

Assessment and Methodological Support Unit

Minutes of the 6th meeting of the Working Group on Request for scientific and technical assistance on the baseline survey of Norovirus in oysters

Held on 13-14 January 2016, Weymouth (United Kingdom)

(Agreed on 3 February 2016)

Participants

- **Working Group Members:**

Micheal O'Mahony

Luigi Lanni

David Lees

Liesbeth Bruckers

Soizick Le Guyader

- **Hearing Experts¹:**

Covadonga Salgado Blanco

Ainhoa Pare

Johne Reimar

Sinead Keaveney

Anna Charlotte Schultz

Elisabetta Suffredini

James Lowther

Anne Thebault

¹ As defined in Article 17 of the Decision of the Executive Director on the selection of external experts:
<http://www.efsa.europa.eu/en/keydocs/docs/expertselection.pdf>.

- **European Commission and/or Member States representatives:**
Not Applicable
- **EFSA:**
AMU: Jane Richardson, José Cortiñas Abrahantes

1. Welcome and apologies for absence

The Chair welcomed the participants.

2. Adoption of agenda

#	Items
1.	Welcome and apologies for absence
2.	Adoption of agenda
3.	Declarations of Interest
4.	Member States comments: <ul style="list-style-type: none"> • Duration/timing of the survey • Dispatch centre survey • Sample transport • Storage of test sample materials • Retesting • PCR screening before qualification • Laboratory cross checking • Molecular characterisation – central repository location and access
5.	AOB

The agenda was adopted without changes.

3. Declarations of Interest of Working Groups members

In accordance with EFSA’s Policy on Independence and Scientific Decision-Making Processes² and the Decision of the Executive Director on Declarations of Interest³, EFSA screened the Annual Declaration of

² <http://www.efsa.europa.eu/en/keydocs/docs/independencypolicy.pdf>

³ <http://www.efsa.europa.eu/en/keydocs/docs/independencerules2014.pdf>

Interest and the Specific Declaration of Interest filled in by the working group members invited for the present meeting. No Conflicts of Interest related to the issues discussed in this meeting have been identified during the screening process or at the Oral Declaration of Interest at the beginning of this meeting.

4. Scientific topic(s) for discussion

Various options for the timing of the survey were discussed considering the number of samples that could be practically taken and analysed in a country over a 1 month period and the fact that there is annual variation in the number norovirus cases and outbreaks in Europe. It was agreed that a survey with samples taken once every two months for a period of two years would be appropriate. This design may reduce the temporal precision but also results in a broader spatial representation.

The definitions for the target populations were discussed. The working group confirmed that relaying areas are not to be included in the survey. With regard to the batch it was agreed that the inspector cannot know if the batch will be re-immersed or re-packaged after leaving the dispatch centre and as a result any batch present in the dispatch centre at the time of visit can be included in the survey.

Options for within country stratification of dispatch centres were discussed, for example seasonality of production, production volumes or region. EFSA will explore (via simulations) whether a simple random sample is sufficient or whether stratification is needed to ensure that the samples selected are representative.

The issue of seasonal availability of oysters at dispatch centres was discussed considering the monthly batch data already collected by the member states. It was agreed to increase the inflation of the sample size to 20% in the dispatch centre survey to ensure the necessary precision can be achieved.

The process for selecting oysters at the dispatch centre was discussed. It was agreed that official control procedures should be followed. The comment from Ireland on the representativeness of 15 oysters from large batches was discussed. It is acknowledged that 15 oysters may not be representative for larger batches however for countries with a large number of dispatch centres there is also a limit on the number of oysters which can be processed by the laboratory.

The only modification to the CEFAS good practice guide requirements for sample transport is the extension of the time to 72 hours between sample collection and the initial processing step at the laboratory.

