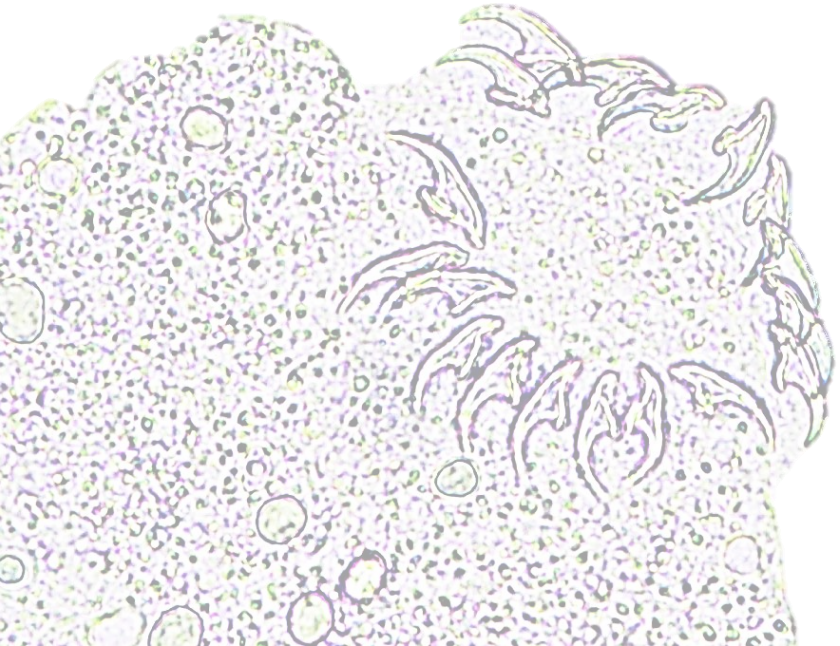
Development of a multiplex TaqMan Real-Time PCR Assay for the detection and differentiation of *Echinococcus granulosus* s.l. and *Echinococcus multilocularis*.

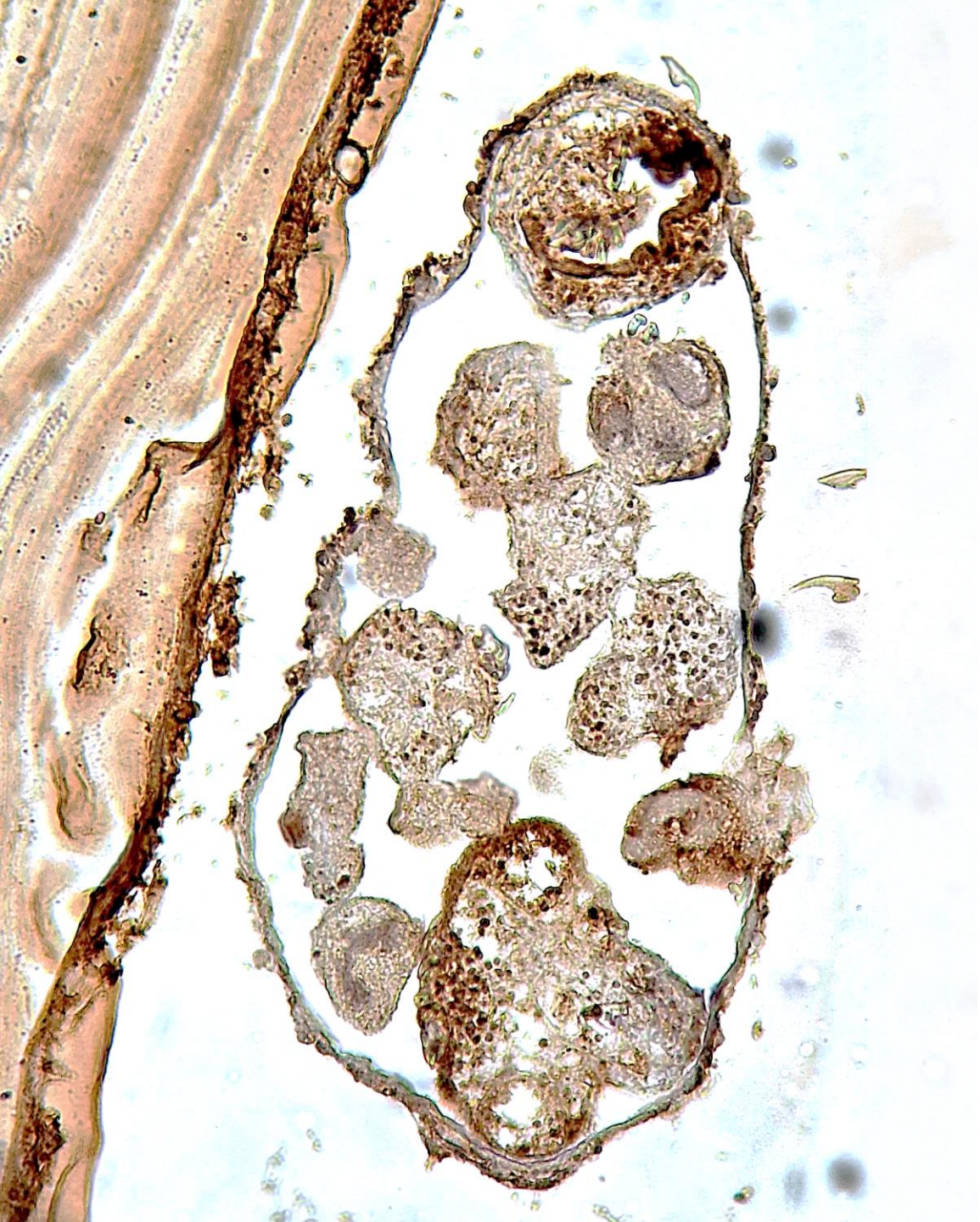
Azzurra SANTORO, Eleonora BELLO, Federica SANTOLAMAZZA, Adriano CASULLI





