ANNEX 1

Questionnaire on Toxoplasma gondii molecular (DNA) detection (with a focus on oocysts)

Dear colleague, In the frame of TOXOSOURCES (Toxoplasma gondii sources quantified; https://onehealthejp.eu/jrp-toxosources/), a Joint Research Project within the H2020 One Health European Joint Programme, we are collecting information on molecular (DNA-based) methods applied to Toxoplasma gondii oocysts detection in laboratories worldwide. This questionnaire will be integrated with literature review data to guide in the selection for a suitable method to be applied for the molecular detection of T. gondii oocysts in fresh produce (i.e. RTE leafy green vegetables) in the TOXOSOURCES project. We would really appreciate your responses to those questions of this questionnaire that are applicable to your laboratory, by February 20th 2020. Thank you in advance for your help! Kind regards, TOXOSOURCES WP3_Task1 team Marco Lalle, Istituto Superiore di Sanità (ISS), Italy Iva Slana, Veterinary Research Institute (VRI), Czech Republic Anne Mayer-Scholl, German Federal Institute for Risk Assessment (BfR), Germany Nadja Bier, German Federal Institute for Risk Assessment (BfR), Germany and TOXOSOURCES Project Leader Pikka Jokelainen, Statens Serum Institut, (SSI), Denmark TOXOSOURCES Project Co-Leader, Joke van der Giessen, Dutch National Institute for Public Health and the Environment (RIVM), the Netherlands The questionnaire has been developed based on the outline of the project IMPACT: Standardising molecular detection methods to IMprove risk assessment capacity for foodborne protozoan Parasites, using Cryptosporidium in ready-to-eat salad as a model (Partnering Grant Project Grant Agreement Number GP/EFSA/ENCO/2018/03 – GA03). This activity is part of the European Joint Programme One Health EJP. This programme has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.

1.Please complete with your details (Name, Institution, Country, Email address)

2.In what type of institution do you work? Please select all that apply

- ☐ Health agency
- ☐ University
- ☐ Research institute
- ☐

3.What is the main focus of your institution? Please select all that apply

- ☐ Human health
- ☐ Animal health
- ☐ Food, water, environment
- ☐

4.Do you currently use molecular (DNA) methods for Toxoplasma gondii oocyst detection?

- ○ Yes
- ○ No

5.Please specify any other methods you use (e.g. microscopy)

6.In which type of samples do you detect Toxoplasma gondii by molecular (DNA) methods? Please select all that apply.

- [ ] Faecal samples
- [ ] Water samples
- [ ] Soil samples
- [ ] Food samples (nother than meat)
- [ ] Meat samples
- [ ]

7.If you use molecular (DNA) detection methods for Toxoplasma gondii in food and/or meat samples, please specify food items (e.g. type of lettuce; type of meat/meat product):

8.If you selected ‘faecal samples’ above: For processing faecal samples for DNA extraction for detection of Toxoplasma gondii, which methods do you use? Please select all that apply

- [ ] Filtration
- [ ] Density gradient purification
- [ ] Centrifugation
- [ ] Stomacher
- [ ] Washing and pelleting
- [ ]

9.If you selected ‘water samples’ above: For processing water samples for DNA extraction for detection of Toxoplasma gondii, which methods do you use? Please select all that apply

- [ ] Filtration
- [ ] Density gradient purification
- [ ] Centrifugation
- [ ] Stomacher
- [ ] Washing and pelleting
- [ ]

10.If you selected ‘soil samples’ above: For processing soil samples for DNA extraction for detection of Toxoplasma gondii, which methods do you use? Please select all that apply

- [ ] Filtration
- [ ] Density gradient purification
- [ ] Centrifugation
- [ ] Stomacher
- [ ] Washing and pelleting
- [ ]

11.If you selected ‘food samples (other than meat)’ above: For processing food samples (other than meat) for DNA extraction for detection of Toxoplasma gondii, which methods do you use? Please select all that apply

- [ ] Filtration
- [ ] Density gradient purification
- [ ] Centrifugation
- [ ] Stomacher
- [ ] Washing and pelleting
- [ ]

12.If you selected ‘meat samples’ above: For processing meat samples for DNA extraction for detection of Toxoplasma gondii, which methods do you use? Please select all that apply

- [ ] Filtration
- [ ] Density gradient purification
- [ ] Centrifugation
- [ ] Stomacher
- [ ] Washing and pelleting
- [ ]

13.If you selected ‘other samples’ above: For processing other samples for DNA extraction for detection of Toxoplasma gondii, which methods do you use? Please select all that apply

- [ ] Filtration
- [ ] Density gradient purification
- [ ] Centrifugation
- [ ] Stomacher
- [ ] Washing and pelleting
- [ ]

14.If you selected “Washing and pelleting”, please report composition of buffer(s) for each sample type

15.Before DNA extraction, do you perform any pre-treatment of the sample (e.g. bead beating; freezing and thawing)?

- ( ) No
- ( ) Yes

16.If YES, please specify

17.How do you extract Toxoplasma gondii DNA for the molecular detection?

- [ ] Manually
- [ ] Automatically

18.For manual extraction, which DNA extraction method do you use for the different matrices (if commercial kit, please specify brand and type)?

19.For automatic extraction, which automated extraction system(s) and reagents do you use? Please specify.

