



DECATHLON



1st Round Expert Webinars Reporting

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1. Introduction

This document is part of the initiative “Development of Cost efficient Advanced DNA-based methods for specific Traceability issues and High Level On-site applications” - Decathlon, a project funded by the European Union Seventh Framework Programme (FP7). In particular, it is part of the activities foreseen within work package (WP) 7 - Communication, dissemination exploitation and training, which are implemented throughout the entire lifetime of the project.

WP7 is the central WP for the external communication and the assessment of the socio-economic aspects of method development in WP3-WP5, as well as for all project-related training activities, also to underpin the validation studies in WP6.

This document presents the 1st round of expert webinars reporting of the Decathlon project. The expert webinars is a part of knowledge exchange with stakeholders in the project. The inputs of external project stakeholders to the proposed solutions and their broader inputs to the applicability of the proposed solutions are essential to the project. This report is based on the expert webinars (WP4 and WP5) and feedback reported by experts (WP3) during the first round of stakeholder consultation. This report is thus structured in the following central sections:

- Section 2: “WP3 - Improved detection of food pathogens expert feedback” – this section includes the feedback collected from stakeholders mainly via email during January 2015;
- Section 3: “WP4 GMO – related issues expert webinars” – this section describes the discussions held during a two hours webinar on the 16th of October, 2014;
- Section 4: “WP5 Customs control analysis methods expert webinars” – this chapter includes a summary of the stakeholders’ views, presented on a two hours webinar on the 19th of January, 2015.

The series of eight webinars with maximum of 4 participants for the three ‘core’ topics of the project (analyzed under WP3, WP4 and WP5) shall be held during the entire lifetime of the project. These webinars will have the objective of presenting the progress of the project and receiving stakeholder inputs. Each webinar will last a maximum of 2 hours.

WP3

Improved detection of food pathogens expert feedback



2. WP3 - Improved detection of food pathogens expert feedback

In January 2015 the first collection of stakeholders' thoughts related to the improved DNA-based detection of food pathogens within the Decathlon project was implemented.

Stakeholder involvement is a crucial aspect to the success of WP3 and allows the DECATHLON partners to receive the stakeholders' opinions on the WP3 work and the methods that should be developed within the project. The stakeholders' feedback allows the DECATHLON partners to understand if the WP3 work meets the expectations of the relevant key actors that will use the DECATHLON results.

Additionally, personal stakeholder engagement contributes to the dissemination of DECATHLON activities among the target group profiles.

The panel of consulted experts included internationally well-known researchers in the field of *Enterohemorrhagic Escherichia coli* (EHEC) genomics and epidemiology as well as food microbiology. Furthermore experts in the field of infection control and surveillance were involved.

The WP leader introduced the stakeholders into the subject and outlined the work programme of WP3 that is devoted to the improved detection of EHEC as a model organism of food borne pathogens. In addition, the stakeholders accessed the DECATHLON website and became familiar with the structure and the goals of the consortium in general. Five participants contributed to the discussion and the collection of stakeholders' opinions.

The opinions and comments of the participants are denoted anonymously. The minutes have been shared with them for their information and approval.

2.1. Summary of feedback – Food Pathogens

The project and the aim of the consultation were explained to stakeholders prior to collecting their views and perspectives on the topic.

1. What are currently the main problems in the detection of food borne pathogens?

- The main problem still is that all methods in food microbiology rely on an enrichment of the contaminating bacteria. Detection and typing of food pathogens directly from food has not yet been accomplished due to the following reasons:
 - the presence of inhibitors,
 - the low number and often heterogenous distribution of contaminating pathogens in the matrix (requires high sensitivity of the detection method),
 - the inability to determine, whether the contamination detected results indeed from living (thus infectious) pathogens or “only” from DNA.
- Another problem is that actually only a small percentage of contaminating bacteria can be cultivated from the food matrices. The success rate of cultivation heavily depends on the food matrix. Whereas bacteria can usually be cultivated without greater problems from meat samples, there is a high discrepancy concerning plant products and fresh produce. It is assumed that only 10-20 % of the bacteria colonizing/contaminating leafy vegetables can be cultivated.
- Furthermore, many contaminating pathogens can enter a so-called „viable, but not culturable“ (VBNC) state. The VBNC state and the inability to cultivate bacteria is not necessarily the same. It is believed that the VBNC state represents a specific physiological resting phase. In contrast, the inability to cultivate bacteria rather results from the lack of knowledge of the required bacterial growth conditions.

