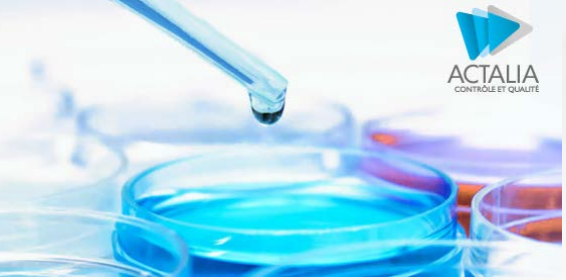
Detection of protozoan parasites in food: progress, challenges, and perspectives

20th Workshop of the National Reference Laboratories for Parasites
28-29 October 2025

European Union Reference Laboratory for Parasites
Unit of Foodborne and Neglected Parasitic Diseases
Department of Infectious Diseases
ISTITUTO SUPERIORE DI SANITÀ

Stéphanie La Carbona, PhD
ACTALIA



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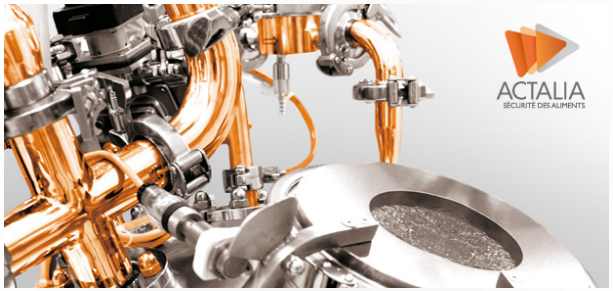
Expertise Center for Food Industries



ACTALIA

- **210 collaborators**
- **12 locations in France**
- **Turnover: 20 M€**
- **Head office: Saint-Lô**

- Non profit organization with private and research activities
- Acknowledged by the french Ministry in charge of Food

MINISTÈRE DE L'AGRICULTURE, DE L'AGRO-ALIMENTAIRE ET DE LA SOUVERAINETÉ ALIMENTAIRE

ITAI INSTITUT TECHNIQUE AGRO-INDUSTRIEL

A service offer dedicated to food companies

ACTALIA CONTRÔLE ET QUALITÉ

Routine analyses

ACTALIA SENSORIEL

Sensory studies

ACTALIA INNOVATION

From a new product idea to the production

ACTALIA SÉCURITÉ DES ALIMENTS

Control of the microbiological quality of food and water through technology

ACTALIA EXPERTISE ANALYTIQUE CECALAIT

ACTALIA PRODUITS LAITIERS

Specialization in the dairy sector

ACTALIA Environnement

Environmental performances

ACTALIA Food Safety Department

Positioning and national specificities

Technological platform of biosafety level 3

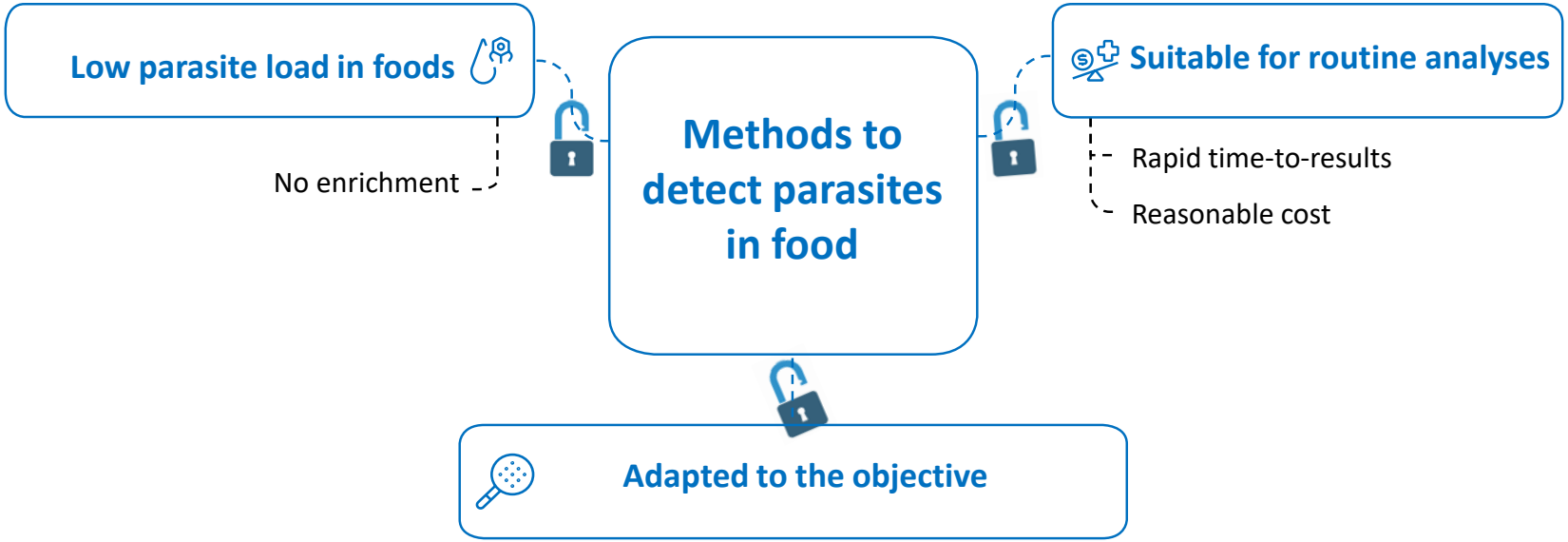
VIROLOGY
(enteric viruses, emerging viruses)
[since 2010]

HYGIENIC DESIGN
(sole french EHEDG certification organism)
[since 2013]

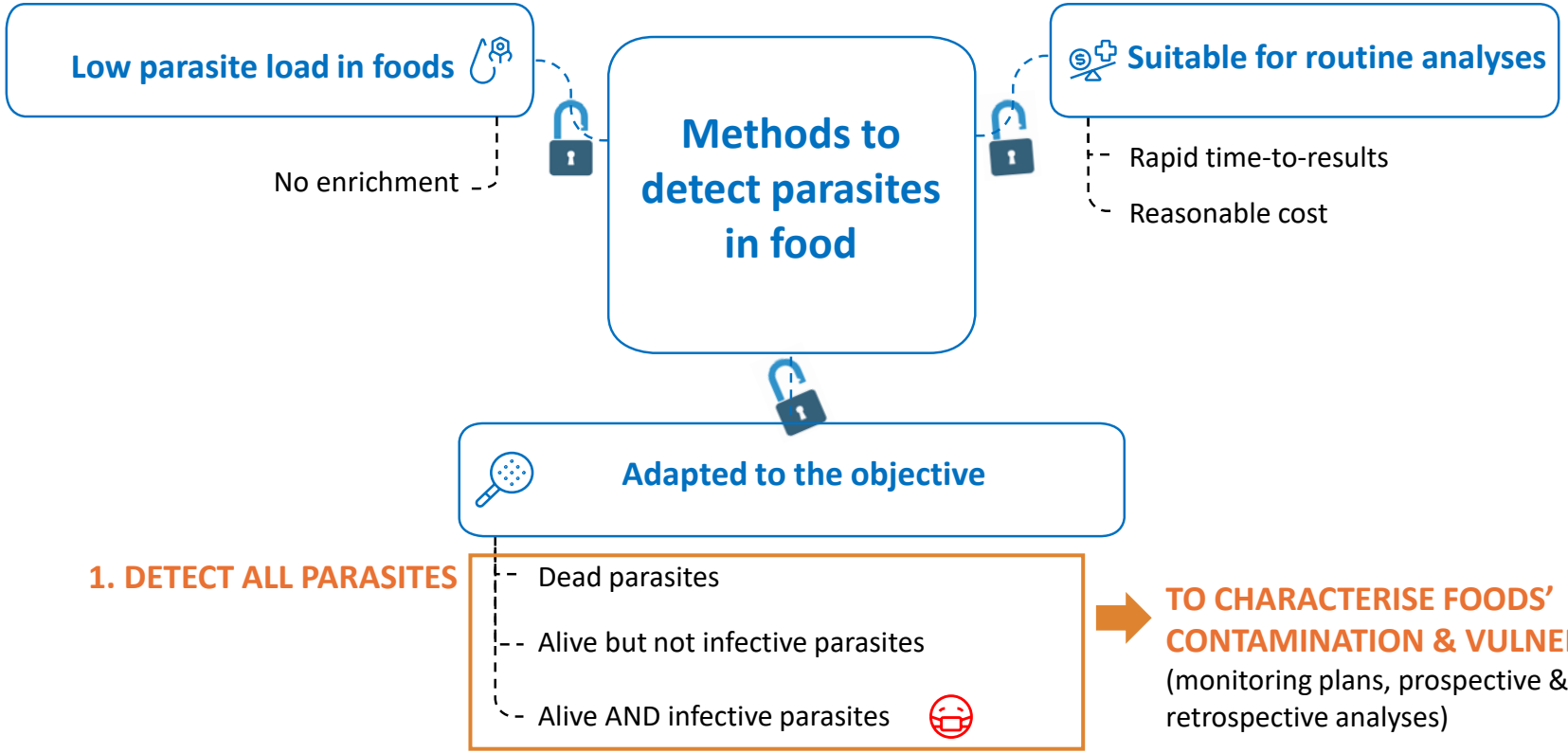
PARASITOLOGY
(protozoa, Anisakidae)
[since 2014]