It was agreed that PCR screening before quantification could be performed as long as the method had a comparable sensitivity to the

quantification method. The requirement for laboratories to be able to report the LOQ for the NoV PCR quantification was also discussed. Since not all laboratories are accredited this could be an issue, this will be raised at the (Live Bivalve Mollusc Working Group) LBM WG. The data model should be updated to record both qualitative and quantitative results.

The generation and maintenance of sampID was discussed by the working group with a requirement for clarification in the protocol.

In order to combine E.coli monitoring data with the baseline survey data more detailed data than simple paired samples would be required. Therefore it is proposed that this activity could be the subject of a procurement one the survey report has been finalised and it will not be included in the protocol.

The working group supported the inclusion of hepatitis A testing in the survey; this will be proposed to the LBM WG for agreement on inclusion in the protocol.

It is proposed to store digestive glands, supernatant and RNA at a single location in each country for further research funding should become available.

The updated protocol will be circulated for final comments and approval for publication should be sought by the end of January.

5. Any Other Business

Not applicable.

Assessment and Methodological Support Unit

Minutes of the 5th meeting of the Working Group on Request for scientific and technical assistance on the baseline survey of Norovirus in oysters

WEB/TELE-conference, 17 November 2015

(Agreed on 13 January 2016)

Participants

- **Working Group Members:**

Micheal O'Mahony

Luigi Lanni

David Lees

Liesbeth Bruckers

Soizick Le Guyader

- **Hearing Experts¹:**

Covadonga Salgado Blanco

Ainhoa Pare

- **European Commission and/or Member States representatives:**

Not Applicable

- **EFSA:**

AMU: Jane Richardson, José Cortiñas Abrahantes

¹ As defined in Article 17 of the Decision of the Executive Director on the selection of external experts:
<http://www.efsa.europa.eu/en/keydocs/docs/expertselection.pdf>.

1. Welcome and apologies for absence

The Chair welcomed the participants.

2. Adoption of agenda

#	Items
1.	Welcome and apologies for absence
2.	Adoption of agenda
3.	Declarations of Interest
4.	Approval of minutes
5.	Review of protocol prior to MSs commenting period: Comments received Clarity and factually correct Omissions
6.	AOB

The agenda was adopted without changes.

3. Declarations of Interest of Working Groups members

In accordance with EFSA's Policy on Independence and Scientific Decision-Making Processes² and the Decision of the Executive Director on Declarations of Interest³, EFSA screened the Annual Declaration of Interest and the Specific Declaration of Interest filled in by the working group members invited for the present meeting. No Conflicts of Interest related to the issues discussed in this meeting have been identified during the screening process or at the Oral Declaration of Interest at the beginning of this meeting.

4. Approval of minutes

The minutes were approved

² <http://www.efsa.europa.eu/en/keydocs/docs/independencepolicy.pdf>

³ <http://www.efsa.europa.eu/en/keydocs/docs/independencerules2014.pdf>

5. Scientific topic(s) for discussion

5.1. Review of protocol prior to MS commenting period

Text describing the rationale for including “(>1000 total NoV PCR copies per gram” in the survey objectives was agreed. The definitions for the target populations in the survey were reviewed and revised. Improvements to the figures, illustrating the production chain and the hierarchies within the target population, to improve clarity and to reflect practice across the EU were agreed. The temporal and spatial allocation of the samples to be taken at dispatch centres was discussed. The statisticians emphasised the importance of sampling in such a way that the seasonal variability and the variability between dispatch centres can be accounted for separately in the data analysis. It was agreed to try and obtain information on the number batches produced monthly but that production volumes (in kgs) could serve as a proxy if this information was not available. For sampling in production areas the longitude and latitude of the representative monitoring point was added to the information to be reported. The detailed bench protocol developed by the EURL expert working group has been added to the protocol in Appendix B.

The draft protocol will now be circulated to the competent authorities for comments.

6. Any Other Business

Not applicable

7. Next meeting(s)

The next meeting will be held on 13-14 January 2016.

