1. Content of the 'Topic Description' document

1.1 Topic area

Diagnostics, field detection, surveillance.

1.2 Topic title

Improvement of diagnostics of quarantine pathogens by digital PCR.

1.3 Description of the problem the research should solve

Laboratory diagnostics is an integral part of plant health management, detecting and identifying material contaminated with pathogens or pests and thus allowing their exclusion from production. Molecular methods are an important component of many diagnostic schemes or even the only method allowing sensitive and high throughput detection of certain plant pathogens. For quarantine plant pathogens, a zero-tolerance concept is in place. In the absence of data on the biological relevance of low concentrations we aim to detect as low a target concentration as possible.

The main challenges encountered in both diagnostics and research are:

(i) The absence of certified (or other) reference materials with defined levels of targets. This requires that laboratories themselves prepare in-house controls for both validation and routine testing. While this is relatively straightforward for some pathogens which can be efficiently grown in artificial media, the preparation of characterized controls for obligate parasite is much more demanding, time consuming or not feasible at all.

(ii) The vast array of different matrices to be tested (leaves, tubers, roots, wood, soil etc.) containing different and often high levels of PCR inhibitors and target background.

The digital PCR is a variant of real-time PCR relying on compartmentalization of reaction into many individual ones. As a method enabling absolute quantification without the need for standards digital PCR is the method currently used as a higher order method in several metrological projects based on nucleic acid detection in the clinical field. It is a suitable method for characterization of reference samples/controls prepared in-house and a normalization method in inter-laboratory testing providing absolute quantification of the target copy numbers at selected stages of the process (reference value). In comparison with real-time PCR the digital PCR has been reported to provide an improved sensitivity in high background, higher resistance to inhibition and accurate, absolute quantification without the need for reference materials. The method is particularly suitable for all difficult samples and for samples where one wants to analyze larger volumes of sample (reactions can be combined without losing sensitivity) so can be used for any critical samples where the expected concentration is low or there is high inhibition.

1.4 Description of the expected results

The project aims to assess and demonstrate the suitability of **digital PCR** for

(i) characterization of in-house prepared control materials

(ii) detection and quantification of selected target organisms in difficult samples of plants, vectors or environmental samples

<u>Intermediate results</u>

- The collaboration will enable exchange of experience, data and increased awareness of the potential benefits of the digital PCR approach

- Selection of the most relevant tests for diagnostics and characterization of reference materials
- Transfer of selected existing real-time PCR tests into digital PCR format and identification of critical points of transfer
- Preparation of in-house reference materials and their characterization with digital PCR

End results

- Quality assurance procedures (SOPs or equivalent) for preparation, characterization and use of in-house, characterized controls for molecular tests
- Update of PM 7/98 (2) tables giving detailed guidance for the validation process by field as regards digital PCR
- Comparison of real-time PCR and digital PCR performance on selected pathogens and matrices
- Strengthened collaboration among participating laboratories interested in the implementation of digital PCR for further activities
- Dissemination of knowledge to stakeholders e.g. NPPOs, other laboratories, producers of diagnostic tests/kits etc.

The activities undertaken in this project will have links to, or build on the following projects:

- 2015-B-115: The biology and epidemiology of *Candidatus* Liberibacter solanacearum and potato phytoplasmas and their contribution to risk management in potato and other crops
- 2015-A-118: Identification and early detection of *Cryphonectria parasitica* and *Ceratocystis* spp. occurring on trees in Europe
- 2015-F-146: Harmonized protocol for monitoring and detection of *Xylella fastidiosa* in its host plants and its vectors
- 2015-F-132: VirusCollect: building an international network of reference collections for regulated and other important plant viruses and viroids

1.5 Beneficiaries of this research product

Direct beneficiaries: diagnosticians and researchers involved in official diagnostics, test kit producers etc.

Indirect beneficiaries: EPPO and National Plant Protection Organizations.

1.6 Research funders and research contribution/ distribution

The participants will work with the pathogens and reference/control materials most relevant to their needs and together, the consortium will assess digital PCR for a range of different harmful organisms from different fields, and with different matrices.

Funding organisation	Research activity and researchers involved
1. Ministry of Agriculture, Forestry and Food, Slovenia Ms. Erika Orešek Erika.oresek@gov.si	-Application of digital PCR to the preparation and characterization of in-house controls for molecular tests. -Assessment of digital PCR in difficult samples (e.g. *Xylella fastidiosa*, *Ca*. Liberibacter solanacearum, *Xanthomonas campestris*). Contact person: Tanja Dreo Tanja.dreo@nib.si

