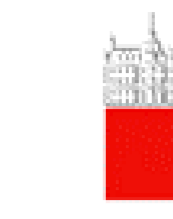


Overview of IMPACT: Standardising molecular detection methods to improve risk assessment capacity using *Cryptosporidium* spp. in ready-to-eat salads

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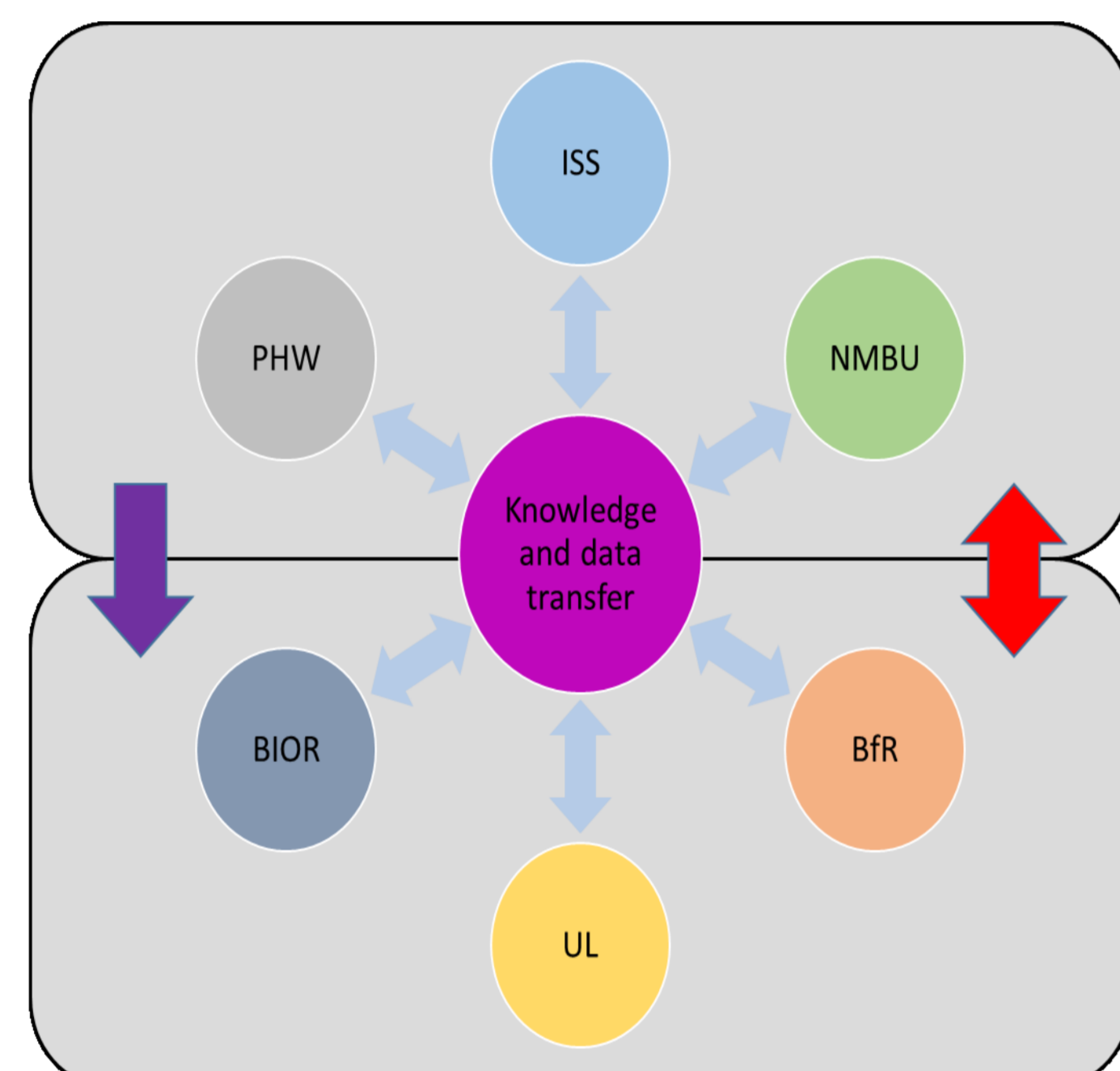


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BACKGROUND

- Several recent cryptosporidiosis outbreaks associated with fresh produce reported in Europe
- Existing ISO 18744:2016 method is expensive, laborious, and not amenable to high throughput testing
- A fast, reliable and standardisable molecular assay for detection of *Cryptosporidium* in food matrices is lacking



↓ Knowledge transfer: molecular detection and quantification of *Cryptosporidium* (evaluation, validation and standardisation of methods and training).
 ↑ Knowledge exchange: spiking standardisation, optimisation of molecular detection and quantification assay, organisation/analysis of ringtrial

Figure 1: Structure of the consortium with the aim to maximise knowledge transfer and sharing

OBJECTIVES

- Exchange of know-how amongst research labs (Figure 1)
- Capacity building for food safety in Europe
- Improvement of risk assessment capacity
- Guidance for artificial contamination studies
- Validation of a real-time PCR assay for *Cryptosporidium*
- Cryptosporidium* as a model organism for other protozoa

