

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

24	89.28
Answers	Mean time to complete (min.)

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

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

Health agency	4
University	11
Research institute	11
Altro	6



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

Human health	13
Animal health	12
Food, water, environment	14
Altro	4



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

Yes	17
No	7



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

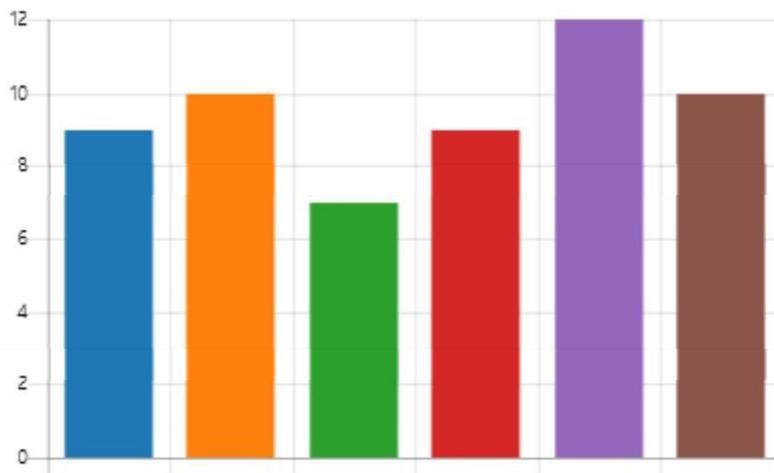
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6. In which type of samples do you detect *Toxoplasma gondii* by molecular (DNA) methods? Please select all that apply.

● Faecal samples	9
● Water samples	10
● Soil samples	7
● Food samples (nother than m...	9
● Meat samples	12
● Altro	10



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):

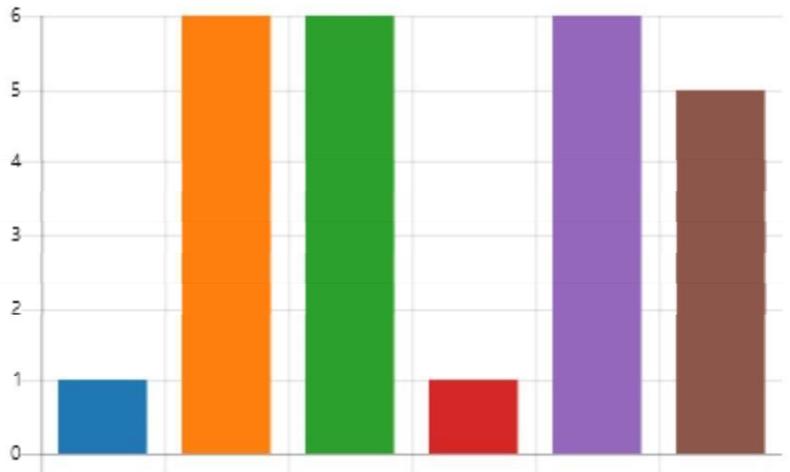
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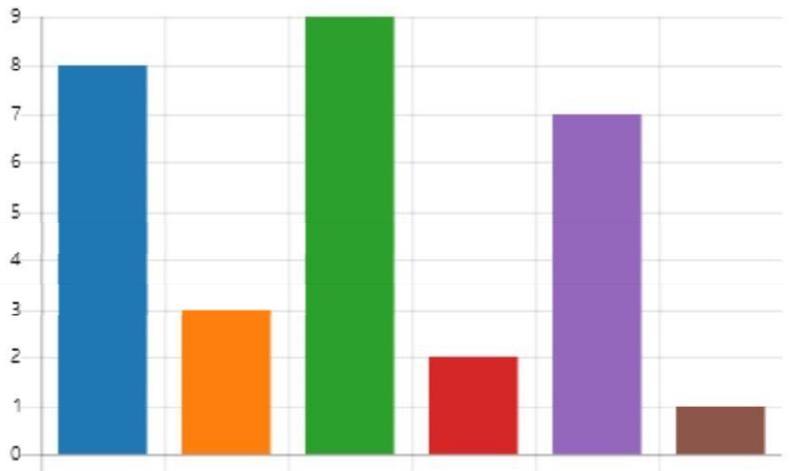
8. If you selected 'faecal samples' above: For processing faecal samples for DNA extraction for detection of *Toxoplasma gondii*, which methods do you use? Please select all that apply

● Filtration	1
● Density gradient purification	6
● Centrifugation	6
● Stomacher	1
● Washing and pelleting	6
● Altro	5