Assessment and Methodological Support Unit

Minutes of the 4th meeting of the Working Group on Request for scientific and technical assistance on the baseline survey of Norovirus in oysters

WEB/TELE-conference, 28 October 2015

(Agreed on 17 November 2015)

Participants

- **Working Group Members:**

Micheal O'Mahony

Luigi Lanni

- **Hearing Experts¹:**

Covadonga Salgado Blanco

Ainhoa Pare

- **European Commission and/or Member States representatives:**

Not Applicable

- **EFSA:**

AMU: Jane Richardson, José Cortiñas Abrahantes

BIOCONTAM: Frank Boelaert

1. Welcome and apologies for absence

The Chair welcomed the participants.

¹ As defined in Article 17 of the Decision of the Executive Director on the selection of external experts:
<http://www.efsa.europa.eu/en/keydocs/docs/expertselection.pdf>.

Apologies were received from David Lees, Soizick Le Guyader and Liesbeth Bruckers.

2. Adoption of agenda

#	Items
1.	Welcome and apologies for absence
2.	Adoption of the agenda
3.	Declarations of interest
4.	Approval of minutes
5.	Sampling and laboratory analysis
6.	AOB

The agenda was adopted without changes

3. Declarations of Interest of Working Groups members

In accordance with EFSA's Policy on Independence and Scientific Decision-Making Processes² and the Decision of the Executive Director on Declarations of Interest³, EFSA screened the Annual Declaration of Interest and the Specific Declaration of Interest filled in by the working group members invited for the present meeting. No Conflicts of Interest related to the issues discussed in this meeting have been identified during the screening process or at the Oral Declaration of Interest at the beginning of this meeting.

4. Approval of minutes

The minutes were approved with minor changes

5. Scientific topic for discussion

5.1. Sampling and laboratory analysis

In production areas it was agreed the method used for E. coli monitoring can be applied for the baseline survey. The procedure to be used when the indicator species is not an oyster was agreed.

² <http://www.efsa.europa.eu/en/keydocs/docs/independencypolicy.pdf>

³ <http://www.efsa.europa.eu/en/keydocs/docs/independencerules2014.pdf>

The timing of surveying in dispatch centres was in relation to days when batches of oyster would be available for sampling. It was agreed that in both locations a sample of 12-15 oysters would be sufficient.

The information to be collected when taking a sample of oysters was agreed to be both necessary for the data analysis and obtainable by the official sampler.

6. Any Other Business

Not applicable

7. Next meeting

The protocol will be circulated for comments from working group and a teleconference arranged in Week 47.

Assessment and Methodological Support Unit

Minutes of the 3rd meeting of the Working Group on Request for scientific and technical assistance on the baseline survey of Norovirus in oysters

Held on 13 October 2015, Milan (Italy)

(Agreed on 28 October 2015)

Participants

- **Working Group Members:**

David Lees

Micheal O'Mahony

Luigi Lanni

Liesbeth Bruckers

- **Hearing Experts¹:**

Covadonga Salgado Blanco

Ainhoa Pare

- **European Commission and/or Member States representatives:**

Not Applicable

- **EFSA:**

AMU: Jane Richardson, José Cortiñas Abrahantes

1. Welcome and apologies for absence

The Chair welcomed the participants.

Apologies were received from Soizick Le Guyader.

¹ As defined in Article 17 of the Decision of the Executive Director on the selection of external experts:
<http://www.efsa.europa.eu/en/keydocs/docs/expertselection.pdf>.

2. Adoption of agenda

#	Items
1.	Welcome and apologies for absence
2.	Adoption of the agenda
3.	Declarations of interest
4.	Survey design
5.	Sample collection
6.	Secondary objectives
7.	Data reporting
8.	AOB

The agenda was adopted without changes.