EFFICACY OF BIOCIDES &
FOOD PROCESS
[platform since 2015]

Detecting protozoan parasites in food: which locks?



Detecting protozoan parasites in food: which locks?



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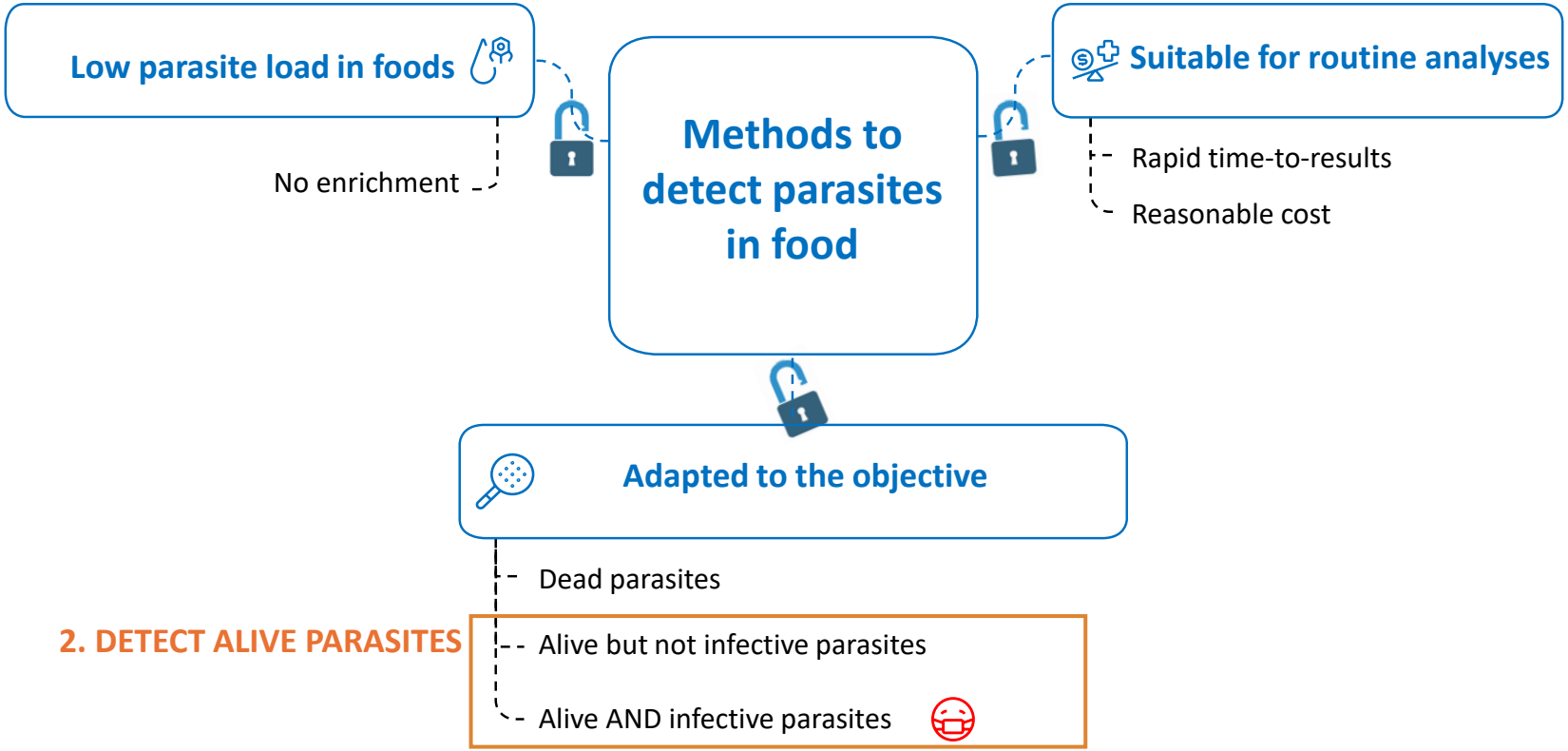

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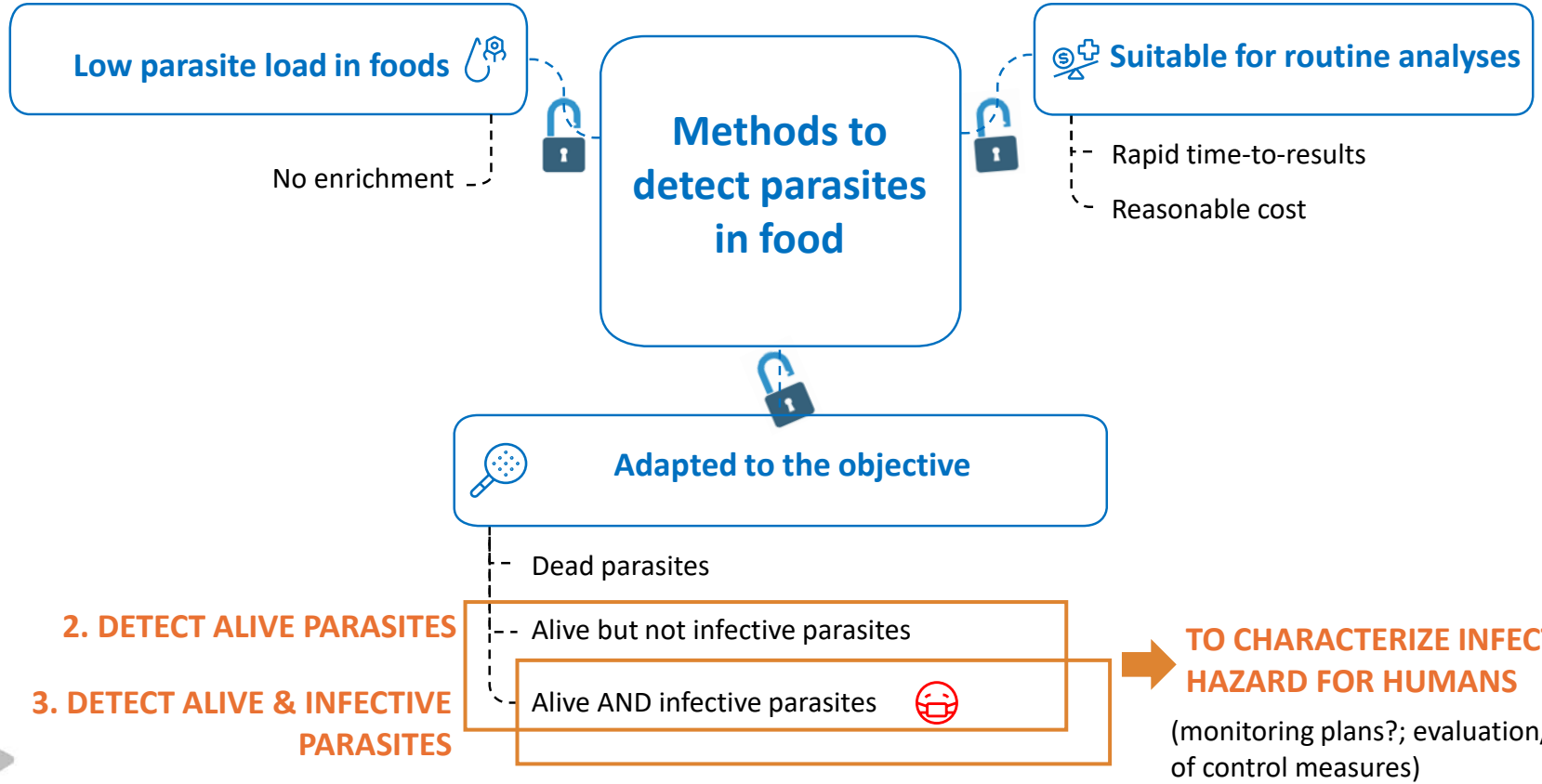