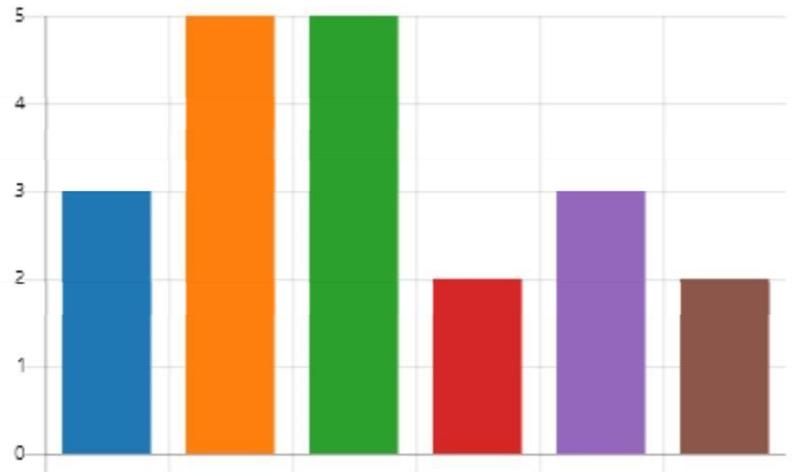
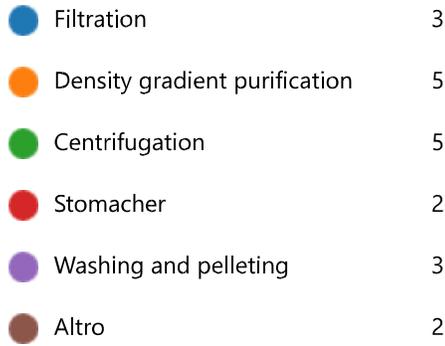


9. If you selected 'water samples' above: For processing water samples for DNA extraction for detection of *Toxoplasma gondii*, which methods do you use? Please select all that apply

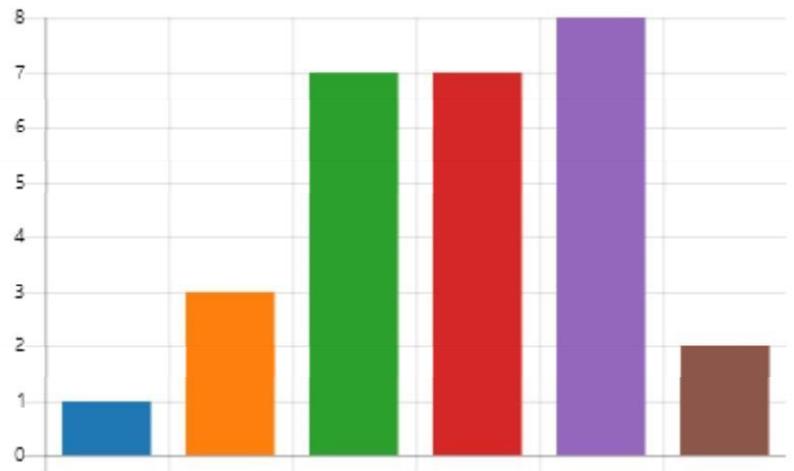
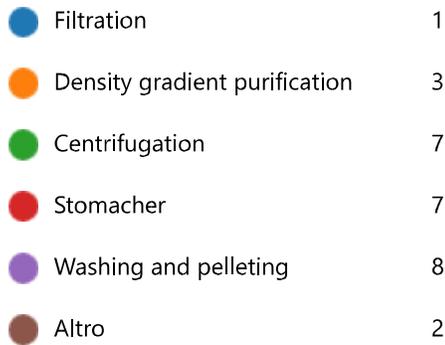
● Filtration	8
● Density gradient purification	3
● Centrifugation	9
● Stomacher	2
● Washing and pelleting	7
● Altro	1