2. How can the detection of food pathogens be improved?

- On-site detection of food pathogens will be a great step forward to meet the consumers’ demands and to facilitate food safety throughout the production chain. Suitable LAMP assays and/or microfluidics-based detection tools will be trendsetting.
- The identification and discrimination of EHEC from other *E. coli* variants can also be accomplished by mass spectrometry.

- We can only detect what is already known. The latest EHEC outbreak in Germany and central Europe in 2011 demonstrated, that our current detection tools and routines only focus on the most prevalent EHEC variants. This hindered the fast and reliable typing and detection of the actual pathogen. Accordingly, the comprehensive knowledge of EHEC variants with a high potential to cause severe disease should be included into improved EHEC detection strategies to close gaps identified from the old methods.
- Improved methods should be robust, simple (suitable for on-site detection purposes), fast and less sensitive to inhibition.
- Improved protocols and detection tools should have been standardized and validated.
- Next-generation sequencing (NGS)-based approaches allow the fast and standardized detection and identification of biomarkers and even the comprehensive analysis of complex bacterial samples.
- Approaches, that allow multiplexing will improve and facilitate the detection of food pathogens.

3. What has the highest priority regarding the development of new DNA-based detection methods for food borne pathogens?

- The on-site detection of EHEC in fresh produce, vegetable and herb-based food, which is often sold as “ready-to-use” should have the highest priority. The number of outbreaks due to e.g. sprouts, spinach, tomatoes, or melons is increasing and there is probably also a great number of sporadic cases. The main problem here will be the processing of the diverse matrices. Standardized protocols are not available. In contrast, there has already been intensive work on the screening of food pathogens in water and meat samples and several guidelines and protocols are available for these matrices.
- Improved DNA extraction protocols from diverse food matrices have to be established that result in sufficient yield and quality for further screening or NGS-based approaches. To include the need for increased sensitivity of detection methods, these protocols should also allow the processing of larger volumes or quantities of food samples.
- To enable the comprehensive typing of clinically relevant EHEC variants, and to support NGS-based detection, new markers for the different EHEC lineages associated with severe human disease have to be identified.

- The exchange of relevant knowledge and data between different partners and sectors should be improved.

4. What are advantages or disadvantages of the DECATHLON approach?

- The multidisciplinary approach of the partners within WP3 and the overall DECATHLON consortium is very promising and interesting. However, this also requires an optimized and very careful communication (“a common language”) between the different partners to avoid misunderstandings and to make sure that the final goals can be achieved.
- Comparative analysis of a large number of *E. coli* genome sequences from different sources (incl. public databases) will allow a comprehensive survey of relevant biomarkers.
- Comparative genomics of EHEC and other pathogenic bacteria aiming at the identification of discriminative markers for improved subtyping is an internationally highly competitive and a rapidly evolving field. The scientific merits to be received from this might be low (due to other groups publishing corresponding results earlier). Their translation into applications is, however, very important and promising. The proposed bioinformatic analysis is from the infrastructural point of view quiet challenging.

2.2. Conclusions

Based on the stakeholders' feedback, the following conclusions were made.

The stakeholders had very similar opinions on most of the issues related to the detection of food borne pathogens. This is interesting, because they have different backgrounds and profiles within the food pathogen and EHEC field. There was, nevertheless, an overall consensus of the problems and possible solutions. This will facilitate the work of the DECATHLON partners involved in WP3.

The most important bottlenecks, which hinder the proper detection of food pathogens are (i) the difficulty to retrieve contaminating bacteria or their DNA from the different relevant matrices and (ii) the lack of approaches to comprehensively address all clinically relevant EHEC variants.

There is a strong need to develop robust, and fast methods that could be standardized and used for on-site detection of food pathogens.

The multidisciplinary approach within WP3 is beneficial for the project.

The stakeholders were interested to continue their involvement in the DECATHLON project, and indicated that they will be available for future discussions.

WP4

GMO – related issues expert webinars



3. WP4 - GMO-related issues expert webinars

On the 16th of October 2014 the first stakeholder webinar related to GMOs methods within the Decathlon project was implemented.

Stakeholder involvement is a very crucial aspect to the successful implementation of the Decathlon project. One of the aims of their involvement is to gather views and opinions on the methods currently being developed within the project, thus understanding better if the work of Decathlon is in alignment with the needs and wishes of the relevant key actors that will use the Decathlon results. Another objective of personal stakeholder engagement is also to promote the project and disseminate its work and activities among the target group profiles.