3. Declarations of Interest of Working Groups members

In accordance with EFSA's Policy on Independence and Scientific Decision-Making Processes² and the Decision of the Executive Director on Declarations of Interest³, EFSA screened the Annual Declaration of Interest and the Specific Declaration of Interest filled in by the working group members invited for the present meeting. No Conflicts of Interest related to the issues discussed in this meeting have been identified during the screening process or at the Oral Declaration of Interest at the beginning of this meeting.

4. Scientific topic(s) for discussion

1. Survey design⁴

The working group clarified that Identification Marks are applied to batches with the primary function of identifying the dispatch centre as the producer of the batches. Unique identifiers for the batches are managed by the internal traceability system of the dispatch centre.

The working group agreed that one representative monitoring point within a production area should be selected. This should be the

² <http://www.efsa.europa.eu/en/keydocs/docs/independencepolicy.pdf>

³ <http://www.efsa.europa.eu/en/keydocs/docs/independencerules2014.pdf>

⁴ <http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2015-00455>

monitoring point with highest levels of E. coli contamination based on the routine monitoring performed under Regulation (EC) No 854/2004.

The working group agreed that live samples of oyster are to be included in the survey.

The proposed sample sizes for sampling in production areas and at dispatch centre were discussed. Information on the number of batches produced in each dispatch centre is required. Since this data is not available, the working group proposed an estimate of 5 batches per week, which results in an annual production per dispatch centre of 260 batches. The working group acknowledged that no information is available to estimate the correlation between batches from the same dispatch centre.

Proposed sample sizes for production areas visited monthly for one year were presented using data from the CEFAS study in the United Kingdom published in 2011 to estimate the correlation between samples from the same production area. By taking monthly samples at a single production area the total number of production areas to be sampled could be decreased, whilst the total number of samples is increased. The working group supported a survey design with regular monthly samples as this design would also allow the temporal variation to be accounted for and at the same time controlling the precision of the final prevalence estimate.

The preservation of RNA extracts was discussed. It was agreed that all RNA extracts should be stored at -80°C until the survey is completed and that should funding become available, samples with a high copy number could be considered for molecular characterisation.

Three secondary objectives remain; analysis for hepatitis A virus and inclusion of E.coli results either from production areas or from both production areas and dispatch centres. The inclusion of these secondary objectives in the final protocol will be dependent on the feedback from the member states on the draft survey protocol

The working group considered that number of samples to be taken by France may exceed their financial capacity and there may be insufficient inspectors to take the samples. The working group discussed extending the survey over two years with alternate monthly sampling of production areas and dispatch centres as an acceptable solution.

5. Any Other Business

The presentation for the European Commission Live Bivalve Molluscs working group was agreed.

6. Next meeting(s)

A teleconference will be arranged on the topic of Sample collection in the next two weeks.

Assessment and Methodological Support Unit

Minutes of the 2nd meeting of the Working Group on Request for scientific and technical assistance on the baseline survey of Norovirus in oysters

Held on 23-24 September 2015, Parma (Italy)

(Agreed on 13 October 2015)

Participants

- **Working Group Members:**

David Lees

Micheal O'Mahony

Soizick Le Guyader (by teleconference on the 23 September 2015)

Luigi Lanni

- **Hearing Experts¹:**

Covadonga Salgado Blanco

- **European Commission and/or Member States representatives:**

Not Applicable

- **EFSA:**

AMU: Jane Richardson, José Cortiñas Abrahantes, Federica Barrucci

1. Welcome and apologies for absence

The Chair welcomed the participants.

Apologies were received from Ainhoa Pare and Liesbeth Bruckers.

¹ As defined in Article 17 of the Decision of the Executive Director on the selection of external experts:
<http://www.efsa.europa.eu/en/keydocs/docs/expertselection.pdf>.

2. Adoption of agenda

#	Items
1.	Welcome and apologies for absence
2.	Adoption of the agenda
3.	Declarations of interest
4.	Survey design
5.	Sample collection
6.	Laboratory analysis
7.	AOB

The agenda was adopted without changes.

