivity were lower.

4.3.2 Investigation of *E. coli* O157 with the real-time PCR method (ABI)

Results of real-time PCR measurements show 100% match with the results of the reference method. In case *E. coli* O157 was investigated by using real-time PCR, I observed neither false positive, nor false negative results.

4.4 Analysis of the matrix effect

I carried out matrix effect analysis and established the factors which hinders the safe detection of pathogens during the alternative microbiological methods. My experiments proved that regarding relative accuracy and specificity data the comparative results of the investigations carried out by using alternative rapid methods show deviation per each matrix. While searching for the reason of this phenomenon I established that seasoned meat, chocolate, feeding stuff, and environmental samples, as well as fruits containing oenocyanin and anthocyanin have an influence on pathogen detection with real-time PCR rapid methods. Apart from the matrix effects verified in the cases of rapid methods the errors of the standardised method were also revealed. False negative result can be measured even by using the conventional method. The reason of this is the inappropriate conduction of the preliminary enrichment and/or the disturbing effect of a microbiological agent being concomitantly present.

4.5 Optimisation of the enrichment procedure

I established the detection limits of the alternative testing methods, based on which I optimised the enrichment procedures. Limitations of the enrichment procedure were demonstrated with a *Campylobacter* example. Certain components of the selective enrichment broths can inhibit the proliferation of *Campylobacter* spp., therefore I compared the four selective broths suitable for the enrichment of *Campylobacter* spp. Based on the obtained results and in cases of the qPCR tests the blood containing Bolton's enrichment broth have proven to be positive for most cases.

I established that the qPCR method is more suitable for the detection of *Campylobacter* species. Culturing testing methods cause information deficit because although these are suitable

for the detection of *Campylobacter* spp., yet these cannot be applied for the detection of the species most frequently causing problems, *Campylobacter jejuni*. With the applied qPRC method *Campylobacter* spp. and *Campylobacter jejuni* can be detected simultaneously. I made an attempt towards quantification from the samples through the qPCR method.

4.6 Selection of testing method with especial regard to the duration of the procedure

Based on my experiments the TaqMan *Salmonella enterica* real-time PCR Kit (ABI) is proved to be the fastest when testing poultry products as opposed to the VIDAS ICS2-SLM and BAX methods. Therefore, in the case of poultry the recommended method is the TaqMan *Salmonella* testing, which is suitable for result communication within 24 hours. This way compliance for all parameters (*Salmonella* spp. *S. aureus*, *E. coli*, microbial count) can be shown within 24 hours. My results proved that the microbiological condition of fresh poultry meat can be determined within 24 hours by using the applied modern and rapid microbiological procedures, thereby providing possibility for fast qualification before marketing.

Comparing the three methods using different working rationales it can be established that:

- Specificity of chromogenic culture media is appropriate, their visual assessment is simpler than the conventional methods and these provide fast results. Their disadvantage is that the microbiologist can read false results because of the enzyme cross reactions.
- The VIDAS technology is a rapid, automated procedure and it is suitable for the detection of two types of micro-organisms at the same time. Its disadvantage is that we may obtain false negative result if cellular density of 10^6/mL is not reached, as well as it has a limited testing capacity.
- The advantage of the real-time PCR tests is that its detection limit is low, and the testing period is shorter. The disadvantage of the ABI real-time PCR method is that even the defunct cells in the sample can be detected, thus false positive result is obtained more frequently, furthermore, inhibition caused by the food matrix is much more common.

In the case of the BAX system the probability of the false positive result is lower due to the subsequent enrichment step, but this is more time-consuming. The BAX system is suitable for the testing of samples with high-level of contamination or samples with inhibition potential, as well as where the exclusion of higher false positivity rate and inhibition embodies a greater economic interest than a testing period being 8 hours shorter. If occurrence of dead cells in large numbers and risk of inhibition cannot be expected, fastest results can be obtained by the application of the TaqMan *Salmonella enterica* Kit (ABI). It is true for both methods that no

false negative results were obtained by using them, which is a reassuring fact from the point of view of food safety.

As a matter of course, the possibilities for automation, data storage and data analysis, the promptness of repairing the equipment, the rapid introduction of developments, modifications, innovations, and last but not least the cost of the procedure are the utmost determinative factors among the reliable methods. Instrumentalised test are characterised by high purchase and low operational costs, therefore these are only economic if exploited on full capacity. The material cost of the solutions is higher when not demanding instrumentalisation.

