WORK PROGRAMME of EURL for FOODBORNE VIRUSES

PERIOD: 2021/2022

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CONTENT

INTRODUCTION

Abbreviations

RT-dPCR	RT digital PCR
HAV	Hepatitis A Virus
HEV	Hepatitis E virus
SFA	Swedish Food Agency
NGS	Next Generation Sequencing
PT	Proficiency Testing
SOP	Standard Operating Procedure
WP	Work Programme
LBM	Live Bivalve Molluscs

The EURL is about to complete its third year and an organisation at the Swedish Food Agency (SFA) has continuously been built up. Moreover, a network of 30 national reference laboratories (NRLs) has been established.

The network of National reference Laboratories (NRLs) has been established and in a complex area like foodborne viruses, several countries have designated two and even three laboratories.

Member states (MS) that still have not designated NRLs for foodborne viruses are:

Croatia, Estonia, Greece (for non-animal matrices), Lithuania, Malta, Poland (for animal matrices) and Portugal.

During 2020, EU decided to close the temporary legislation EU 2017/2298, amending EU669/2009, which stated increased level of official control of norovirus and hepatitis A virus (HAV) in frozen raspberries from Serbia. There are currently no microbiological criteria for foodborne viruses in the EU legislation, but a discussion about microbiological criteria for norovirus and HAV in molluscan shellfish, eaten raw, has been initiated by the Commission.

Annual PT distributions of molluscan shellfish spiked or bioaccumulated with norovirus and HAV are planned. Virus spiked soft fruits PT is also planned. Other matrices included in ISO 15216 have to be developed at the EURL and development and verification of swabbing techniques of food surfaces are included in this WP, starting in 2021. New for this work programme is also the participation in the standardisation process for hepatitis E virus detection and quantification in animal products.

The covid-19 pandemic has hindered the possibility to perform some of the activities in the previous Work Programme. Such activities will be included in this WP.

The plan for this WP is that 2021 is a year where we hopefully can have physical meetings at least in late autumn and that things have gone back to normal in 2022.

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)

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: In the matrix group, soft fruit and leaf stem and bulb vegetables the EURL has distributed raspberries and leaf vegetables. There has been requests from the NRLs to also cover other matrix groups included in ISO 15216, especially when it comes to swabbing techniques used for surfaces.

There are some previous experience of swabbing techniques at the EURL, but there is a need to implement and verify swabbing techniques according to ISO 15216. Ultimately, this method should be accredited at the laboratory. Implementation is planned to be done during 2021 and distribution of a surface PT is planned for 2022. More details about the implementation and verification can be found under 1.3.

The ongoing discussions about microbiological criteria for noroviruses in oysters implies the importance to include this matrix in the PT scheme every year.

During the last two years there have been two PT distributions of virus spiked oyster hepatopancreas. Due to the pandemic, we were not able to organise any bioaccumulation of oysters as planed in the previous WP. If not the pandemic hinders, we will start collaboration with a Swedish marine institute at the University of Gothenburg during 2021. One travel during 2021 will aim to the agreement of facilities and further activities to have all the permissions needed to conduct bioaccumulation of viruses at the Marine Institute.

As bioaccumulation will not be possible in 2021, we intend to conduct a PT using oyster hepatopancreas artificially spiked with HAV and norovirus (similar to what was done during validation of ISO 15216). In 2022, we plan to distribute virus bioaccumulated oysters. Bioaccumulation in 2022 means that there is a need for two persons to visit the facilities at three occasions.

Furthermore, we will distribute virus contaminated soft fruits (strawberries) in 2021.

For each new matrix an SOP will be produced, meaning that during the beginning of 2022 a new SOP describing swabbing techniques for virus extraction from surfaces will be produced and published on the EURL website.

All PT analyses will be based on ISO 15216. In the informative text to this standard the use of a miniMag® equipment from bioMérieux is indicated. BioMérieux will not produce this equipment anymore and instead will provide a device called GENE-UP®. As several NRLs already have this equipment the EURL bought this device in order to evaluate PT samples with this alternative equipment. Initial tests indicate that the new equipment is interchangeable with the miniMAG®. Further verifications have to be performed and such verifications are included in the budget for 2021 and 2022.