3. Declarations of Interest of Working Groups members

In accordance with EFSA's Policy on Independence and Scientific Decision-Making Processes² and the Decision of the Executive Director on Declarations of Interest³, EFSA screened the Annual Declaration of Interest and the Specific Declaration of Interest filled in by the working group members invited for the present meeting. No Conflicts of Interest related to the issues discussed in this meeting have been identified during the screening process or at the Oral Declaration of Interest at the beginning of this meeting.

4. Scientific topic(s) for discussion

4.1. Survey design⁴

The proportional allocation of production areas and dispatch centres to be sampled per country were discussed. An updated table of norovirus prevalence data prepared for the BIOHAZ WG on heat treatment of bivalve molluscs was presented. It was proposed that the sample size could be further refined using this information.

A survey design to estimate a point prevalence for norovirus in the two settings and accounting for temporal variation in norovirus levels was discussed. Other secondary objectives potentially to be included

² <http://www.efsa.europa.eu/en/keydocs/docs/independencepolicy.pdf>

³ <http://www.efsa.europa.eu/en/keydocs/docs/independencerules2014.pdf>

⁴ <http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2015-00455>

in the survey of scientific relevance were discussed; estimation of monthly prevalence values, inclusion of routine *E. coli* monitoring results, laboratory testing for hepatitis A virus and molecular analysis of norovirus samples. It was agreed to draft the protocol focusing on the key elements to allow estimation of the EU prevalence of norovirus in the two settings and to describe the benefits and costs of including the secondary objectives and the modification required to the protocol to address these objectives.

In order to effectively select dispatch centres for sampling information on the number of batches produced by a dispatch centre per month would be needed. This could be collected by the member states when preparing their sampling plans. EFSA proposed to organise a workshop for the member states with training on sample selection and data reporting. Definitions for the sampling units were agreed.

4.2. Sampling and laboratory analysis

It was considered whether to sample all monitoring points in a production area and that a new fixed monitoring point would be needed in production areas where the indicator species is not oysters. At dispatch centres batches with an assigned Identification Mark should be sampled and the information linked to that batch recorded.

The European Reference Laboratory (EURL) will update the protocol for quantitative analysis of norovirus RNA in the areas of storage, sample transport and improvements to quantification. This protocol will be included in the Annex of the technical report.

The requirements for laboratories designated by the competent authority to attend a training session organised by the EURL and participate in a proficiency test were agreed.

4.3. Data reporting and analysis

The EFSA data collection framework was presented. It was agreed that two data models should be prepared, one for each setting. It was agreed that when selecting variables to be reported the focus should be on the variables required for analysis

5. Any Other Business

Not applicable.

6. Next meeting(s)

The next meeting is scheduled for 13 October 2015 if a meeting room can be found in Milan.

Assessment and Methodological Support Unit

Minutes of the 1st meeting of the Working Group on Request for scientific and technical assistance on the baseline survey of Norovirus in oysters

Held by WEB/TELE-conference, 08 September 2015

(Agreed on 24 September 2015)

Participants

- **Working Group Members:**

David Lees

Micheal O'Mahony

Soizick Le Guyader

Luigi Lanni

- **Hearing Experts¹:**

Covadonga Salgado Blanco

- **European Commission and/or Member States representatives:**

Not Applicable

- **EFSA:**

AMU: Jane Richardson, José Cortiñas Abrahantes

SCER: Ana Afonso

1. Welcome and apologies for absence

The Chair welcomed the participants.

Apologies were received from Ainhoa Pare and Liesbeth Bruckers.

¹ As defined in Article 17 of the Decision of the Executive Director on the selection of external experts:
<http://www.efsa.europa.eu/en/keydocs/docs/expertselection.pdf>.

2. Adoption of agenda

#	Items
1.	Welcome and apologies for absence
2.	Adoption of the agenda
3.	Declarations of interest
4.	Survey design
5.	Sample size considerations for norovirus survey
6.	AOB

The agenda was adopted without changes.