2. Austrian Agency for Health and Food Safety, Austria Ms. Sylvia Bluemel sylvia.bluemel@ages.at	-Application of digital PCR to the preparation and characterization of in-house controls for molecular tests. -Assessment of dPCR for *X. fastididosa*, *Ca*. Liberibacter solanacearum in different hosts, Flavescence dorée in grapevine and vectors, tobamoviruses in tomatoes, viroids in fruit trees. Contact person: Helga Reisenzein Helga.reisenzein@ages.at
3. Research and Breeding Institute of Pomolology Holovousy, Ltd, Czech Republic Ms Jana Suchá Jana.sucha@vsuo.cz	- Application of digital PCR for the monitoring and diagnosis of *Flavescence dorée*, *Xylella fastidiosa*, *Peach latent mosaic virus*. -Provision of DNA samples and plant material. Contact person: Jana Suchá Jana.sucha@vsuo.cz Contact person: Jana Kloutvorová Jana.kloutvorova@vsuo.cz
4. French Agency for Food, Environmental and Occupational Health & Safety, France Ms. Géraldine Anthoine geraldine.anthoine@anses.fr	-The nematology unit will focus on the comparison of dPCR with real time PCR used as detections method for *Meloidogyne chitwoodi*, *M. fallax* and *Bursaphelenchus xylophilus* on complex matrices. Contact person: Anne-Marie CHAPPE anne-marie.chappe@anses.fr -The bacteriology group will focus on the assessment and improvement of detection methods for *Xylella fastidiosa* (efficiency evaluation of dPCR: in comparison with real time PCR, on complex matrices and, for samples at the limit of detection). -The virology and phytoplasmology group will focus on the absolute quantification of reference samples used in proficiency test and the assessment of new detection methods (e.g.: Tomato yellow leaf curl virus (TYLC) on leaves, Pepino mosaic Virus (PepMV) on seeds, phytoplasma on leaves). Contact person: Amandine Cunty amandine.cunty@anses.fr
5. Department of Agriculture Food and the Marine, Ireland Mr. James Choiseul James.Choiseul@agriculture.gov.ie	-The Plant Health Laboratories (Ireland) will focus on procedures for the preparation of in-house controls for the fungal pathogens e.g. *Phytophthora ramorum* and *Hymenoscyphus pseudoalbidus.* -dPCR will also be investigated as a method for direct detection of pathogens in woody material and matrices containing high levels of inhibitors.

	Contact person: Maria Destefanis Maria.Destefanis@agriculture.gov.ie
6. The Council for Agricultural Research and Economics, Italy Mr. Luca Riccioni luca.riccioni@crea.gov.it	-Application of digital PCR to the preparation and characterization of in-house controls for molecular tests (in collaboration with the other partners). -Assessment of digital PCR as detection methods for bacteria, virus and fungus pathogens (to be decided). Contact person: Luca Riccioni Luca.riccioni@crea.gov.it
7. Science and Advice for Scottish Agriculture, United Kingdom Mr. David Kenyon David.Kenyon@sasa.gsi.gov.uk	-Assessment of digital PCR in difficult samples such as insect vectors (e.g. *Xylella fastidiosa*, *Ca*. Liberibacter solanacearum). -Transfer of existing tests to dPCR for: *Clavibacter michiganensis* subsp. *sepedonicus*, *Ralstonia solanacearum*, *Phytophthora lateralis*, *Synchytrium endobioticum*, *Globodera pallida*, *Globodera rostochiensis*, Tomato chlorosis virus, Tomato infectious chlorosis virus. Contact person: Vince Mulholland vince.mulholland@sasa.gsi.gov.uk
8. US Department of Agriculture, Animal and Plant Health Inspection Service, United States of America Ms. Laurene Levy laurene.levy@aphis.usda.gov	-CPHST lab will preliminarily investigate dPCR as a platform for sensitive detection of *Ca.* Liberibacter asiaticus; later the platform may be expanded to other plant pathogens. dPCR will also be explored as a means to quantify reference materials for distribution. Contact person: John Rascoe John.rascoe@aphis.usda.gov
1. Alma Mater University of Bologna, Italy Claudio Ratti claudio.ratti@unibo.it	-Contribution to be detailed Contact person: Claudio Ratti claudio.ratti@unibo.it
2. Universidade do Algarve, Portugal Raquel Campos-Herrera rcherrera@ualg.pt	-Use of digital PCR to identify and quantify micro-organisms in soil Contact person: Raquel Campos-Herrera rcherrera@ualg.pt

1.7 Research project partnership outside Euphresco

Euphresco funding ensures a certain level of transnational collaboration among Euphresco member countries. It is possible, if the funding consortium is interested, to contact funding organisations or research groups outside the geographical area covered by Euphresco members. The Euphresco coordinator could advertise the research topic in order to have an enlarged collaboration. If funders are interested in this possibility, please check the case below:

☒ The funding consortium of the topic mentioned in section 1.2 requires to advertise the topic outside the Euphresco network

1.8 Any other relevant information on content

The topic will be advertised to involve members outside of the Euphresco network. Interest from non-Euphresco members has already been identified (section 1.7).

2. Euphresco management aspects of the project

2.1 Indication of the topic budget

Funding organisation [a]	Mechanism [b]	Total Budget [c]
1. MKGP (SI)	NC	€ 30 000
2. AGES (AT)	NC	€ 13 750
3. VSUO (CZ)	NC	€ 30 000
4. ANSES (FR)	NC	€ 19 000
5. DAFM (IE)	NC	€ 10 000
6. CREA (IT)	NC	€ 3 000
7. SASA (GB)	NC	€ 39 600
8. USDA (USA)	NC	€ 4 806
9. UNIBO (IT)	NC	€ 5 000
10. UOA (PT)	NC	€ 500
total		€ 155 656

2.2 Expected duration of the project (only for non-competitive topics)

24 months.

2.3 Any other relevant information on topic organisation and management

The use of digital PCR is on the increase and is a part of several on-going or planned Euphresco projects. Duplication will be avoided through close collaboration with these projects.

[a] First member is project coordinator. A minimum of two partners are necessary for each proposal. Add lines as needed.
[b] Please indicate the preferred mechanism (e.g. real pot RP; virtual pot VP; non-competitive NC), or several mechanisms if there is flexibility.
[c] Optional, as this amount can still change in the next phase. In-kind contribution should also be indicated in this column.