WORK PROGRAMME of EURL for FOODBORNE VIRUSES

PERIOD: 2019/2020

Version 1.2
(date 28/01/2019

Magnus Simonsson
magnus.simonsson@slv.se
+46 72 272 20 52
National Food Agency
Box 622
751 26 Uppsala
Sweden

SUMMARY

INTRODUCTION

Abbreviations

RT-dPCR	RT digital PCR
HAV	Hepatitis A virus
NFA	National Food Agency
NGS	Next Generation Sequencing
PT	Proficiency testing
SOP	Standard operating procedure

Increased awareness of the public health impact of foodborne viruses has led to the establishment of a EURL for foodborne viruses. The EURL has almost finished its first year and an organisation at the National Food Agency (NFA) has been built up. A network of 24 national reference laboratories (NRLs) (EU 20, 2 EFTA, plus Serbia and Germany, soon to be designated) has been established. In a complex area like foodborne viruses, several countries have designated two and even three laboratories. In total 17 MS has designated NRLs, meaning that there are still 11 MS that are in the process of designating laboratories. Due to the delay in designating laboratories, the EURL decided to concentrate the NRL activities to late 2018.

We anticipate that eight more MS will designate laboratories early 2019. In the budget estimates we will include those eight potential NRLs.

There are currently no microbiological criteria for foodborne viruses in the EU legislation but EU 2017/2298, amending EU669/2009, states that there should be an increased level of official control of norovirus and hepatitis A virus (HAV) in frozen raspberries from Serbia. Virus inoculated raspberries was therefore the first matrix based PT to be sent out and we intend to repeat this PT during 2019. As the import route for raspberries from Serbia primarily goes through Hungary, most sampling and analyses are done there. Unfortunately, Hungary has no designated NRL for foodborne viruses.

During 2018 we organised a workshop for the NRLs as well as two courses addressing detection of norovirus in raspberries. 2018 was the first year for this EURL and the work programme for 2019 and 2020 contain similar activities but with courses addressing production of control materials. In addition to the raspberry PT, a PT for the quantification of viruses in bivalves will be conducted on a yearly basis, starting in 2019. During 2020 we plan to replace the raspberry PT with a leafy green matrix.

Regulation (EU) 625/2017 Art 94(2):
European Union reference laboratories designated in accordance with Article 93(1) shall be responsible for the following tasks insofar as they are included in the reference laboratories' annual or multiannual work programmes that have been established in conformity with the objectives and priorities of the relevant work programmes adopted by the Commission in accordance with Article 36 of Regulation (EU) No 652/2014:
(taking into account Art 147 of (EU) 625/2017)

1 TO ENSURE AVAILABILITY AND USE OF HIGH QUALITY METHODS AND TO ENSURE HIGH QUALITY PERFORMANCE BY NRLs.

Please, provided activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- *Art. 94.2.a* ***Providing national reference laboratories with details and guidance on the methods of laboratory analysis, testing or diagnosis, including reference methods.***
- *Art. 94.2.b* ***Providing reference materials to national reference laboratories***
- *Art. 94.2.c* ***Coordinating the application by the national reference laboratories and, if necessary, by other official laboratories of the methods referred to in point (a), in particular, by organising regular inter-laboratory comparative testing or proficiency tests and by ensuring appropriate follow-up of such comparative testing or proficiency tests in accordance, where available, with internationally accepted protocols, and informing the Commission and the Member States of the results and follow-up to the inter-laboratory comparative testing or proficiency tests.***
- *Art. 94.2.l* ***Where relevant for their area of competence, cooperate among themselves and with the Commission, as appropriate, to develop methods of analysis, testing or diagnosis of high standards.***

Sub-activity 1.1 *Development, distribution of Proficiency tests (PT)*

Objectives: To ensure harmonised analysis results among NRLs through the distribution of homogenous samples for PT and assess and report the results.
Description: As the EURL started up during 2018, the PT for 2018 was distributed very late in 2018. Hence, the assessment and the report will be finished early 2019.

The PT for detection of norovirus and hepatitis A virus (HAV) in raspberries will be repeated during spring 2019. During the development of improved methods for the detection of norovirus and HAV in raspberries the laboratory experienced problems with homogeneity between samples, probably due to freezing procedures of viruses in a raspberry matrix. We have seen similar problems during the development of the PT. There is a need to solve this problem to be able to better assess the performance of the NRLs. This problem will be addressed in the beginning of 2019.

Late spring we intend to distribute the second PT with norovirus- and HAV contaminated raspberries.