The EURL has performed comparisons between RT digital PCR (RT-dPCR) and RT-qPCR in oyster matrix. The work has been published and RT-dPCR was concluded to be fit for purpose, especially if high precision was desired. 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.

Methods providing precise and accurate quantification of noroviruses in oysters are highly valuable for the implementation of future microbiological criteria. Therefore, the EURL together with NRL Netherlands have planned to include, in an oyster PT distribution, the possibility to compare RT-dPCR with RT-qPCR method described in ISO 15216-1. Ten NRLs registered to participate in this pilot study, but due to lock down and restrictions the EURL decided to cancel this part of the PT distribution. It was decided to include this in an oyster PT distribution planned to be distributed in Mars 2021. Expenses for this exercise are included in the budget for 2021.

Quality assurance of the EURL is important to maintain high quality and accreditation. The participation in external PT distribution is therefore of high importance. FAO Reference Centre for Bivalve Mollusc Sanitation, CEFAS, UK distribute PTs with norovirus an HAV contaminated Live Bivalve Molluscs (LBM). A contract between the FAO centre and the EURL will declare the exchange of PT distributions between the two laboratories. No charge will be incurred between the laboratories. The extra costs for distribution to the FAO reference centre and the performance of the PT analyses are included in the EURL budget. This is foreseen to be an annually recurring exercise.

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 of hard surfaces.
3. Available facilities for bioaccumulation of bivalves, 2021.
4. PT distribution of virus-contaminated strawberries 2021.
5. PT distribution of virus-contaminated oyster hepatopancreas 2021.
6. Assessment of the comparison between quantification of norovirus in oyster samples by RT dPCR and RT qPCR, 2021.
7. PT distribution of virus-contaminated hard surfaces 2022.
8. PT distribution of virus-bioaccumulated oysters 2022.
9. An SOP for hard surfaces, 2021.
10. Assessment and reports for each PT distribution and follow up of NRLs if necessary, 2021, 2022.
11. Quality assurance and maintained accreditation for the EURL through PT participation.

Duration

2021 and 2022

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 recombinations, 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. RT-PCR based methods have been developed and implemented for clinical samples, but the sensitivity is too low to be used on food samples. Further optimizations are therefore needed.

Typing through the cap region is implemented at the EURL and environmental samples has been analysed through Next Generation Sequencing (NGS), showing the appearance of several genotypes in a single sample.

An application based on R programming language has been developed for the sake of finding conserved primer regions in highly variable viruses as noroviruses. The application is due for publication in 2021.

This type of bioinformatics analyses in combination with laboratory testing of different norovirus types will be used in order to optimise a broader norovirus typing method suitable for food samples with low levels of norovirus.

Many food samples, such as 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 NGS it is possible to find more or less all types in a specific sample. With current technology it seems most reasonable to use, so called, amplicon sequencing to facilitate the detection of more than one norovirus type in a single food sample This will improve our capability for source attribution and tracing in outbreak situations, which, in turn, will help us to better understand the epidemiology, evolution and transmission of foodborne viruses.

In 2021 we will continue the work with the standard cap-sequence and start the work with a description of a workflow that could be shared with the NRL Working Group for NGS and the Inter EURL WG on NGS. The work towards a sensitive pol-cap sequencing method to also enable the identification of recombinant noroviruses will include the use of NGS for sequencing this target.

There is a need for both bioinformatics and platform competence.

During 2021 and 2022 we will continue the work to increase the EURL skills in collaboration with the NRL WG for NGS and the inter EURL WG for NGS.

Expected Output:

1	A sensitive pol-cap typing method is developed to enable the identification of recombinant noroviruses, 2021
2	NGS technology has been tested for typing of multiple noroviruses in a sample, targeting the cap-region, 2021,2022.