The panel of experts consisted of policy makers and researchers. Moreover, the project coordinator, the WP leader and the partner responsible for communication and dissemination within Decathlon also participated in the workshop. A total of 8 people were present during the webinar.

The opinions and comments of the participants are denoted anonymously. The minutes have been shared with the participants for their information and approval.

The webinar lasted approximately two hours, and was implemented through an online communication platform.

The programme of the webinar was as follows:

1. Introduction to the Decathlon project
2. Presentation of GMO methods within the framework of Decathlon
3. Discussion session among all the participants
4. Conclusions - final remarks

3.1. Minutes from expert webinar - GMOs

All the participants were welcomed and the programme and structure of the webinar was outlined. Following this all the participants introduced themselves and their organization.

1. Introduction to the Decathlon project

Esther introduced the project, its objectives, foreseen results, main activities and impact. Presentation has been provided to the participants together with the minutes.

2. GMO methods within the framework of Decathlon

Jeroen presented the overview of the GMOs within the Decathlon project and the possible methods for detection. Presentation has been provided to the participants together with the minutes.

3. Discussion session (based on selected questions)

Question 1: What are the bottlenecks in your current detection methods?

- Getting good quality DNA out of complex matrices, that will allow subsequent analysis for the presence (and quantity) of GMOs;
- Dedicated detection strategies, there is not a single one to cover all kinds of GMOs;
- Multiplexing of methods to reduce costs, if feasible;
- Costs per sample, in the light of an increasing number of GMOs to be tested;
- There are gaps in the current methods.

2. What should be the criteria for new methods?

- The overall objective should be that the new methods will fill gaps identified from the old methods;
- New methods should be economically feasible, robust, and reduce -or be less sensitive to- inhibition;
- The new methods should also, if feasible, capture the next generation of GMOs, such as stacked GM events, retransformation events and new plant breeding techniques;

- There is a lack of international protocols and standards for innovative approaches such as NGS, including the validation of these methods;
- Screening methods are indeed very important;
- One criterion could be to identify the different parameters for the desired outcomes of the methods;
- Regarding NGS, the stakeholders recognize that many different technologies and softwares are available, and it will require careful selection to determine the best strategies for the GMO issues at stake.

3. What do you see as the highest priority when discussing new methods for GMO detection, identification and quantification?

- Address the identified bottlenecks, cost efficiency is a very high priority, innovative DNA isolation approaches will need to be taken into account;
- New developments in terms of methods for detection and identification should preferably contribute to the harmonization of detection methods between laboratories;
- One suggested priority could be to develop a method for data sharing between the laboratories, so there would an increased dataflow more cost efficient;
- Multiplexing techniques are important in new detection methods as well.

4. What do you see as advantages / disadvantages with relation to the methods to be developed in Decathlon?

- The approach of Decathlon seems positive, yet it is a bit difficult to say more specific, before more results are being developed. The multi-sectorial approach is very interesting, and it can also function as an optimization of existing methods;
- The multidisciplinary approach of Decathlon is very interesting, and also the fact that the project tries to predict the needs of the future within these selected areas both at a European and international level;
- The project has a very practical approach, which is very good, and the methods will actually be used in practice, which is very useful;

- The project should be careful not to duplicate work that's already being done in the ENGL. Despite the importance of making current methods more cost-effective, Decathlon should maximize the opportunity to explore new methods such as NGS.

3.2. Conclusions

Based on the whole webinar and the discussions that evolved from the four different questions, a series of conclusions were identified.

The participants were much in agreement about many of the issues that involve GMO detection methods, this is interesting as the experts had different backgrounds and profiles within the GMO area however, there was an overall consensus of the problems and possible solutions. This is a very positive finding, as an agreement of what the problems are and what can be done to surpass them is somewhat easier to address if experts have the same views overall.

A bottleneck that really complicates the analysis of GMOs is the difficulty of getting good quality DNA out of complex matrices, and thus the whole detection process might suffer.

The need to develop methods that are more cost efficient was seen as of utmost importance by the stakeholders as well.

The multidisciplinary approach of Decathlon i.e. in areas others than GMOs was considered to be an asset for the project, and the experts agreed that it will be interesting to follow how the different methods developed could add value for a different area.

All stakeholders were interested to continue their involvement in the Decathlon project, and are available for more discussions in the future, for instance other webinars.