When it comes to PT distributions with live molluscan shellfish (LBM), like oysters, the EURL does not have facilities for bioaccumulation. During 2019 we will come to agreement with the University of Gothenburg to be able to use facilities at one of the Marine stations on the Swedish West Coast. Initial contacts have been taken but the actual costs for such an agreement are still difficult to predict. Two travels for two persons are anticipated. One to Gothenburg to agree on facilities and one to CEFAS, UK to learn about bioaccumulation of oysters.

As bioaccumulation will not be possible in 2019 we intend to conduct a PT using oyster hepatopancreases that have been artificially spiked with HAV and norovirus (similar to what was done during validation of ISO 15216). The following year it will, most probably, be possible to distribute bioaccumulated samples. The bivalve matrix for 2020 will also be oysters, as this will become an increasingly important matrix, if there will be a regulation on microbiological criteria for norovirus and HAV in oysters. We will travel to Gothenburg. Firstly for agreement on the bioaccumulation facilities, secondly for bioaccumulated test samples and finally for bioaccumulation of PT samples.

Furthermore, during 2020 we will distribute virus contaminated leafy green PT samples. The development of a procedure for producing such samples will mainly be done in 2019.

For each new matrix an SOP will be produced and distributed to the NRLs for the corresponding method.

All PT analyses will be based on ISO15216. In the informative text to this standard the use of a miniMag® equipment from bioMérieux is indicated. BioMérieux will not produce this equipment any more instead they will provide something they call GENE-UP®. As several NRLs already have this equipment the EURL intend to by such machine to be able to evaluate PT samples with this alternative equipment. It is also important for the EURL to be able to do troubleshooting if NRLs have problems with GENE-UP®

During the workshop in 2018 the NRLs requested the inclusion of extra samples to assess variations of standard methods, such as the use of inhibitor removal kits and the use of RT digital PCR for the quantification of viral genomes. Therefore the cost of a certain amount of extra samples in all PT distributions will be added to the budget.

Expected Output:

1. Evaluation of the state of harmonisation of qualitative and quantitative results by the NRLs regarding detection and quantification of HAV and norovirus in bivalve and vegetable matrices.
2. Development of methods for production of homogenous virus-contaminated samples in soft fruit, vegetables and LBM.
3. PT distribution of virus-contaminated raspberries 2019.
4. PT distribution of virus-contaminated oyster hepatopancreas 2019.
5. PT distribution of virus-contaminated leafy greens 2020.
6. PT distribution of virus-bioaccumulated oysters 2020.
7. A SOP for each new matrix.
8. Assessment and reports for each PT distribution and follow up of NRLs if necessary.

Duration: 2019 and 2020

Sub-activity 1.2 *Implementation and development of typing methods for norovirus*

Objectives: To improve the typing methods used for norovirus.
Description: Typing methods of norovirus are of high importance both for tracing sources in outbreaks and to be able to follow the epidemiological development. The typing methods for norovirus are to some extent harmonised but there is a need for further development. Many noroviruses are so called recombinant viruses (one virus is a combination of two different types). Norovirus type GII.4 Sydney 2012 is suggested to have had a prolonged time as a pandemic virus due to such recombination, thereby overcoming heard immunity.
Since current standard typing methods (cap sequence) do not capture recombinant noroviruses, an extended part of the norovirus genome (pol-cap sequence) has to be sequenced. Methods based on RT-PCR have been suggested for this, but they seem to be less sensitive and not suitable for food samples. This method therefore needs to be optimised.
We intend to use bioinformatic analyses and laboratory testing of different norovirus types in order to optimise a broader norovirus typing method suitable for food samples with low levels of norovirus.

Many food samples, like oysters, tend to harbour multiple types of norovirus. Conventional RT-PCR in combination with conventional sequencing (the method above) will only capture the most common type. With Next Generation Sequencing (NGS) it is possible to find more or less all types in a sample. With current technology it seems most reasonable to use, so called, amplicon sequencing to facilitate the detection of more than one type in a food sample.
In 2019 we will work with the standard cap-sequence and, if sensitive pol-cap sequencing has been developed, we will develop next generation sequencing targeting this larger part of the norovirus genome to also enable the identification of recombinant noroviruses.
The EURL currently has limited experience of the NGS technique, but we have installed an Illumina platform at the laboratory and assigned several persons (one specifically for viruses) to develop their skills. There is a need for both bioinformatics and platform competence. During 2019 and 2020 we will continue the work to increase the EURL skills through participation in courses and hands-on activities. There will also be support from the EURL working group on NGS (see also 3.2). We will start the laboratory work using NGS for typing of human faecal samples and combinations of different faecal samples to demonstrate the possibility to find more than one type of norovirus in a sample. A natural continuation will be the investigation of food matrices spiked with norovirus positive faecal samples but also naturally virus contaminated food samples.
Expected Output:

1	A sensitive pol-cap typing method is developed to enable the identification of recombinant noroviruses
2	NGS technology has been developed for typing of multiple noroviruses in a sample, targeting the standard cap-region.
3	NGS technology has been developed for typing of multiple noroviruses in a sample, targeting the pol-cap-region. Enabling identification of recombinant noroviruses.

Duration: 2019 2020

Sub-activity 1.3 *Quantification of foodborne viruses using RT digital PCR (RT-dPCR) methods*

Objectives: To increase the possibility for laboratories to use RT digital PCR (RT-dPCR) as an alternative to RT-qPCR for quantification of viruses in food.

Description: Previous studies, including studies conducted at the EURL, demonstrate that RT-dPCR offers better repeatability (precision in quantification within the same laboratory) than RT-qPCR. RT-dPCR uses a technology that can quantify viral genomes without the use of a standard curve, which is the case for quantitative RT-qPCR. Reverse transcription dPCR is also less affected than RT-qPCR by suboptimal PCR efficiency. Suboptimal PCR efficiency can be caused by e.g. inhibitory substances in the sample and primer-template mismatches. Food matrices often contain inhibitory substances for RT and/or PCR, causing underestimation of the viral load or false negative results. Furthermore, norovirus and HAV are RNA viruses and are prone to a high mutation rate, and thereby display great sequence diversity between strains. Mismatches between primers/probes and their target sequences can also cause underestimation or false negative results. Previous studies, including studies conducted at the EURL, demonstrate that RT-dPCR quantification is more resilient than RT-qPCR to inhibitory substances from food and to primer-template sequence mismatches.
The EURL aims to compare the differences between standard quantitative RT-PCR methods and RT-dPCR when it comes to resistance to inhibitory substances from different food matrices. This will be done during the setup of different PT distributions.
The EURL will also compare the quantification of norovirus using quantitative RT-PCR or RT-dPCR to answer the question if a simple factor can translate results between the two methods.

The reproducibility (precision in quantification between laboratories) of RT-dPCR has not yet been investigated. In a questionnaire to the NRL laboratories, more than one third stated the use or the possibility to use RT-dPCR.
At the workshop for NRLs in Uppsala 2018, there were requests to include RT-dPCR in the work program. The EURL will therefore include extra samples in PT distributions. In the long run the EURL aim to (outside this work program) arrange oysters contaminated with norovirus, to allow for an interlaboratory comparison of e.g. reproducibility and matrix effects, to elucidate whether the method is suitable to use as an alternative to RT-qPCR.

Expected Output:
In the long run RT-dPCR can be used as an alternative to standard methods, leading to more robust analysis and less variable results between laboratories.
Duration: 2019 2020

2 TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO NRLs

Please, provided activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- *Art. 94.2.d* ***Coordinating practical arrangements necessary to apply new methods of laboratory analysis, testing or diagnosis, and informing national reference laboratories of advances in this field.***
-
- *Art. 94.2.e* ***Conducting training courses for staff from national reference laboratories and, if needed, from other official laboratories, as well as of experts from third countries.***
-
- *Art. 94.2.g* ***Providing information on relevant national, Union and international research activities to national reference laboratories.***

Sub-activity 2.1 *Annual workshop*

Objectives:
To inform NRLs about PT results, development of the standardisation process and scientific progress concerning foodborne viruses and the detection and quantification of those in food matrices. Further, to discuss with NRLs the actual needs among NRLs and future work that can be done in the network.

Description:
The first workshop for NRLs for Foodborne Viruses was held in Uppsala November 2018. It was decided that the workshop 2019 also will be held in Uppsala, and that, from there on, the workshop should be hosted by one of the NRLs every other year. The dates of the upcoming workshops have not been decided, as many laboratories are NRLs for different topics, and several suggested dates already were blocked by other EURL workshops. It was decided at the 2018 workshop that DG SANTE, EFSA and ECDC always should be invited to future workshops. On their own initiative ECDC already indicated their interest to participate.
In general, the workshops will contain information on PT results, information about standardisation processes, updates on relevant legislation, and progress in method development. Further, discussions will be held about how NRL network collaboration promotes scientific progress and how the network cooperation may facilitate harmonisation of analytical results amongst Member States.