3. Declarations of Interest of Working Groups members

In accordance with EFSA's Policy on Independence and Scientific Decision-Making Processes² and the Decision of the Executive Director on Declarations of Interest³, EFSA screened the Annual Declaration of Interest and the Specific Declaration of Interest filled in by the working group members invited for the present meeting. No Conflicts of Interest related to the issues discussed in this meeting have been identified during the screening process or at the Oral Declaration of Interest at the beginning of this meeting.

4. Scientific topic(s) for discussion

4.1. Survey design⁴

The working group discussed the objectives of the survey for norovirus in production areas. The group consensus was that the survey should estimate the likelihood that a classified production area is contaminated with norovirus RNA. Different thresholds based on number of PCR copies of norovirus detected in samples will be used to categorise an area as contaminated in the analysis phase. Members of the working group proposed that data related to monitoring for E. coli in production areas and human health data should be considered at the analysis phase. An inclusion criterion for classified production area was proposed "production areas actively producing market size

² <http://www.efsa.europa.eu/en/keydocs/docs/independencepolicy.pdf>

³ <http://www.efsa.europa.eu/en/keydocs/docs/independencerules2014.pdf>

⁴ <http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2015-00455>

oysters” to ensure that it would be possible to obtain a suitable sample for laboratory analysis. Issues related to seasonal production areas and changes in production area status require further discussion. The status of a production area can change however at a given time point it can have only one status (A, B or C).

The working group discussed the objectives of the survey for norovirus in approved dispatch centres. The group consensus was that the survey should estimate the likelihood that a batch of final product handled by a dispatch centre is contaminated with norovirus RNA. Different thresholds based on number of PCR copies of norovirus detected in samples will be used to categorise a batch of final product as contaminated in the analysis phase. This would make batches of final product the potential sampling unit for this survey. Information on the approved dispatch centres in each country is already available but annual production volume data is not available and it is believed that this would take more than a month to obtain. This information could be sought at a later date and used by the member states to finalise their sampling plans. An alternative, based on freezing samples from dispatch centres and randomly selecting from these samples for laboratory analysis was discussed. However this would make planning difficult as the number of samples to be analysed would not be known prior to starting the survey. Issues including seasonal availability of batches, mixing of oysters from different production areas in a single consignment, off-shore dispatch centres, sampling frequency and the role of food business operators in taking samples require further discussion.

In both cases this will be a European baseline survey and the sampling units should be representative for Europe. Risk-based sampling should not be applied. The timing of the two surveys was discussed considering available laboratory capacity (parallel one year survey or separate surveys on consecutive years or parallel two year survey with samples taken on alternate months). There is a larger proportion of sampling units in one member state and laboratory capacity needs to be considered. The group agreed that the same protocol will need to be implemented in all countries at the same time.

4.2. Sample size considerations for norovirus survey

The statistical basis of sample size calculations was presented. Since the objectives described above require EU prevalence estimation of norovirus in production areas and batches in dispatch centres it was agreed that in both cases the appropriate methodology for sample size calculation specific for estimation purposes should be used. The parameters proposed in the mandate of a level of confidence of 95% and a level of precision (also described as margin of error) of 5% were

accepted by the working group. The expected prevalence for samples with >1000 total norovirus PCR copies and the implication on sample size were discussed. Data on prevalence of norovirus was available in a previous EFSA opinion on oysters but there was wide variation between the three countries for which data was available. The selection of an expected prevalence of 50% results in the largest sample size needed and would allow an estimation of lower or higher prevalence values, while the margin of error is kept the same for any prevalence level. It was agreed to use the expected prevalence of 50% as specified in the mandate, however other scenarios can also be investigated.

The group agreed that there is no need to consider differences between different oyster species.

5. Any Other Business

Not applicable

6. Next meeting(s)

The next meeting is scheduled for 23-24 September. The availability of experts for a working group meeting at the end of October will be explored.