WP5

Customs control analysis methods expert webinars



4. WP5 - Customs control analysis methods expert webinars

On the 19th of January 2015 the first stakeholder webinar related to customs control analysis methods within the Decathlon project was implemented.

Stakeholder involvement is a very crucial aspect to the successful implementation of Decathlon project. One of the aims of their involvement is to gather views and opinions on the methods currently being developed within the project, thus understanding better the work of Decathlon is in alignment with the needs and wishes of the relevant key actors that will use the Decathlon results. Another objective of personal stakeholder engagement is to promote the project and disseminate its work and activities among the target group profiles.

The panel of experts consisted of researchers, custom laboratory specialists and a member of the Advisory Board of Decathlon (a full list of participants is included in Annex 1). Moreover, the project coordinator, the WP leader and the partner responsible for communication and dissemination within Decathlon also participated in the workshop. A total of 11 people were present during the webinar.

The opinions and comments of the participants are denoted anonymously. The minutes have been shared with the participants for their information and approval.

The webinar lasted approximately two hours, and was implemented through an online communication platform.

The programme of the webinar was as follows:

1. Introduction to the Decathlon project;
2. Presentation of customs control analysis methods within the framework of Decathlon;
3. Discussion session among all the participants;
4. Conclusions - final remarks.

4.1. Minutes from expert webinar – customs control analysis methods

All the participants were welcomed and the programme and structure of the webinar was outlined. Following this all the participants introduced themselves and their organization.

1. Introduction to the Decathlon project

Esther introduced the project, its objectives, foreseen results, main activities and impact. Presentation has been provided to the participants together with the minutes.

2. Customs control analysis methods within the framework of Decathlon

Tamara presented the overview of the customs control analysis methods within the Decathlon project, highlighting the work accomplished in the project to date (in particular under WP5 – Customs issues). Presentation has been provided to the participants together with the minutes.

Comments and Discussion

- In the USA there are currently some difficulties in collecting samples of protected species. There is a particular way of collecting samples for court room purposes. If there is no established custody chain, the actual testing in the court of Law could be a problem.
- The biggest problem in USA with non-human DNA is canned seafood, such as tuna, given that it is a mixture, rather than what is claimed on the label.
- It is very important to know the chain of custody of a specific sample, namely the place and time at which samples are collected, by whom, how many transfers and in which conditions were made, among other aspects. It would this be important to create a file for each samples collected, in order to show it is authentic, if necessary in Court of Law.
- Tobacco is not as big of a problem in the USA as in the European Union. There are a lot of tobacco varieties and it is beyond the scope of the project to make analysis to all tobacco varieties. What will be done in Decathlon is to see whether there are more general profiling methods, and to see if it is possible to compare DNA profiles from different batches.

- The reference materials will be based on the Genbank and BOL databases, although the consortium is aware that the database of endangered species is still limited. Furthermore, the partnership has as a subcontractor Naturalis Biodiversity Centre, which is an expert in identifying endangered species and has a large collection of well-defined reference species.
- Regarding can fish samples, Decathlon aims to also be able to identify not only individual samples, but also different fish species in canned samples.

3. Discussion session (based on selected questions)

Question 1: What do you experience as bottlenecks of current detection methods?

- Develop standardized methods that could be used in several different areas including tobacco, seafood, etc.;
- Develop a method which can identify more than one species in a sample;
- Define a methodology for sample preparation and collection, including the combination of samples to form a single batch, how many samples to take to mix. After processing sample by PCR, how to detect the profile is also a bottleneck of the current methods (it depends on the equipment, and is not suitable for screening work, it is only suitable for middle scale analysis not multiple sample analysis);
- Further developments on hydrogen and oxygen isotopes would be relevant in the IRNS method, for analysis of Tobacco species (currently Carbon and Nitrogen are analysed in France, which is not enough discriminating).

How is Decathlon already addressing some of these aspects:

- Documents on standardization – proposed minimum performance parameters (part of the Decathlon project on WP6) will be sent to all and input from all parties are welcomed as many inputs will be better and also will be working on multi methods;
- Aspect of sample preparation is an important part of the project. The project is analysing methods that are sensitive for identifying tobacco data but they also have to be sensitive to analyse reconstituted tobacco.

2. What would be the criteria for recommending/applying new detection methods for customs issues?

- Standardization of methods is very important. Methods could be registered/ shared with the World Customs Organization (WCO) so as to receive feedback from a recognisable worldwide organisation;
- It is important that consumables are not too expensive;
- The methods should be easily performed by regular custom laboratories and customs specialists;
- EFSA should analyse which methods should be operated routinely by all customs laboratories;
- The European Network of Customs Labs should also provide inputs regarding the methods that should be used.