Expected Output:

1	A well informed NRL network about harmonisation and standardisation progresses.
2	An NRL network that shares information on method development for improved analysis of foodborne viruses in complex matrices.

3	An NRL network functioning as a facilitator of progresses in the scientific field of foodborne viruses with the aim to decrease public health burden of foodborne virus transmission.

Sub-activity 2.2 *Technical training courses*

Objectives:
To give strong theoretical background and hands-on training in analytical methods and techniques to less experienced NRLs.

Description:
During 2018 the EURL gave hands-on training courses about detection of norovirus in raspberries on two occasions. In total ten laboratories participated. In a questionnaire to the NRLs we identified that there are many laboratories with no or low experience of RNA transcription, a technique necessary for the production of control RNA used in ISO 15216. In 2019 we therefore intend to give training courses on two occasions on RNA transcription with a maximum of totally 16 participants. During the 2018 workshop there was a discussion about training courses for 2020. We plan to include two technical training course occasions in the EURL Work Program of 2020, also here with a total maximum of 16 participants. The topic of these courses will be decided at the workshop 2019.
Expected Output: Better method performance among the NRLs.
Duration: 2019 and 2020

Sub-activity 2.3 *Preparedness of staff and ad hoc assistance to NRLs*

Objectives:
To follow the national and international scientific development in the area of foodborne viruses and to inform about the EURL and its activities. Support NRLs with scientific and methodological knowledge in a timely manner. The EURL website should be continuously updated and improved as a means of communication with NRLs and other stakeholders.

Description:
This activity seeks to keep the staff knowledgeable in the area of foodborne viruses to be able to support NRLs in the best way with such knowledge. Workshops and training courses organised by the EURL will be continuously evaluated to improve those activities over the years. Preparation of work programs and budget is included as well as the internal organisation of the EURL.
It is important to follow scientific publications as well as to participate in conferences. Two staff members will participate in the biennial Food and Environmental Virology Conference in Spain during 2020. Relevant abstracts will be presented about progress in method development achieved at the EURL. The Swedish Society for virology has a yearly symposium. One staff member will participate to enable good contact with the Swedish virology community and the hospital laboratories on which we depend for the collection of reference materials, especially faecal samples used for contamination of food matrices in method development, validation studies and PT distributions.
During the workshop 2018 there was a request to give guidance on validation/verification for the accreditation of ISO 15216. The production of such a guidance document is included in the work program for 2020. Another request was about bioinformatic surveillance of the inclusivity of norovirus and HAV primers and probes recommended in ISO 15216. This surveillance aims to show if new sequence variants of these viruses published in GeneBank are indicated to not be detected with current primers and probes and therefore require new primer- and/or probe designs

The EURL promised to investigate the possibility to have such surveillance. Bioinformatic surveillance is not included in the budget for 2019-2020.

Expected Output:

1	Improved competence among and organisation of staff members to enable improved support to the NRLs.
2	Opportunities to inform the scientific society about the EURL activities.
3	The creation of a Guidance Document for the validation/verification for accreditation of ISO15216, to facilitate NRL accreditation process.

Duration: 2019 2020

Sub-activity 2.x *(name of Sub-activity)*

Objectives:
Description:
Expected Output:
Duration:

3 TO PROVIDE SCIENTIFIC AND TECHNICAL ASSISTANCE TO THE EUROPEAN COMMISSION AND OTHER ORGANISATIONS

Please, provided activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- *Art. 94.2.f* ***Providing scientific and technical assistance to the Commission within the scope of their mission.***

- *Art. 94.2.h* ***Collaborating within the scope of their mission with laboratories in third countries and with the European Food Safety Authority (EFSA), the European Medicines Agency (EMA) and the European Centre for Disease Prevention and Control (ECDC).***

- *Art. 94.2.i* ***Assisting actively in the diagnosis of outbreaks in Member States of foodborne, zoonotic or animal diseases, or of pests of plants, by carrying out confirmatory diagnosis, characterisation and taxonomic or epizootic studies on pathogen isolates or pest specimens.***

Sub-activity 3.1 *Participation in the EURL Working Group for NGS.*

Objectives:
To promote the use of next generation sequencing (NGS), across the EURLs´ network.