3	A workflow for NGS targeting the cap region will be shared with the NRL WG on NGS, 2022.
4	NGS technology has been developed for typing of multiple noroviruses in a sample, targeting the pol-cap-region. Enabling identification of recombinant noroviruses, 2021, 2022.

Duration:

2021 2022

Sub-activity 1.3 Implementation and verification of method for detection and quantification on food surfaces according to ISO 15216.

Objectives:

To accredit the ISO 15216 swabbing method for detection and quantification of HAV and norovirus on food surfaces to enable PT distributions for this methodology.

Description: The EURL has some previous experience of swabbing methods but there is a need for testing different matrices like bell pepper and tile, artificially contaminated with norovirus and HAV. Initially the recovery and inhibition will be analysed. When satisfactory results and experience have been achieved in the laboratory, verification of the method will be done to determine LOD and LOQ for the method on two different matrices.

In short, two different matrices will be chosen and 10 samples for each of 10 different dilution levels will be analysed. Anticipated levels of viral genomes will be statistically analysed towards obtained results. A verification report will be written to be used for accreditation of the method.

An SOP will be written to be shared with the NRLs before PT distribution with this new matrix.

Expected Output:

Proficiency test distributions of surfaces to be analysed with the ISO 15216 swabbing method to detect and quantify norovirus and HAV, 2022.

Duration:

2021 2022

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:

A physical workshop was held in Uppsala in 2019 and a virtual workshop was held in 2020. The value of virtual workshops are less than the physical workshops and the goal is to hold a physical workshop in 2022. The 2021 workshop is scheduled for June and will be held as a digital meeting. The budget will include physical meetings for 2022. As requested from the NRLs, DG SANTE, EFSA and ECDC will be invited to both workshops.

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 theoretical background and hands-on training in analytical methods and techniques to less experienced NRLs.

Description:

During 2019 the EURL gave training courses on two occasions on RNA transcription with a total of 16 participants and 4 course leaders. In 2020, a course in the method for norovirus and HAV quantification in LBM was planned. Due to the pandemic it was cancelled and replaced by a webinar on verification of the ISO 15216 method as a basis for accreditation. The meeting contained discussions on specific laboratory and national demands to obtain accreditation. It served as a very good input to the guide for verification of ISO 15216 in single laboratories that is under development at the EURL (see 2.2). The cancelled technical course on norovirus and HAV quantification in LBM will be held late autumn 2021. The technical course for 2022 will be decided together with the NRLs during the workshop, 2021.

The inter EURL WG on NGS is organising joint training courses that will be held in 2022. The EURL will include four persons from the NRL network to participate in such a course.

Expected Output:

1	Better method performance among the NRLs.

Duration:

2021 and 2022

Sub-activity 2.3 Preparedness of staff and 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 are 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 2022. 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 strengthen the 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 provide a guidance on validation/verification for the accreditation of ISO 15216. The production of such a guidance document was planned to 2020 but has been delayed due to that the leader of the project was assigned to organise the Swedish national programme for testing of covid-19. The produced guide will be a document that continuously can be updated for new matrices and performance parameters. An R programming language based application is under development to be used by the NRLs for the calculation of parameters such as LOD and LOQ. The continuation of this work will be included in the budget for 2021 and 2022. The ISO update of the verification and validation ISO standard 16140 are under revision. A new part of ISO 16140 is under development to be used for non-cultivable microorganisms. This is especially relevant for the ISO 15216 method. The EURL should be part of the development of this new part of ISO 16140 updates. Workdays for this activity are included in the budget.

Another request was about bioinformatics 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 design. Negotiations with Public Health Sweden will be introduced during 2022 to facilitate the possibility for such surveillance through applications developed at their institute.

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:

2021 2022

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 have formed a WG for NRLs for Foodborne Viruses on NGS. The WG had its first virtual meeting in 2020. The goal for the WG is to increase the possibility for a faster development of NGS techniques used in the area of foodborne viruses and to promote collaboration and knowledge transfer between laboratories. The group will have one physical/virtual 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. Production of a workflow document for NGS for cap typing of norovirus will be part of the assignments of the WG.