20.What kind of molecular detection method(s) do you use? Please select all that apply.

- [ ] Conventional PCR
- [ ] Nested PCR
- [ ] Semi-nested PCR
- [ ] Real-time PCR with non-specific fluorescent dyes
- [ ] Real-time PCR with sequence-specific probes
- [ ] Digital PCR
- [ ] LAMP
- [ ]

21.If you perform qPCR or digital PCR, please report details of the instrument(s) (e.g. brand, model)

22.Which is the target gene(s) you use for Toxoplasma gondii molecular (DNA) detection? Please select all that apply.

- [ ] SSU (18S) rRNA gene
- [ ] B1 gene
- [ ] 529bp RE
- [ ]

23.If you apply different molecular (DNA) procedures for Toxoplasma gondii detection , please specify the target gene(s) used for each method (e.g. B1 gene for conventional PCR)

24.What is the amplicon size for each target gene?

25.Do you use the Toxoplasma gondii molecular detection method for quantification?

- Yes
- No

26.If YES, please could you provide the algorithm? (quantification is not simply Ct values)

27.Do you use different molecular detection methods for Toxoplasma gondii for different sample types?

- Yes
- No

28.If YES, please specify

29.Do you use an internal amplification control (IAC) in Real-time PCR for Toxoplasma gondii detection?

- Yes
- No

30.If YES, please specify

31.Do you add the control for PCR inhibitors in a separate PCR reaction?

- Yes
- No

32.If YES, please specify

33. Do you use a multiplex assay for Toxoplasma gondii molecular detection?

- [ ] No
- [ ] Yes including non-Toxoplasma gondii genes
- [ ] Yes only on multiple Toxoplasma gondii genes

34.If you use multiple Toxoplasma gondii target genes, please specify which ones

35.If NON-Toxoplasma gondii genes are included, please specify which ones

36.Do you use a molecular method for typing of Toxoplasma gondii?

- Yes
- No

37.What kind of molecular method for Toxoplasma gondii genotyping do you use? Please specify and report reference(s), if available

38.Have you tested the analytical sensitivity of the molecular (DNA) method(s) for Toxoplasma gondii detection?

- Yes
- No

39.What is the analytical sensitivity (in %) of the Toxoplasma gondii molecular detection method(s) you use (i.e. the ability of a method to detect the target, calculated as the number of positive results relative to expected positive results)?

40.How you define your limit of detection (LoD)?

- Based on DNA (the limit of DNA amount that can be distinguished from the absence of the DNA)
- Based on oocysts (the minimum number of oocysts of Toxoplasma gondii that can be distinguished from the absence of oocysts)
- Based on tachyzoites/bradyzoites (the minimum number of Toxoplasma gondii tachyzoites/bradyzoites that can be distinguished from the absence of tachyzoites/bradyzoites)
- I do not define LoD
-

41.What is the LoD of the Toxoplasma gondii molecular detection method(s) you use?

42.What is the analytical specificity (in %) of the Toxoplasma gondii molecular detection method(s) you use (i.e. the ability of an assay to exclusively identify a target substance or organism rather than similar but different substances)?

43.How have the molecular methods you use for detection of Toxoplasma gondii been developed?

- In-house
- Based on published method(s)
-

44.Please provide references to the detection method(s)

45.Is the molecular method you use validated?

- Yes
- No

46.If YES, how was the molecular method validated?

- According International guidelines (e.g . ISO standard for microorganism test validation).
- According to in house guidelines.
-

47.Please provide reference for the validation procedure or a brief description of the parameters chosen for validation

48.Are you satisfied with the Toxoplasma gondii molecular method you use in the laboratory?

- Yes
- No

49.If not satisfied, which challenges are you facing? Please select all that apply

- The method is time-consuming
- Invalid runs
- Poor specificity (cross-reactivity with unintended parasites or matrices)
-

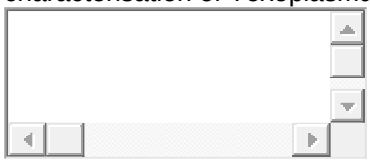
50.If you observe poor specificity, please specify cross-reactions

51.With regards to Toxoplasma gondii oocysts molecular detection ONLY, what do you think could be done to improve the method(s)? Please specify

52.With regards to Toxoplasma gondii oocysts molecular detection ONLY, please think about the attributes of the molecular detection methods for Toxoplasma gondii in food (other than meat). Please score each attribute (7 = most important, 1 = least important).

	7	6	5	4	3	2	1
High level of characterisation (proportion of samples characterised)	○	○	○	○	○	○	○
High level of staff expertise needed	○	○	○	○	○	○	○
Specialist equipment and infrastructure needed	○	○	○	○	○	○	○
Low costs	○	○	○	○	○	○	○
High sensitivity	○	○	○	○	○	○	○
Portability (for standardisation and comparability between labs)	○	○	○	○	○	○	○
Biological robustness (including repeatability and reproducibility)	○	○	○	○	○	○	○

53.With regards to Toxoplasma gondii oocysts molecular detection ONLY, what do you think is currently missing for the development of standard methods for the molecular detection, quantification, and characterisation of Toxoplasma gondii in food (other than meat)? Please comment



54.Thank you for your contribution. If you wish to add any further comment, please do it here.