5. SUGGESTIONS

Investigation of *Salmonella* with chromogenic culture media

Based on our results all three culture media (COMPASS *Salmonella*, SM2 and HARLEQUIN *Salmonella* Agar) can be chosen as secondary optional culture media according to the MSZ EN ISO 6579 standard.

Investigation of *Listeria monocytogenes* with chromogenic culture media

My comparative analyses showed that parameters of all tested chromogenic media for the detection of *Listeria monocytogenes* set forth in the MSZ EN ISO 16140 standard are matching in 100% in comparison with the reference method, therefore I recommend all of these for the purpose of secondary culture media. The RAPID' *L. mono* Agar is more selective, it can be evaluated more easily, but the cost of this culture medium is the highest.

Investigation of *Salmonella* with the VIDAS method

From the experiments I carried out it was also revealed that the VIDAS SLM is more suitable for the detection of *Salmonella* species, than the VIDAS ICS2-SLM. The reason for this may be that *Salmonella* species cannot proliferate to a necessary level during the short enrichment step in the M-broth. If the cellular density of 5×10^5-10^6 cells/mL is not reached in the M-broth, we may obtain a negative result by using the VIDAS ICS2-SLM method. This alternative rapid method can be successfully applied for the food safety control of slaughterhouses.

Investigation of *Listeria monocytogenes* with the VIDAS method

No false negative results were observed, which is very important from the point of view of

food safety. Based on the results this method was introduced in our laboratory for purposes of routinely performed tests.

Investigation of *E. coli* O157 with the VIDAS method

During my experiments I measured no *E. coli* O157 positive results with naturally contaminated environmental and meat samples. In accordance with the literature data the values of performance characteristics determined according to the MSZ EN ISO 16140 standard show significant deviations, probably arising from the samples, the sampling (type, place, initial level of bacterial infection), the environmental factors, the seasonality and the differences of the detection methods. Despite the low incidence the monitoring of this microbial group is recommended because it is associated with a high mortality rate.

Investigation of *Salmonella* with the real-time PCR method

Compared with the standardised "gold standard" method I obtained more positive *Salmonella* results with the real-time PCR method.

The reason for this may be the detection of the dead *Salmonella* cells. This phenomenon was significant in the cases of the raw meat, marinated meat, the environmental, and the feeding stuff samples. Due to the antibacterial effect of the spices or during steps of food processing a part of the *Salmonella* cells is damaged or completely killed..

The character of the feeding staff and hygiene samples may provide a reason to detecting the false positive samples. The viable *Salmonella* cells are destroyed at high temperatures or if subjected to chemical treatment, but the dead cells may be present in these samples in large numbers. The detection of these dead cells provides the possibility to draw conclusions on the conditions of the raw materials before their processing. Based on our results we set up the BAX system for the routinely performed feeding stuff tests.

In my experience when testing poultry samples the BAX system measured fewer false *Salmonella* positive samples than the TaqMan *Salmonella enterica* Kit. The reason of this phenomenon is that a subsequent enrichment step is incorporated in the BAX system's testing protocol, with which the detection of dead cells can be reduced to the minimum. The price for this, however, is the longer detection time.

Investigation of *E. coli* O157 with the real-time PCR method

Opposed to the culturing method the real-time PCR method is more suitable for the detection of the presence of the O157:H7 serotype of *E. coli* being undetectable with conventional

methods and unable to proliferate. The introduction of this method is especially recommended in light of the incertitude of the conventional method and of the increased demand on the testing of pathogenic *E. coli* groups. The Directive of the European Union also recommends this method since 2013.

It can be established on the basis of the numerous test results that the used and commercially available detection methods a method of appropriate sensitivity, accuracy and specificity can be selected with high certitude if the sampling plan (sample count and frequency), time period intended to be provided for the tests, the technological procedures and the effect exerted on the method by the matrices are known.