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:

2021 2022

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.

The use of methods for detection and quantification of norovirus and HAV in foods like soft fruits varies around the world despite the fact that a validated ISO method is available. Interpretation of results (detected/not detected) seems also to vary. To address these questions the American Frozen Food Institute (AFFI) has initiated an international expert group with members from academia (University of Barcelona), national agencies (CDC), industry (Nestle) and others. The EURL for Foodborne Viruses has been engaged through Magnus Simonsson as a facilitator of the expert group. So far Magnus has been involved as a representative from the Swedish Food Agency. The expert group will produce a white paper. It seems sensible that the EURL should be represented in this expert group as the publication of the white paper will be an important and internationally published opinion. Workdays for facilitation of the expert group is included in the budget to state the support of the EURL to participate in this group.

Expected Output:

1. A well informed and trained staff that can give support to the Commission and EU agencies in a timely manner, 2021, 2022.
2. A white paper addressing the problem with diverse methods and interpretation of results applied globally, will be written.

Duration:

2021 2022

Sub-activity 3.3 Standardisation of methods for the detection and quantification of hepatitis E virus (HEV) in animal products.

Objectives:

To ensure the availability of standardized methods, for the detection and quantification of hepatitis E virus (HEV) in animal products, as a basis for harmonisation in the NRL network and official control in EU MS.

Description:

Hepatitis E virus (HEV), a faecal-orally transmitted virus, is the most common cause of acute hepatitis in the world. HEV types 1 and 2 only infect humans, and are endemic in tropical areas where they

cause large waterborne outbreaks. In Europe, there is a dominance of HEV type 3, which is zoonotic. Its transmission to humans is mainly through food products from pigs and wild boar.

A large part of HEV-3 infections is mild or asymptomatic, but common symptoms are jaundice, fever fatigue, nausea, vomiting and loss of appetite. Immunocompromised individuals can develop chronic infection with liver failure as a serious consequence. In recent years, a causal relationship between HEV-3 and a number of neurological conditions have been identified, in particular Guillain-Barré syndrome and neuralgic amyotrophy (severe pain and weakness). The HEV-3 related disease burden is therefore likely underestimated.

Cases of hepatitis E have drastically increased in most EU MS during the last 15 years. The seroprevalence varies between countries and regions but are generally around 10 %, but in areas with specific eating habits, over 50 % has been recorded.

In 2017 EFSA published a scientific opinion on HEV. The first conclusion states “The validation and standardisation of methods for detection and quantification of HEV from meat and meat products should be a high priority”. ISO/TC34/SC9 are creating a working group to standardise a method on HEV. The WG will be led by Nigel Cook (UK) and a work plan and timeline is suggested and is currently under ballot. The EURL have through national grants been able to develop and in house validated methods for HEV in animal products: The methods could be an important input to the standardisation process.

Harmonisation in the NRL network and thereby the official control in EU MS, would in the long run enable us to reduce the disease burden through management measures.

After the Workshop for NRLs in 2021, it was announced by the technical officer, Paolo Caricato that the Commission intend to support the EURL participation in the ISO standardisation process for HEV in animal products.

The EURL budget includes participation in ISO/TC34/SC9 HEV WG meetings and a limited amount of working days for the participation.

Expected Output:

1. A standardised method for the detection and quantification of hepatitis E virus in animal products.
2. In the long run a harmonisation process in the NRL network and thereby national official control laboratories.
3. Ultimately, reduced disease burden through management measures.

Duration:

2021 2022

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 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 and included in PT distributions.

Expected Output:

1. Collection of standards and controls to increase uniformity of method performance.
2. Overall improved quality assurance of the analyses performed at the NRLs for detection of foodborne viruses.

Duration:

2020 2022

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 purchased 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. Hepatitis A virus will be cultivated in cooperation with the Swedish National Veterinary Institute.

Expected Output:

1. 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:

2021 2022