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

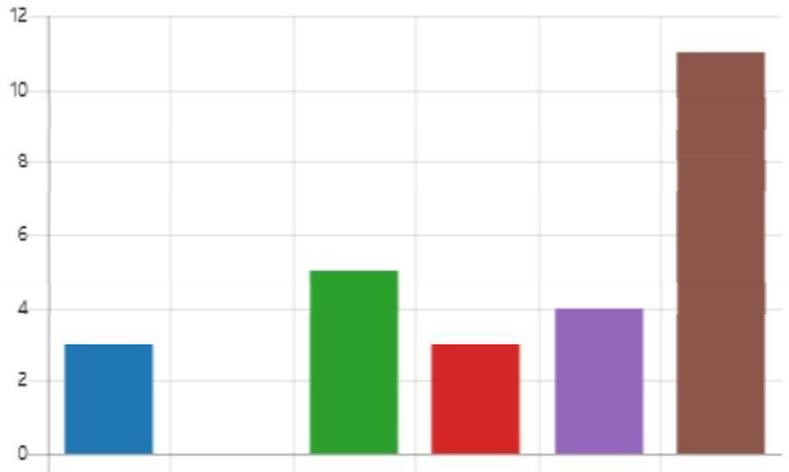


11. If you selected 'food samples (other than meat)' above: For processing food samples (other than meat) for DNA extraction for detection of *Toxoplasma gondii*, which methods do you use? Please select all that apply



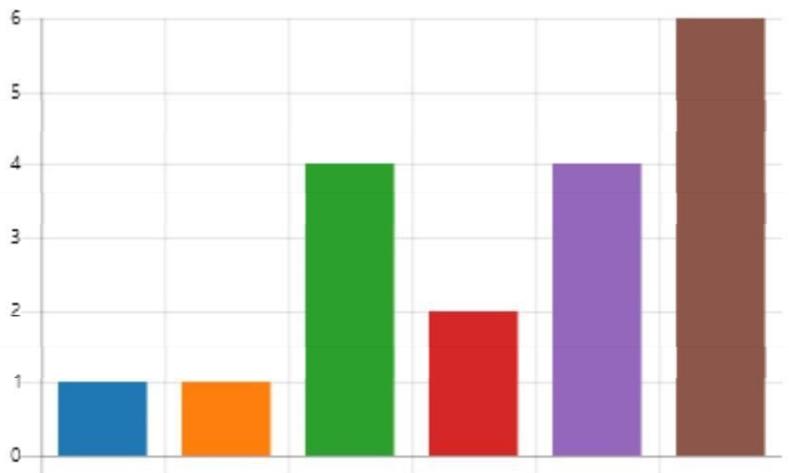
12. If you selected 'meat samples' above: For processing meat samples for DNA extraction for detection of *Toxoplasma gondii*, which methods do you use? Please select all that apply

● Filtration	3
● Density gradient purification	0
● Centrifugation	5
● Stomacher	3
● Washing and pelleting	4
● Altro	11



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	1
● Density gradient purification	1
● Centrifugation	4
● Stomacher	2
● Washing and pelleting	4
● Altro	6



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

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

● No	4
● Yes	18



16. If YES, please specify

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

● Manually	21
● Automatically	5



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

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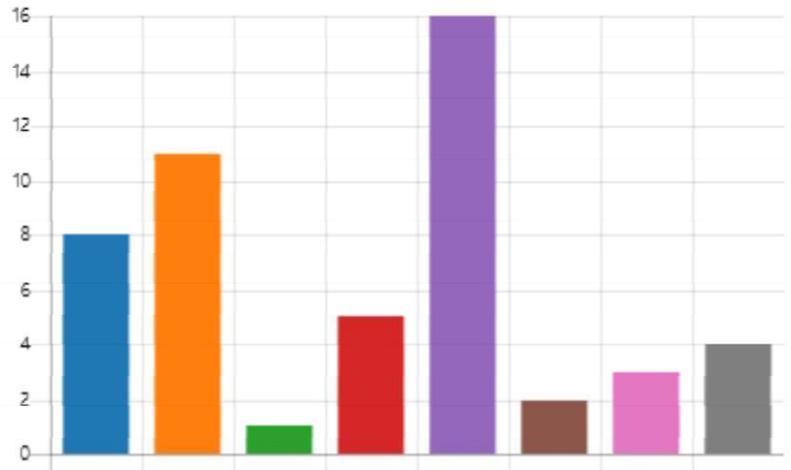
19. For automatic extraction, which automated extraction system(s) and reagents do you use? Please specify.

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

Conventional PCR	8
Nested PCR	11
Semi-nested PCR	1
Real-time PCR with non-specif...	5
Real-time PCR with sequence-...	16
Digital PCR	2
LAMP	3
Altro	4



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

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

SSU (18S) rRNA gene	5
B1 gene	9
529bp RE	17
Altro	4



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)

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

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

● Yes	7
● No	16



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

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

● Yes	6
● No	17



28. If YES, please specify

6
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29. Do you use an internal amplification control (IAC) in Real-time PCR for Toxoplasma gondii detection?