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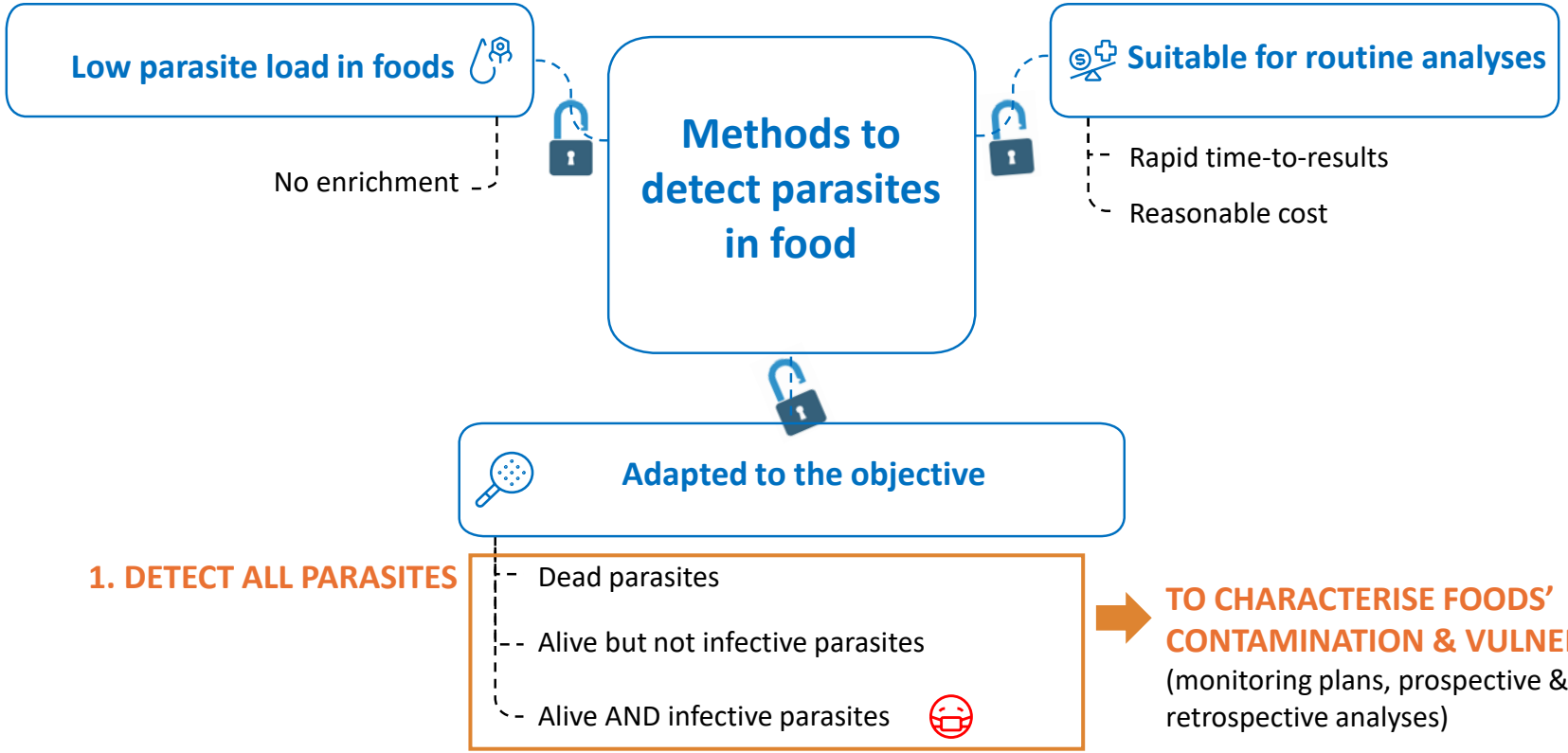
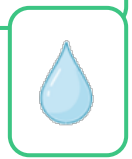


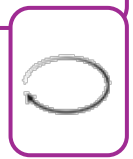
Methods to detect all parasites

ISO 18744 (2016): Microbiology of the food chain — Detection and enumeration of *Cryptosporidium* and *Giardia* in fresh leafy green vegetables and berry fruits

Amendment ...

ELUTION → CONCENTRATION → ISOLATION → (OO)CYSTS DETECTION





Methods to detect all parasites

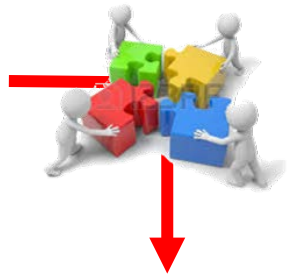
ISO 18744 (2016): Microbiology of the food chain — Detection and enumeration of *Cryptosporidium* and *Giardia* in fresh leafy green vegetables and berry fruits

Amendment ...

BAM Chapter 19: (2004) BAM Chapter 19a: Detection of Cyclospora and Cryptosporidium from Fresh Produce: Isolation and Identification by Polymerase Chain Reaction (PCR) and Microscopic analysis → Microscopy, nPCR

(2024) BAM Chapter 19b: Molecular Detection of Cyclospora cayetanensis in Fresh Produce Using Real-Time PCR → qPCR

No standard for *T. gondii*: → In-house molecular methods (nPCR, PCR, qPCR)



MOLECULAR METHODS (qPCR)

BUT no consensus at the moment!