6. NEW SCIENTIFIC ACHIEVEMENTS

1. According to the MSZ EN ISO 16140 standard (relative accuracy, relative specificity, and relative sensitivity) I established the performance characteristics of the methods suitable for the detection of certain pathogens. In the cases of routinely performed tests a trustworthy result issued within the least possible time is essential. The international bodies of validation elaborate validations for certain food matrices, but these do not cover for all food types which may occur in a laboratory performing routine tasks. In case of missing matrices I established the given method's performance characteristics along with the already validated methods.
2. I carried out matrix effect analysis and established the factors which hinders the safe detectability of pathogens I investigated by using alternative microbiological methods. My experiments proved that regarding relative accuracy and specificity data the comparative results of the investigations carried out by using alternative rapid methods show deviation per each matrix. While searching for the reason of this phenomenon I established that seasoned meat, chocolate, feeding stuff, and environmental samples, as well as fruits containing oenocyanin and anthocyanin have an influence over pathogen detection through real-time PCR rapid methods. Apart from the matrix effects verified in the cases of rapid methods the errors of the standardised method were also revealed. False negative result can be measured even by using the conventional method. The reason of this is the inappropriate conduction of the preliminary enrichment and/or the disturbing effect of a microbiological agent being concomitantly present.
3. I established the detection limits of the alternative testing methods, based on which I

optimised the enrichment procedures. Limitations of the enrichment procedure were demonstrated with a *Campylobacter* example. . Certain components of the selective enrichment broths can inhibit the proliferation of *Campylobacter* spp., therefore I compared the four selective enrichment broths suitable for the enrichment of *Campylobacter* spp. Based on the obtained results and in cases of the qPCR tests the blood containing Bolton's enrichment broth have proven to be positive for most cases.

4. I established that the qPCR method is more suitable for the detection of *Campylobacter* species. Culturing testing methods cause information deficit because although these are suitable for the detection of *Campylobacter* spp., yet these cannot be applied for the detection of the species most frequently causing problems, *Campylobacter jejuni*. With the applied qPRC method *Campylobacter* spp. and *Campylobacter jejuni* can be detected simultaneously. I made an attempt towards quantification from the samples through the qPCR method.
5. Based on my experiments the TaqMan *Salmonella enterica* real-time PCR Kit (ABI) has proven to be the fastest when testing poultry products as opposed to the VIDAS ICS2-SLM and BAX methods. Therefore, in the case of poultry the recommended testing method is the TaqMan *Salmonella* testing, which is suitable for result communication within 24 hours, with which it could be achieved to indicate compliance within 24 hours for all limit parameters (*Salmonella* spp. *S. aureus*, *E. coli*, microbial count) stipulated for poultry. My results proved that the microbiological condition of fresh poultry meat can be determined within 24 hours by using the applied modern and rapid microbiological procedures, thereby providing possibility for fast qualification before marketing.

7. PUBLICATIONS

JOURNALS

In journals with impact factor:

1. **Rohonczy, K.,** Zoller, L., Hermann, Zs, Fodor, A., Mráz, B., Tabajdi-Pintér, V. (2013) Coparison of automated ELISA and two different real-time PCR techniques for *Salmonella* detection in poultry samples. *Acta Microbiologica et Immunologica Hungarica, (in press)* IF: 0.79

2. **Rohonczy, K.,** Rantsiou, K., Cocolin, L. (2013) Modified enrichment strategies coupled with molecular and conventional methods to detect and quantify *Campylobacter jejuni* in chicken meat from the market. *Journal of Food Safety*, 33 (4), pp. 497-502. IF: 0.72

CONFERENCE PROCEEDINGS

Hungarian (abstract):

1. **Rohonczy K**., Mohácsiné Farkas C., Kiskó G., Taczman-Brückner, A., Farkas Á. (2005) Impedimetriás elven alapuló gyors módszer kifejlesztése zöldségeken előforduló *L. monocytogenes* kimutatására. *HUNGALIMENTARIA 2005.* 2005. április 19-20., Debrecen.

2. Fodor A., **Rohonczy K**., Tabajdiné Pintér V. (2006) E. coli vizsgálatának összehasonlító elemzése, különös tekintettel az EU mikrobiológiai kritériumokra. *EOQ 2006.* 2006. március 29-30., Debrecen.

3. **Rohonczy K**., Fodor A., Tabajdiné Pintér V. (2006) Patogén mikroorganizmusok kimutatására szolgáló korszerű gyorsmódszerek összehasonlító vizsgálatai. *EOQ 2006*. 2006. március 29-30., Debrecen.

4. Tabajdiné Pintér V., **Rohonczy K**. (2006) Gyors mikrobiológiai vizsgálatok jelentősége az ivóvíz vizsgálatok területén. *Ökotech Mavíz Szakmai Nap*, 2006. október 1., Budapest.