Description:
Participate in two group meetings each year and give continuous input to the EURL working group. As the use of NGS technique is not very well developed for foodborne viruses there is a need for cooperation amongst laboratories in this area. Beside the EURL working group for NGS the EURL for Foodborne Viruses intend to form a group inside the NRL network to increase the possibility for a faster development of NGS techniques used in the area of foodborne viruses. The group will have one physical meeting each year, preferably connected to the yearly workshop. Discussions will be held about diversity of platforms and bioinformatics pipelines and the possibility for a harmonised process when it comes to NGS typing of especially noroviruses.

Expected Output:
Support to the commission, EFSA and ECDC in their work to ensure the development of NGS as a typing method in EU MS.
Duration: 2019 2020

Sub-activity 3.2 *Scientific assistance and emergency support to the Commission, EU agencies and third countries.*

Objectives:
To ensure that the EURL staff is well trained, up-dated and knowledgeable in the area of foodborne viruses so that appropriate expertise can be provided to the Commission and that adequate support will be given in emergency situations, for example during foodborne viral outbreaks.

Description:
Sub-activity 2.3 will support this activity and requests from the Commission and EU agencies for scientific and technical assistance will have priority and be handled by the EURL scientific staff in a timely manner. Serbia specifically needs support, due to problems with norovirus contamination of raspberries. If requested, we will continue our support to Serbia.
Expected Output:
A well informed and trained staff that can give support to the Commission and EU agencies in a timely manner.
Duration: 2019 2020

Sub-activity 3.x *(name of Sub-activity)*

Objectives:
Description:
Expected Output:
Duration:

4 REAGENTS AND REFERENCE COLLECTIONS

Please, provided activities related to Regulation (EU) 2017/625:
(Number of Sub-activity boxes can be adjusted by EURL)

- *Art. 94.2.*j ***Coordinating or performing tests for the verification of the quality of reagents and lots of reagents used for the diagnosis of foodborne, zoonotic or animal diseases and pests of plants.***

- *Art. 94.2.k* ***Where relevant for their area of competence, establishing and maintaining:***
  - ***i. reference collections of pests of plants and/or reference strains of pathogenic agents;***
  - ***ii. reference collections of materials intended to come into contact with food used to calibrate analytical equipment and provide samples thereof to national reference laboratories;***
  - ***iii. up-to-date lists of available reference substances and reagents and of manufacturers and suppliers of such substances and reagents.***

Sub-activity 4.1 *Production of standard DNA, control RNA and process control virus*

Objectives:
To ensure that NRLs have access to standards and controls to increase uniformity of method performance between laboratories. To provide a means for quality assurance and method evaluations to laboratories.

Description:
DNA standards and control RNA as well as process control virus (mengo virus) recommended in ISO 15216 will be produced (mengo virus will be bought from the Swedish National Veterinary Institute), and shared with the NRLs.

Expected Output:
Collection of standards and controls to increase uniformity of method performance.
Overall improved quality assurance of the analyses performed at the NRLs for detection of foodborne viruses.
Duration: 2019 2020

Sub-activity 4.2 *Organise and collect reference materials such as faecal samples and cultivated viruses.*

Objectives:
To have an up-to-date collection of reference substances and reagents and to store these in an accessible manner.

Description:
During 2018 we bought two freezers (-70 and -20) and one refrigerator to be able to store reference materials such as faecal samples and cultivated viruses. In cooperation with hospitals and other partners we will continuously collect norovirus faecal samples and other relevant enteric viruses to be registered and stored. Typing of such virus stocks will be done. Viruses like HAV will be cultivated in cooperation with the Swedish National Veterinary Institute.

Expected Output:
A collection of stored and available substances and reagents is maintained and continuously updated. Relevant reference materials may be provided to NRLs and other stakeholders.
Duration: 2019 2020

Sub-activity 4.3 *(name of Sub-activity)*

Objectives:
Description:
Expected Output:
Duration:

Sub-activity 4.x *(name of Sub-activity)*

Objectives:
Description:
Expected Output:
Duration:

5 REQUIREMENTS RELATED TO OTHER LEGISLATION

Please specify applicable legislation:
(Number of Sub-activity boxes can be adjusted)

Sub-activity 5.1 *(name of Sub-activity)*

Objectives: Description: Expected Output: Duration:

Sub-activity 5.2 *(name of Sub-activity)*

Objectives: Description: Expected Output: Duration:

Sub-activity 5.3 *(name of Sub-activity)*

Objectives: Description: Expected Output: Duration:

Sub-activity 5.x *(name of Sub-activity)*

Objectives: Description: Expected Output: Duration:

REMARKS

(if necessary)