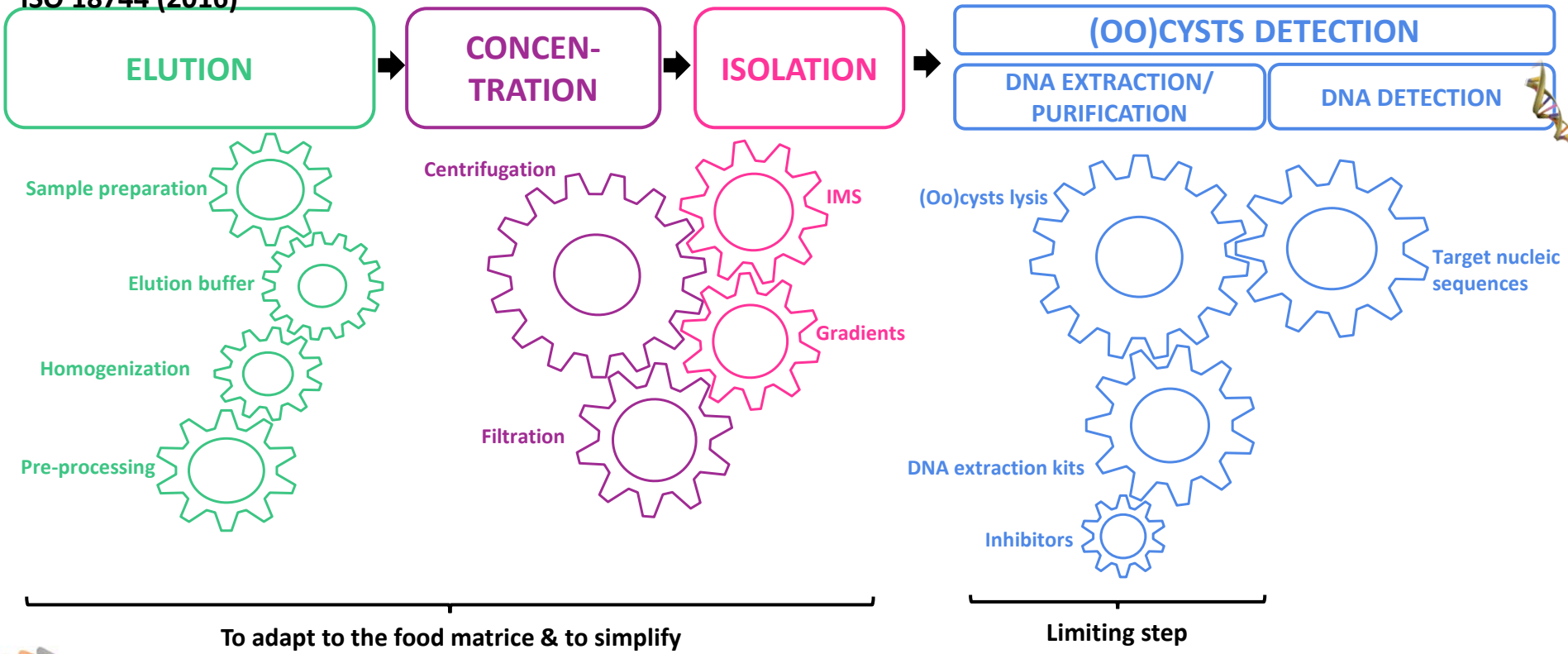
Detection of all parasites by qPCR: need for harmonization

1. Method's development and selection

- **Best performances**
- **Easy-to-implement**

Detection of all parasites by qPCR: need for harmonization

ISO 18744 (2016)



Detection of all parasites by qPCR: need for harmonization

1. **Method's development and selection**

2. **Performance validation of the selected method**

➡ **Which performance criteria?**
How to determine them?

ISO 17468:2016
Microbiology of the food chain — Technical requirements and guidance on establishment or revision of a standardized reference method

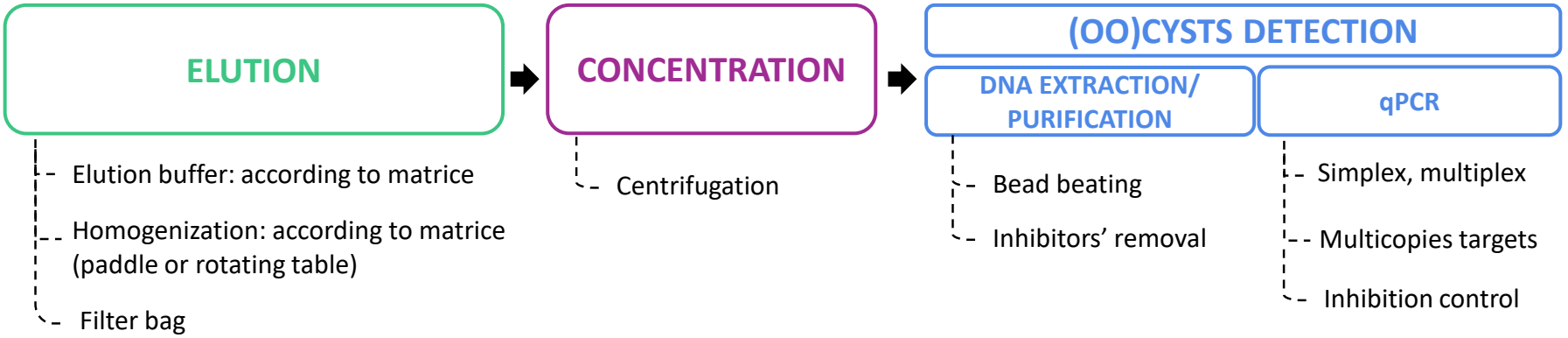
ISO 16140-2:2016
Microbiology of the food chain — Method validation — Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method

ISO 16140-3:2021
Microbiology of the food chain — Method validation —
Part 3:
Protocol for the verification of reference methods and validated alternative methods in a single laboratory

ISO/AWI 16140-8
Microbiology of the food chain — Method validation
Part 8: Protocol for the validation of alternative methods against a reference method for viruses or parasites

➡ **LOD_{95}, LOD_{50}**
Sensitivity
Inclusivity/Exclusivity

Detection of all parasites by qPCR: promising standardized method



Detection of all parasites by qPCR: promising standardized method

Some examples of LD_{95} ((oo)cysts/g or oocysts/L)

	FRUITS AND VEGETABLES						SHELLFISH	DAIRY PRODUCTS
	FRESH				FROZEN		Mussels tissues	Raw milk [cow]
	Lettuce	Coriander	Parsley	RTE salads	Raspberries	Parsley		
T. gondii	< 1	< 1 (Berrouch, 2021)	< 1	< 1 (Calero-Bernal, 2025)	< 1 (Unpublished, pers. comm.)	2 < LD < 20 (Unpublished, pers. comm.)	4 < LD < 20 (Cazeaux, 2022)	ND
C. parvum	2 < LD < 4 (Unpublished, pers. comm.)	< 1 (Berrouch, 2021)	1 < LD < 4 (Berrouch, 2021)	< 1 (Mayer-Scholl, 2022)	< 1 (Unpublished, pers. comm.)	10 < LD < 20 (Unpublished, pers. comm.)	40 < LD < 400 (Cazeaux, 2022)	50 < LD < 100 (Unpublished, pers. comm.)
G. duodenalis	< 1	< 1 (Berrouch, 2021)	< 1	ND	< 1 (Unpublished, pers. comm.)	2 < LD < 20 (Unpublished, pers. comm.)	4 < LD < 20 (Cazeaux, 2022)	ND

Detection of all parasites by qPCR: promising standardized method

EXTRACTION CONTROL: *E. acervulina* oocysts ➔ Easy to obtain; not pathogens to humans; do not interfere with the target analysis

ELUTION

- Elution buffer: according to matrice
- Filter bag
- Homogenization: according to matrice (paddle or rotating table)

CONCENTRATION

- Centrifugation

(OO)CYSTS DETECTION

DNA EXTRACTION/ PURIFICATION

- Bead beating
- Inhibitors' removal

qPCR

- Simplex, multiplex
- Multicopies targets
- Inhibition control

Detection of all parasites: alternatives to qPCR?