How is Decathlon already addressing some of these aspects:

- Decathlon includes two persons from the European Network of Customs Labs. The coordinator of the CLEN. Mr Schepers is member of the advisory Board who could not participate in the webinar but will certainly provide his views in this regard.

3. Do you see added value of detection and on-site application methods currently being developed within Decathlon?

- Although the project is still developing the methods, participants are optimistic that the methods developed under Decathlon could be very useful and could also be applied in the USA;
- More customs laboratories could be included in the discussion.
- Laboratory analysis is to be performed as approval of on-site screening.

How is Decathlon already addressing some of these aspects:

- Decathlon will include more in depth discussions in the customs issue with other custom specialist during the stakeholder meeting in Lisbon and, if possible, during meetings of the European Network of Customs Laboratories.

4. Concerning the Customs issues of tobacco and species identification, which part should have priority?

- From endangered species point of view, fish products are wide spread around the world, so this could be a priority to the project. It might be relevant to have an on-site analysis and to have a further analysis on the laboratories afterwards;
- Tobacco is not priority in the USA but rather fish species identification;
- Species identification, especially of endangered species, is very important;
- In some regions the identification of tobacco is very relevant (e.g. Bulgaria), especially regarding the identification of legal and illegal tobacco (related to the potential presence of GMOs in tobacco or to the species that are present and that can have a different impact on human health).

How is Decathlon already addressing some of these aspects:

- The project will continue working on the identification of tobacco and also endangered and fish species and would aim to develop a flexible method (adjustable).

4.2. Conclusions

The whole webinar and the discussions on the four different questions identified were successfully held, evolving into a series of relevant conclusions for the consortium. The participants had different backgrounds and profiles within the custom analysis area and had experience with methodologies adopted in different regions of Europe and USA.

There was an overall consensus of the problems and possible solutions, which is a very positive finding, as an agreement of what the problems are and what can be done to surpass them is somewhat easier to address if experts have the same views overall.

Some of the bottlenecks that hamper the custom method analysis are the standardization of the methods and sample preparation, both of which are expected to be tackled within the project.

The methods to be developed and implemented should be more cost efficient (mainly regarding the price of consumables) and should be easy to perform in any customs laboratory.

The Decathlon project will continue working on both tobacco and species identification as both are important and need more inputs from all stakeholders to validate the methods that should be used.

All stakeholders were glad to contribute to the project, interested to continue their involvement in the Decathlon project, and available for more discussions in the future, for instance during other webinars.

Conclusions of the 1st round webinars



5. Conclusion of the 1st round webinars

The 1st round of expert feedback and webinars on the three main issues of the project were successfully held, evolving into a series of relevant conclusions for the consortium. The participants had different backgrounds and profiles within the three main fields and had experiences with related issues and methodologies adopted in different regions.

There was an overall consensus of the problems and possible solutions for each question on the webinars and feedback session which was a very positive finding, as an agreement of what the problems are and what can be done to surpass them is very relevant. Some of the bottlenecks, methods to be developed and implemented of three fields were identified during the 1st round of webinars/stakeholder consultation. On the basis of the stakeholders' input some adjustments were made to the programme, or rather that the focus was slightly shifted. One example of such a slight shift of focus is the fact that the stakeholders in WP5 indicated that for them not only the CITES species are of importance, but also species identification in a broader sense (with fish species identification being mentioned more often). Although species identification with relation to CITES species is clearly in the DoW, this will not change, but at the same time in the selection of the primer sequences for NGS multiplex analysis it can be kept in mind that the method should preferably work also for other, non CITES, species. Another example is the fact that in WP4 the aspect of cost effectiveness and the availability of methods for all types of laboratories, also the less advanced, may lead to a clearer focus also on ready-to-use protocols that may be applied in all labs, together with more advanced methods that may be necessary to detect especially unknown, unauthorised GMOs. It was very good to notice that all stakeholders were glad to contribute to the project, interested to continue their involvement in the Decathlon project, and available for more discussions in the future, for instance during other webinars.

Continuous improvement and further discussion would be held in the next series of webinars. The Decathlon project will continue on the next series of webinars with the specific dates to be confirmed and invite all relevant stakeholders to continuously join the discussion. The project also welcomes new organizations to be involved in the webinars in order to successfully implement the project findings.