5. **Rohonczy K.**, Fodor A., Tabajdiné Pintér V., Mohácsiné Farkas Cs. (2007) Patogén mikroorganizmusok kimutatására szolgáló korszerű gyorsmódszerek összehasonlító vizsgálatai. *HUNGALIMENTARIA 2007*. 2007. október 25-26., Budapest.

6. Fodor A., **Rohonczy K**., Tabajdiné Pintér V. (2007) Egységesítési feladatok az élelmiszer-mikrobiológiaigyakorlatban *HUNGALIMENTARIA 2007*. 2007. október 25-26., Budapest.

7. **Rohonczy K.**, Fodor A., Tabajdiné Pintér V., Zoller L. (2008) Patogén mikroorganizmusok vizsgálata molekuláris biológiai módszerekkel *EOQ 2008.* 2008. április 24-25., Tihany.

8. **Rohonczy K.**, Fodor A., Tabajdiné Pintér V.,Zoller L. (2008) Húsipari termékek biztonságos eltarthatósági idejének meghatározása. *EOQ 2008.* 2008. április 24-25., Tihany.

9. **Rohonczy K.**, Fodor A., Tabajdiné Pintér V., Zoller L. (2009) Real time PCR módszerrel végzett patogén vizsgálatok tapasztalati. *HUNGALIMENTARIA 2009.* 2009. április 22-23., Budapest.

10. **Rohonczy K.**, Fodor A., Tabajdiné Pintér V., Zoller L**.** (2009) Húsipari termékek minőségmegőrzési időtartamának validálása az EU ajánlás tükrében. *HUNGALIMENTARIA 2009.* 2009. április 22-23., Budapest.

11. Tabajdiné Pintér V., **Rohonczy K.**, Zoller L. (2010) Patogén mikroorganizmusok kimutatása rekombináns fágfehérjékkel. *HUNGALIMENTARIA 2009.* 2009. április 22-23., Budapest.

12. Zoller L., **Rohonczy K.,** Tabajdiné Pintér V. (2010) Patogén mikroorganizmusok Real-Time PCR módszerrel történő kimutatása során szerzett tapasztalatok *Wessling szakmai nap.* 2010. április 16., Budapest.

13. Zoller L., **Rohonczy K.**, Tabajdiné Pintér V. (2010) Patogén mikroorganizmusok Real-Time PCR módszerrel történő kimutatása során szerzett tapasztalatok. *Élelmiszer mikrobiológia szeminárium*. 2010. június. 17., Budapest.

International (abstract):

1. Mohácsi-Farkas Cs., Kiskó G., **Rohonczy K.**, Taczman-Brückner, A. (2004): Detection of Listeria monocytogenes by impedimetric method from raw vegetables. *2nd Central European Congress on Food*. 2004. április 26-28. , Budapest. Hungary.

2. Kiskó G., Mohács- Farkas Cs., **Rohonczy K.**, Taczman-Brückner, A. (2004): Detection of Listeria monocytogenes by impedimetric method from raw vegetables and fruit. 2nd Central European Congress on Food. 26-28 April 2004, Budapest, Hungary.

3. Mohácsi-Farkas Cs., Kiskó G., Taczman-Brückner, A., **Rohonczy K.** (2004): Impedimetric detection of Listeria monocytogenes from vegetable and fruit products. The International ICFMH Symposium Foodmicro 2004. 12-16th September 2004, Portoroz, Slovenia.

4. Zoller, L, **Rohonczy, K**, Mráz, B., Tabajdiné Pintér, V. (2012) Comparison of applicability rapid methods for Salmonella detection in poultry meat. The IAFP's European Symposium on Food Safety 2012. 21-23 May Warsaw, Poland

NATIONAL R&D PROJECT

1. Baromfi húsok Salmonella szennyezettségének elemzése, gyors mikrobiológiai módszer fejlesztése (Innovációs Fejlesztési Program 2009)

2. Húsüzemi környezetben előforduló *Listeria monocytogenes* okozta kockázat elemzése (Innovációs Fejlesztési Program 2007)

3. Darabolt sajtok biztonságos eltarthatósági idejének meghatározása (Innovációs Fejlesztési Program 2007)

4. Húsipari termékek biztonságos eltarthatósági idejének meghatározása (Innovációs Fejlesztési Program 2008)

5. Húsüzemi környezetben előforduló *E. coli* O157 okozta kockázat elemzése (Innovációs Fejlesztési Program 2009)