Detection by PCR:

- Sequencing confirmation and/or genotyping
- - Sensitivity
  - Agarose gel for routine analyses

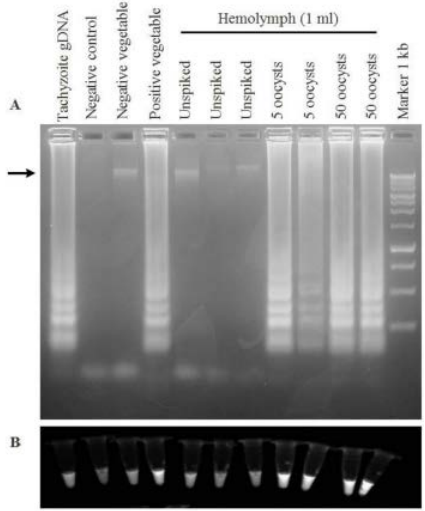
Loop-Mediated Isothermal Amplification-Lateral-Flow Dipstick (LAMP-LFD) to detect *Toxoplasma gondii* oocyst in ready-to-eat salad

Marco Lalle [a], Alessia Possenti [a], Jitender P. Dubey [b], Edoardo Pozio [a]

Detection by LAMP (Loop-Mediated Isothermal Amplification):

- - No need for specific and expensive equipments
  - Sensitivity
- - Potential of false positive
  - Agarose gel for routine analyses

Mussels



Durand *et al.*, 2020

Detection of all parasites: alternatives to qPCR?

Detection by DNA-based NGS approaches:

- targeted metagenomics: sequencing of specific markers (mainly 18S rDNA)
- untargeted metagenomics: sequencing of total DNA from a sample

- Simultaneous detection of different parasites
- Genotyping

Competition from eukaryotic sequences of food matrices

- Sensitivity
- False positives

Detection of parasites in food and water matrices by shotgun metagenomics: A narrative review☆

Paolo Vatta, Simone M. Cacciò *

Application of next generation sequencing for detection of protozoan pathogens in shellfish

Catherine DeMone [a,b], Mei-Hua Hwang [c], Zeny Feng [b], J. Trenton McClure [d], Spencer J. Greenwood [e], Rebecca Fung [f], Minji Kim [g], J. Scott Weese [c], Karen Shapiro [c,f,*]

Establishing the Performance of Next-Generation Amplicon Sequencing for Detection of *Giardia duodenalis* in Ready-to-Eat Packaged Leafy Greens

Holly Nichols [†], Monica Santin, Jenny G. Maloney [*]

Methods to quantify all parasites

qPCR:

Combine sensitivity and quantification

Variability of quantification results according to used standards

Methods to quantify all parasites

qPCR:

Combine sensitivity and quantification

Variability of quantification results according to used standards

Digital droplet PCR:

- Absolute quantification & no need for standards
- Less affected by inhibitors

Sensitivity relative to qPCR

Droplet generation depends on quality of DNA extract

J Food Prot. 2025 Aug 22;88(9):100568. doi: 10.1016/j.jfp.2025.100568. Epub 2025 Jun 24.

Optimized Molecular Detection of Cryptosporidium Within the Water–Soil–Plant–Food Nexus: Advancing Surveillance in Agricultural Systems

Robyn Marijn Schipper [1], Loandi Richter-Mouton [1], Lise Korsten [2]

Front Microbiol. 2023 Sep 8:14:1238689. doi: 10.3389/fmicb.2023.1238689. eCollection 2023.

First application of a droplet digital PCR to detect *Toxoplasma gondii* in mussels

Andrea Mancusi [1], Yolande T R Proroga [1], Angela Giordano [1], Santa Girardi [1], Francescantonio D'Orilia [2], Renato Pinto [3], Paolo Sarnelli [3], Laura Rinaldi [2 4], Federico Capuano [1], Maria Paola Maurelli [4]

Unpublished, pers. comm.

Methods to quantify all parasites

qPCR:

Combine sensitivity and quantification

Variability of quantification results according to used standards

Digital droplet PCR:

- Absolute quantification & no need for standards
- Less affected by inhibitors

Sensitivity relative to qPCR

Droplet generation depends on quality of DNA extract

Digital PCR?

Acta Trop. 2025 Oct:270:107804. doi: 10.1016/j.actatropica.2025.107804. Epub 2025 Aug 25.

Digital PCR-based detection of Cryptosporidium in Pancreatic Tissue and Saliva Samples of Cancer patients; Pancreatic cryptosporidiosis

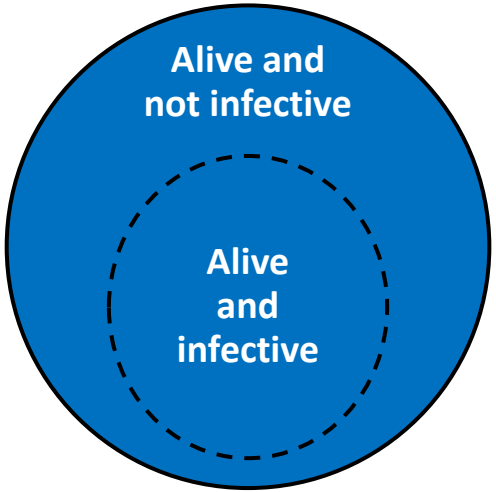
Tufan Gumus [1], Deniz Ece [2], Can Muftuoglu [2], Ufuk Mert [3], Ecem Kalemoglu [4], Goksever Akpinar [5], Milad Asadi [2], Tolga Coskun [2], Hamid Alizadeh [6], Alper Uguz [7], Ayse Caner [8]

PLoS Negl Trop Dis. 2024 May 20;18(5):e0012153. doi: 10.1371/journal.pntd.0012153. eCollection 2024 May.

Employing digital PCR for enhanced detection of perinatal Toxoplasma gondii infection: A cross-sectional surveillance and maternal-infant outcomes study in El Salvador

Mary K Lynn [1], Marvin Stanley Rodriguez Aquino [2], Pamela Michelle Cornejo Rivas [2], Xiomara Miranda [3], David F Torres-Romero [4], Hanson Cowan [1], Madeleine M Meyer [1], Wilber D Castro-Godoy [2 4], Mufaro Kanyangarara [1], Stella C W Self [1], Berry A Campbell [5], Melissa S Nolan [1]

Methods to detect alive parasites

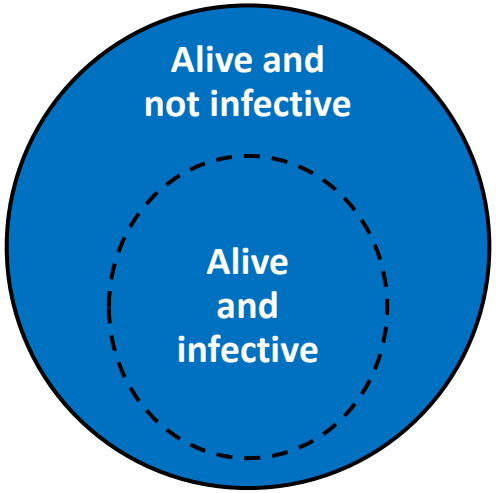


Methods to detect all alive parasites

UMT PARASAFE PARASITES DANS LES ALIMENTS

Assessing viability and infectivity of foodborne and waterborne stages (cysts/oocysts) of *Giardia duodenalis*, *Cryptosporidium* spp., and *Toxoplasma gondii*: a review of methods

Angélique Rousseau[1,2,5], Stéphanie La Carbona[2,*], Aurélien Dumètre[3], Lucy J. Robertson[4], Gilles Gargala[5], Sandie Escotte-Binet[1], Loïc Favennec[5], Isabelle Villena[1], Cédric Gérard[6], and Dominique Aubert[1]