- Yes 9
- No 12



30. If YES, please specify

7

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

- Yes 4
- No 18



32. If YES, please specify

2

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

● No	16
● Yes including non- <i>Toxoplasma</i> ...	5
● Yes only on multiple <i>Toxoplasma</i> ...	2



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

3
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35. If NON-*Toxoplasma gondii* genes are included, please specify which ones

6
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36. Do you use a molecular method for typing of *Toxoplasma gondii*?

● Yes	15
● No	8



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

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

● Yes	12
● No	10



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)?

15

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

● Based on DNA (the limit of D...	6
● Based on oocysts (the minimu...	13
● Based on tachyzoites/bradyzoi...	6
● I do not define LoD	3
● Altro	1



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

16

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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)?

15

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

● In-house	10
● Based on published method(s)	18
● Altro	2



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

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45. Is the molecular method you use validated?

● Yes	15
● No	6



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

● According International guidel...	4
● According to in house guideli...	10
● Altro	4



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

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

● Yes	18
● No	5



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

● The method is time-consuming	2
● Invalid runs	0
● Poor specificity (cross-reactivit...	3
● Altro	3



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

3

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

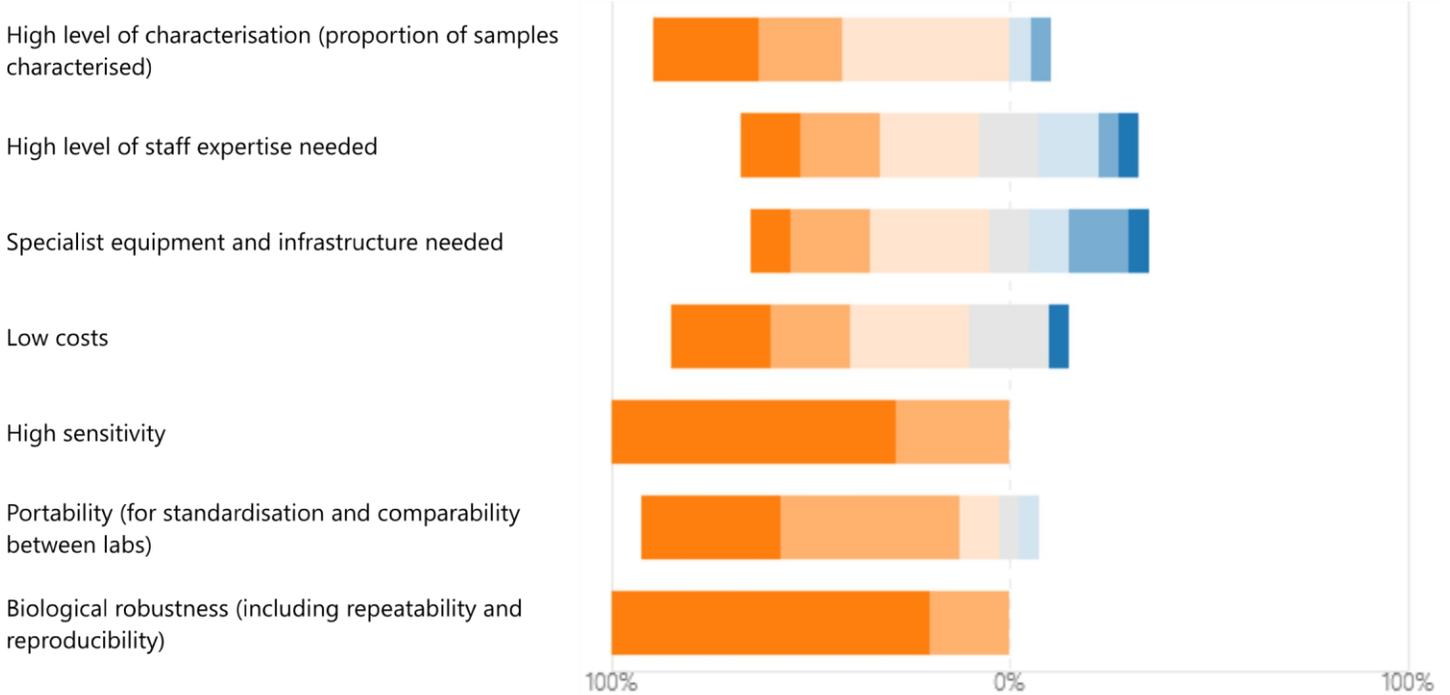
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52. With regards to *Toxoplasma gondii* oocysts molecular detection ONLY, please think about the attributes of the molecular detection methods for *Toxoplasma gondii* in food (other than meat). Please score each attribute (7 = most important, 1 = least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

7 6 5 4 3 2 1



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

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

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