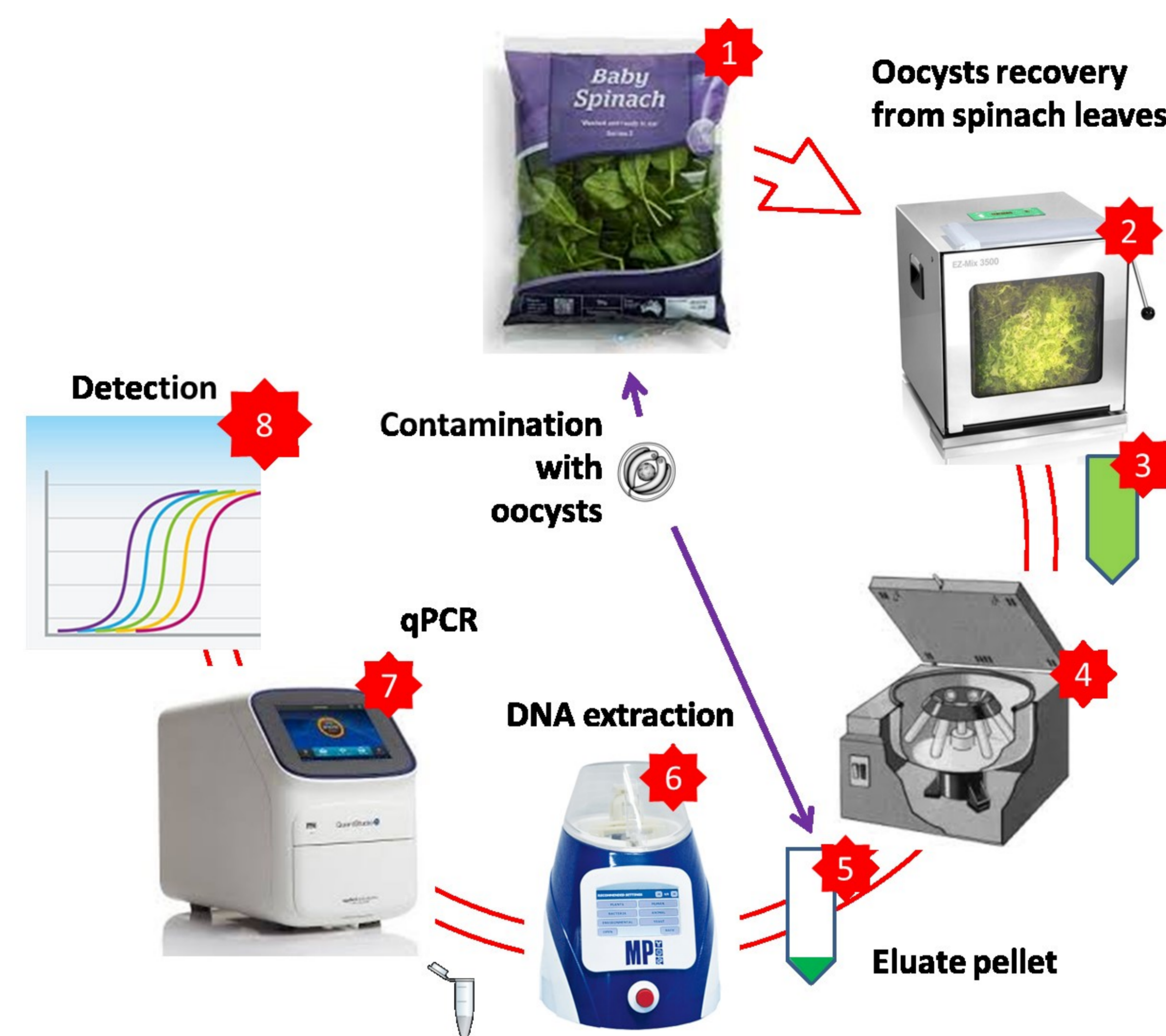


Figure 2: Molecular detection of *Cryptosporidium* spp. baby spinach leaves. 18S qPCR by Elwin et al. (unpublished) targeting all *Cryptosporidium* species

MATERIALS AND METHODS

- Review of current procedures for spiking and detection of *Cryptosporidium* oocysts in fresh produce
- Market survey of oocyst suppliers and collection of expert opinions
- Implementation of an SOP using 18S qPCR assay for the detection of *Cryptosporidium* in salad leaves (Figure 2)
- Dissemination of optimised SOP and video-tutorials to partner laboratories
- Validation of SOP by a ring trial in 12 food-testing labs ongoing

OUTPUTS

- Establishment of spiking guidance for *Cryptosporidium* in salad
- Manuscript submitted: Chalmers et al. A guide to standardised artificial contamination procedures for fresh produce and fruits with protozoan parasite oocysts or cysts using *Cryptosporidium* as a model
- Manuscript for a literature review on PCR-based molecular detection methods for *Cryptosporidium* on leafy greens in preparation
- Method validated, SOP delivered and implemented among consortium partners

CONCLUSIONS

- SOP for *Cryptosporidium* spp. can be extrapolated to other protozoans in food matrices
- Finalized SOP to be disseminated among NRLs, EFSA focal points, COST Action Euro-FBP network, and the OHEJP network
- IMPACT enables exchange of knowledge between EU participants contributing to the strengthening of food testing networks.

References: McKerr C et al. 2015. An outbreak of *Cryptosporidium parvum* across England & Scotland associated with consumption of fresh pre-cut salad leaves, May 2012. PLoS One 10(5):e0125955.
 Åberg R et al. 2015. *Cryptosporidium parvum* caused a large outbreak linked to frisée salad in Finland, 2012. Zoonoses Public Health. 62(8):618-24.
 Utaaker KS, Huang Q, Robertson, LJ. 2015. A reduced-cost approach for analyzing fresh produce for contamination with *Cryptosporidium* oocysts and/or *Giardia* cysts. Food Res. Int. 77: 326-332.