Overestimation of the infective hazard

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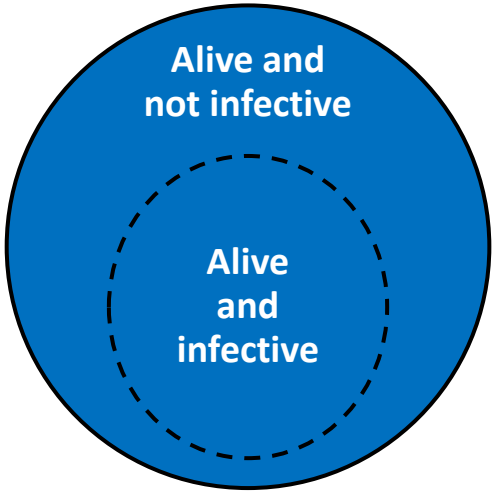
Methods to detect all alive p

Overestimation of the infective hazard



Methods	Parasites	Applications: Detection in naturally contaminated samples	Applications: Agreement with infectivity assays	Applications: Determination of the efficacy of control measures[a]	Applications: Agreement with infectivity assays
Excystation Ability of trophozoites / sporozoites to escape from the cysts / oocysts after stimulation	Cp	Drinking water [80]	NA	**Simple matrix[b]:** H_2O_2-based disinfectants [176] Chlorine [29], Chlorine dioxide, Monochloramine [129] Ozone [29,34,73,129] Ammonia [116] Liming, alum and ferric sulfate flocculation	– – – – NA
Vital dye-exclusion (propidium iodide) Ability of cells to exclude the dye (membrane intact cells) & staining of dead cells	Cp	Wastewater [6] Surface water [190] Marine water [191] Drinking water [6] Cockles, mussels, clams [90]	NA – [§] NA NA NA	**Simple matrix:** H_2O_2-based disinfectants [176] Chlorine [29] Ozone [29,34] Ammonia [116] Liming, alum and ferric sulfate flocculation [183]	– – – – NA
RT-PCR Ability of cells to produce mRNA					NA –
FISH Ability of cells to produce rRNA	Cp G	Water [138] Oysters [93] Water [138]	NA NA NA	**Simple matrix:** Gamma irradiation [185] Storage 15 °C (9 m) [93] NA	NA – NA
PMA-PCR Ability of cells to exclude PMA (membrane intact cells) & no amplification in dead cells	Cp G	Water [149] Water [149]	NA NA	**Simple matrix:** H_2O_2 [144] Storage at RT (14 m) [33] Storage 4 °C (up to 48 m) [*hsp70*] [144] NA **Food matrix:** Storage 4 °C (8 d) – Basil [*β-giardin*] [104]	NA NA NA NA NA NA NA NA

Suitability with routine analyses ...!?

Suitability with routine analyses ...!?

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Molecular methods to detect all alive parasites

RT-qPCR: ALIVE PARASITES ARE METABOLICALLY ACTIVE ➔ PRODUCTION OF mRNA

- Applicable to foods
- Quantitative

Variable LoD depending on food

Control measure assessment (underestimation of the efficiency = securization of the product)

Surveillance and prospective studies

Development of a qRT-PCR method to assess the viability of *Giardia intestinalis* cysts, *Cryptosporidium* spp. and *Toxoplasma gondii* oocysts

Emmanuelle Travaillé [a], Stéphanie La Carbona [b], Gilles Gargala [c], Dominique Aubert [d], Karine Guyot [e], Aurélien Dumètre [f], Isabelle Villena [d], Maryline Houssin [a, *]

Simultaneous detection of the protozoan parasites *Toxoplasma, Cryptosporidium* and *Giardia* in food matrices and their persistence on basil leaves

Jeanne Hohweyer [a], Catherine Cazeaux [b], Emmanuelle Travaillé [c], Emilie Languet [b], Aurélien Dumètre [d], Dominique Aubert [a], Christine Terryn [e], Jitender P. Dubey [f], Nadine Azas [d], Maryline Houssin [c], Favennec Loïc [g], Isabelle Villena [a], Stéphanie La Carbona [b, *]

Molecular detection and viability discrimination of zoonotic protozoan pathogens in oysters and seawater

Minji Kim [a], Lezlie Rueda [a], Andrea Packham [b], James Moore [c,d], Stefan Wuertz [e,f,g], Karen Shapiro [a,*]

Quantification of viable protozoan parasites on leafy greens using molecular methods

Minji Kim [a,b], Karen Shapiro [a], Verónica B. Rajal [c,d], Andrea Packham [a], Beatriz Aguilar [a], Lezlie Rueda [a], Stefan Wuertz [b,d,e,*]

Molecular methods to detect all alive parasites

PMA-qPCR: **ALIVE PARASITES ARE NOT PERMEABLE TO PMA ➔ DNA AMPLIFICATION**

☹ - No discrimination between live and dead parasites
- Not easily applicable to foods

☒ **Control measure assessment**

☒ **Surveillance and prospective studies**

Evaluation of propidium monoazide–based qPCR to detect viable oocysts of *Toxoplasma gondii*

Angélique Rousseau[1,2,3] · Isabelle Villena[2] · Aurélien Dumètre[4] · Sandie Escotte-Binet[2] · Loïc Favennec[3] · Jitender P. Dubey[5] · Dominique Aubert[2] · Stéphanie La Carbona[1]

Methods to assess the effect of meat processing on viability of *Toxoplasma gondii*: towards replacement of mouse bioassay by in vitro testing

Marieke Opsteegh, Cecile Dam-Deisz, Paulo de Boer, Stephane DeCraeye, Andrea Faré, Paul Hengeveld, Ruud Luiten, Gereon Schares, Conny van Solt-Smits, Bavo Verhaegen, Theo Verkleij, Joke van der Giessen, Henk J. Wisselink

Quantification of viable protozoan parasites on leafy greens using molecular methods

Minji Kim[a,b], Karen Shapiro[a], Verónica B. Rajal[c,d], Andrea Packham[a], Beatriz Aguilar[a], Lezlie Rueda[a], Stefan Wuertz[b,d,e,*]

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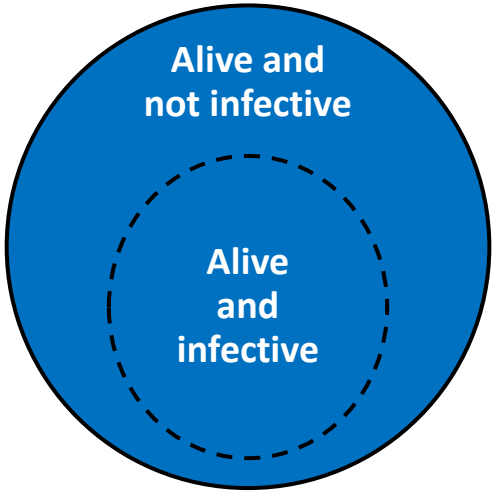
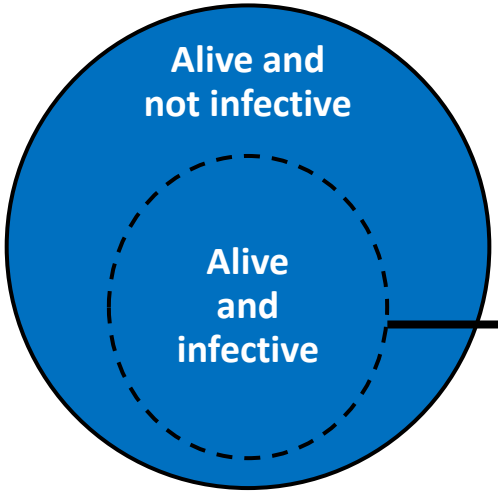


« Real » estimation of infective hazard

Methods to detect alive and infective parasites

« Real » estimation of infective hazard



Methods	Parasites	Applications	
		Detection in naturally contaminated samples	Determination of the efficacy of control measures[a]
Bioassays – Ability of (oo)cysts to induce infection of animals	Cp	River water [210] Wastewater [109] Mussels, clams, oysters, cockles [67,69,70,71,88,90,143]	**Simple matrix[b]:** H_2O_2-based disinfectants [176] Chlorine dioxide [198] Ozone [141,187], ozone + monochloramine/ + chlorine [27,28] Salinity: 10, 20 and 30 ppt of salt at 10 °C or 20 °C (12 w) [68] UV [11,46,148,154,187,195,198] US [172] Pasteurization [99] Storage 15 °C (9 m) [115] Storage 4 °C and 10 °C (8 w) [142] Storage −10 °C to + 35 °C (24 w) [68] **Food matrix:** UV – Fresh apple cider [98] PUV – Raspberries [131] HHP, e-beam, microwaves – Oysters [42,43] Heat (steam cooking) – Mussels [91] Pasteurization – Milk [99] Storage 6 °C (4 w) – Apple [150]
	G	Wastewater [82,109,140] Raw and chlorinated drinking water [111]	**Simple matrix:** Ozone [74] UV [37,139,140,154,196] Gamma irradiation [203]
	T	Water [12,110,219] Oysters, mussels [63]	**Simple matrix:** Chlorine [222] Ozone [59,222] UV [59,220,223] HHP [145] Gamma-irradiation [55] Radiofrequency [221] Storage −10 °C to 70 °C (up to 54 m) [53] **Food matrix:** HHP – Raspberries [146] Storage 4 °C (up to 8 w) – Raspberries and blueberries [126]

= reference method

- Reliable & Sensitive
- Applicable to foods

Semi-quantative

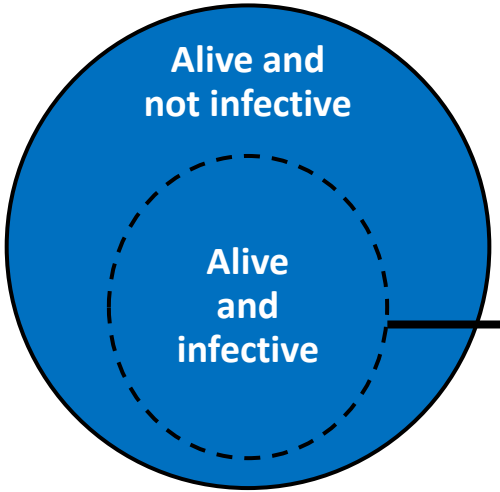
Not applicable to large scale

Control measure assessment

Surveillance and prospective studies

Methods to detect alive and infective parasites

« Real » estimation of infective hazard



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Cell culture infection – Ability of (oo)cysts to invade and infect cells	Cp	Wastewater [85] Water [133,190]	**Simple matrix:** Chlorine [15,193], Chlorine dioxide [198], MOS [122] Ozone [122,187] UV [83,122,123,186,187,195,198] Heat 38 °C to 70 °C [193] Storage 15 °C (9 m) [115] **Food matrix:** Organic acids and hydrogen peroxide – Fruit juices [127]
	Ch	NA	**Simple matrix:** UV [118]
	T	NA	**Simple matrix:** Chlorine [218] Iodophore-based disinfectants, formalin, acidified ethanol [218] Ozone [59] UV [59,223]

➔ **applicability to food matrices?**

Molecular-based methods: CC-qPCR

➔ **Reduce the number of steps for application to foods**

CC-qPCR to detect alive and infective parasites

CC-qPCR *T. gondii*: Which stage to use?

- Additional steps
- Variable cell infection rate

sporozoite

Using quantitative reverse transcriptase PCR and cell culture plaque assays to determine resistance of *Toxoplasma gondii* oocysts to chemical sanitizers

Eric N. Villegas [a,b,*], Swinburne A.J. Augustine [a], Leah Fohl Villegas [a], Michael W. Ware [a], Mary Jean See [b], H.D. Alan Lindquist [c], Frank W. Schaefer III [c], J.P. Dubey [d]

Determining UV Inactivation of *Toxoplasma gondii* Oocysts by Using Cell Culture and a Mouse Bioassay[▽]

Michael W. Ware,[1]§ Swinburne A. J. Augustine,[1]§ David O. Erisman,[1] Mary Jean See,[3]† Larry Wymer,[1] Samuel L. Hayes,[2] J. P. Dubey,[4] and Eric N. Villegas[1,3]*

CC-qPCR to detect alive and infective parasites

CC-qPCR *T. gondii*: Which stage to use?

sporozoite

V/S

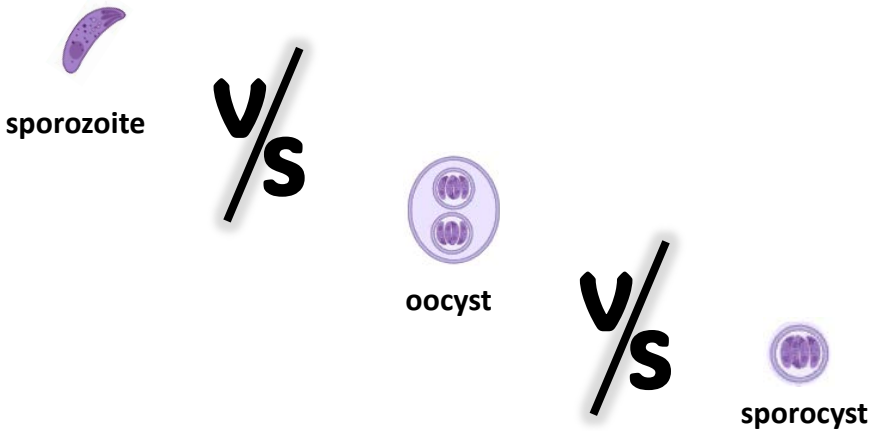
oocyst

Low spontaneous excystation rate
➔ Low cell infection rate

(Unpublished, pers. comm.)

CC-qPCR to detect alive and infective parasites

CC-qPCR *T. gondii*: Which stage to use?



- Ability to spontaneously excyst and release sporozoites (reduced number of steps)
- LD_{100} in mussels =10 infective oocysts
- Short time to results (3 days)

- Not quantitative in mussels
- Transfer to- and performance in- other foods

Toxoplasma gondii Oocyst Infectivity Assessed Using a Sporocyst-Based Cell Culture Assay Combined with Quantitative PCR for Environmental Applications

Angélique Rousseau,[a,b,c] Sandie Escotte-Binet,[a] Stéphanie La Carbona,[b] Aurélien Dumètre,[d,e] Sophie Chagneau,[a] Loïc Favennec,[c] Sophie Kubina,[a,b,c] Jitender P. Dubey,[f] Didier Majou,[g] Aurélie Bigot-Clivot,[h] Isabelle Villena,[a] Dominique Aubert[a]

Control measure assessment

Surveillance and prospective studies

CC-qPCR to detect alive and infective parasites

CC-qPCR *C. parvum*: Which stage to use?

sporozoite

☹ Additional steps

Detection of Infectious *Cryptosporidium parvum* Oocysts from Lamb's Lettuce: CC–qPCR's Intake

Sophie Kubina [1,2,*], Damien Costa [2,3], Loïc Favennec [2,3], Gilles Gargala [2,3], Angélique Rousseau [1,4], Isabelle Villena [4], Stéphanie La Carbona [1] and Romy Razakandrainibe [2,3,*]

CC-qPCR to detect alive and infective parasites

CC-qPCR *C. parvum*: Which stage to use?

sporozoite

V/S

oocyst

Detection of Infectious *Cryptosporidium parvum* Oocysts from Lamb's Lettuce: CC–qPCR's Intake

Sophie Kubina [1,2,*], Damien Costa [2,3], Loïc Favennec [2,3], Gilles Gargala [2,3], Angélique Rousseau [1,4], Isabelle Villena [4], Stéphanie La Carbona [1] and Romy Razakandrainibe [2,3,*]

- Ability to spontaneously excyst and release sporozoites with high cell infection rate (reduced number of steps)
- Applicable to different foods
- Low limits of detection in foods (e.g. LD_{50} = 10 oocysts/g in lamb's lettuce)
- Quantitative
- Short time to results (2 days)

- Need for IMS
- Applicability to all *Cryptosporidium* species and genotypes?

Persistence and survival of *Cryptosporidium parvum* oocysts on lamb's lettuce leaves during plant growth and in washing conditions of minimally-processed salads

Sophie Kubina [a,b,*], Damien Costa [b,c], Catherine Cazeaux [a], Isabelle Villena [d], Loïc Favennec [b,c], Romy Razakandrainibe [b,c], Stéphanie La Carbona [a]

☑ **Control measure assessment**

❓ **Surveillance and prospective studies**

Detect alive and infective parasites: other approachs?

Intestinal organoids:

☑ **Study of parasite development and host-parasite interactions**

? **Application to foods and risk assessment?**

Rapid review: Recent advances in in vitro models for the study of **Cryptosporidium** parvum.
Varegg MS, Woolsey ID, Robertson LJ, Jiménez-Meléndez A.
Curr Res Parasitol Vector Borne Dis. 2025 May 15;7:100269. doi: 10.1016/j.crpvbd.2025.100269. eCollection 2025.

In vitro cultivation methods for coccidian parasite research.
Feix AS, Cruz-Bustos T, Ruttkowski B, Joachim A.
Int J Parasitol. 2023 Aug;53(9):477-489. doi: 10.1016/j.ijpara.2022.10.002. Epub 2022 Nov 15
PMID: 36400306 Free article. Review.

Organoid-based in vitro system and reporter for the study of **Cryptosporidium** parvum sexual reproduction.
Korwin-Mihavics BR, Dews EA, Miller P, Cameron A, Martorelli di Genova B, Huston CD.
Microbiol Spectr. 2025 Aug 5;13(8):e0050225. doi: 10.1128/spectrum.00502-25. Epub 2025 Jun 25.
PMID: 40558094 Free PMC article.

Methods to detect parasites: perspectives

Monitoring plans
Prospective/Retrospective studies
[low level of parasites]

Control measures assessment

[high level of parasites]

Methods to detect parasites: perspectives

Monitoring plans
Prospective/Retrospective studies
[low level of parasites]

Control measures assessment

[high level of parasites]

Standardized DNA-based detection methods

qPCR

- Sensitivity
- Ability to quantify (required if criteria …one day)
- Accessible to analytical labs with reduced cost and time-to-result

- Standards

------→ **dPCR?**

Methods to detect parasites: perspectives

Monitoring plans
Prospective/Retrospective studies
[low level of parasites]

Control measures assessment

[high level of parasites]

Standardized DNA-based detection methods

qPCR

- Recovery procedures adapted to each food category and/or item
- Standardized DNA extraction procedure
- Standardized qPCR assays for each parasite + Inhibition control (duplex)
- Standardized validation of the procedure and performance criteria

Methods to detect parasites: perspectives

Monitoring plans
Prospective/Retrospective studies
[low level of parasites]

Control measures assessment

[high level of parasites]

Standardized DNA-based detection methods

qPCR

- **Project in progress: *Cyclospora* and fresh produce**
- **Next projects?**
  T. gondii and fresh produce
  Alternative to ISO 18744; *Cryptosporidium* spp. and fresh produce

Methods to detect parasites: perspectives

Monitoring plans
Prospective/Retrospective studies
[low level of parasites]

Control measures assessment

[high level of parasites]

Standardized DNA-based detection methods

qPCR

Complementary strategies to gain information about the associated risk

NGS
- Exhaustive and open-minded view
- Becomes accessible

Need to be optimized to be compatible with low amounts of targeted DNA

Methods to detect parasites: perspectives

Monitoring plans
Prospective/Retrospective studies
[low level of parasites]

Standardized DNA-based detection methods

qPCR

Complementary strategies to gain information about the associated risk

NGS

Control measures assessment

[high level of parasites]

RT-qPCR or CC-qPCR methods

RT-qPCR

- Applicable to all parasites and foods
- Quantitative ⇒ Determine inactivation levels, i.e. control measure efficiency

ALL alive parasites ⇒ underestimation of the effiency
⇒ « worst case scenario » approach

Methods to detect parasites: perspectives

Monitoring plans
Prospective/Retrospective studies
[low level of parasites]

Standardized DNA-based detection methods

qPCR

Complementary strategies to gain information about the associated risk

NGS

Control measures assessment

[high level of parasites]

RT-qPCR or CC-qPCR methods

RT-qPCR

CC-qPCR ⛔ Only for *Cryptosporidium* for now

➕ - Applicable to all foods
- Quantitative ⇒ Determine inactivation levels, i.e. control measure efficiency
- Real estimation of the infective hazard

Methods to detect parasites: perspectives

Monitoring plans
Prospective/Retrospective studies
[low level of parasites]

Standardized DNA-based detection methods

qPCR

Complementary strategies to gain information about the associated risk

NGS

Control measures assessment

[high level of parasites]

RT-qPCR or CC-qPCR methods

RT-qPCR

CC-qPCR

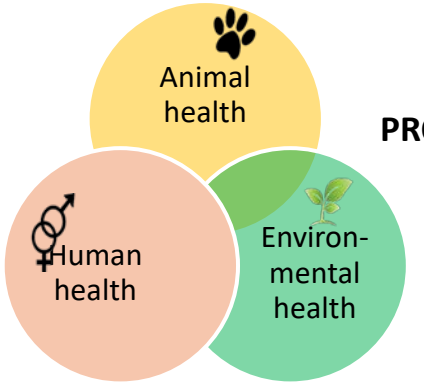
To identify new infectivity markers or inactivation markers to propose new tools?

Methods to detect parasites: conclusion

METHODS TO DETECT PARASITES IN FOOD AND WATER

IMPROVING THE SURVEILLANCE AND DATA COLLECTION

PROVIDING PUBLIC HEALTH AUTHORITIES WITH THE MEANS FOR RISK ASSESSMENT



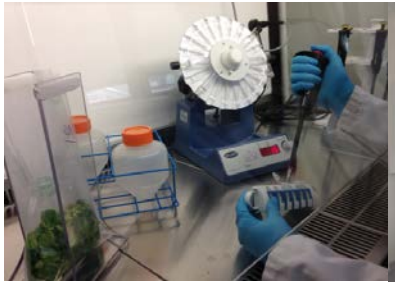
ACTALIA Food Safety Dpmt

Parasitology service:

C. Cazeaux - M. Le Blond – E. Martin

Biosafety level 3 pilot plant

Thank you for listening!



Perspectives

- Duplex qPCR
- NGS
- Crypto : intérêt du 18S
- Stratégie : qPCR + NGS
- Viabilité en surveillance ?

⇨ **Gather efforts** to propose **standardized methods accessible to analytical labs**, with **reduced cost and time-to-result**

Feasibility of a one-suit-in-all method?

⇨ **Harmonize performance and validation requirements** for parasites detection in foo

⇨ Need to validate an alternative method to ISO 18744 (according to ISO 16144-2?)