European Union Reference Laboratory for Parasites (EURL-P; food safety)

Workshop of the National Reference Laboratories for Parasites, Rome, Italy, 28-29 October 2026



The need?

Accurate detection of *Echinococcus granulosus* s.l. species/genotypes and *Echinococcus multilocularis* from different matrices

What we did?

Development of
a Multiplex TaqMan Real-Time PCR (qPCR)

Why qPCR approach?

- ✓ High sensitivity
- ✓ High specificity
- ✓ Rapid turnaround time
- ✓ Multiplex capability

Multiplex TaqMan Real-Time PCR Assay

Assay design and workflow

Sample



Tissue / Faeces / Other matrices

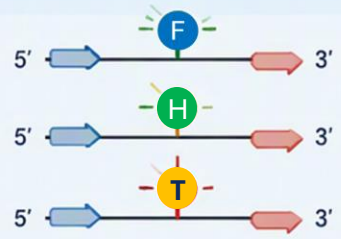
DNA extraction



Multiplex Reaction 1

Targets: *E. granulosus s.s.*, *E. multilocularis*, *E. canadensis* (G6–G7)

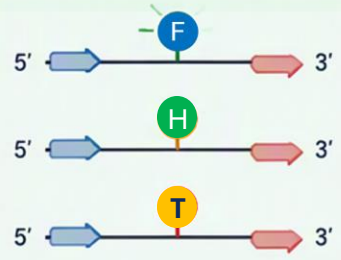
FAM	*E. multilocularis*
HEX	*E. granulosus s.s.*
Texas Red	*E. canadensis* (G6–G7)

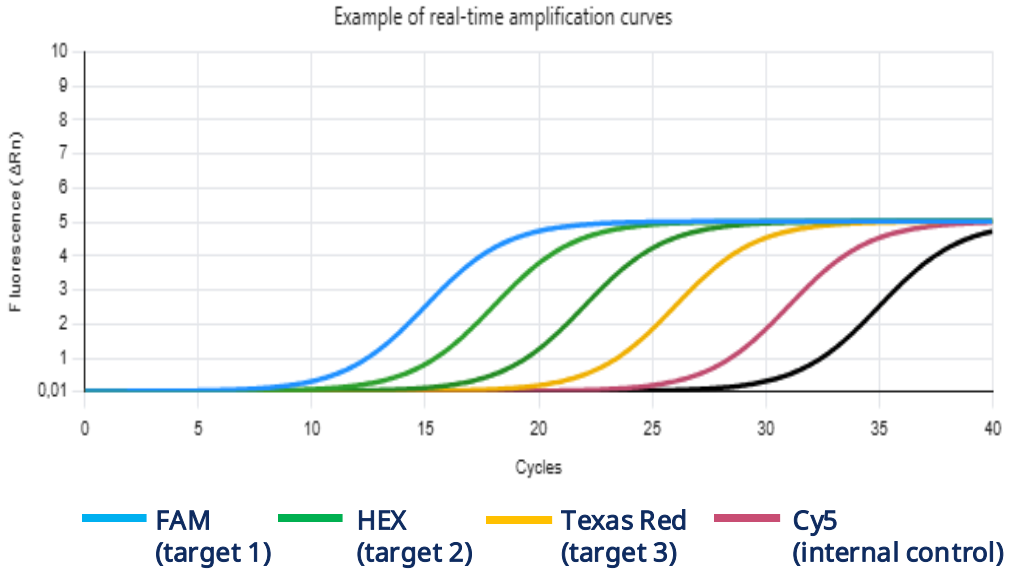


Multiplex Reaction 2

Targets: *E. canadensis* (G8–G10), *E. equinus*, *E. ortleppi*

FAM	*E. canadensis* (G8–G10)
HEX	*E. equinus*
Texas Red	*E. ortleppi*







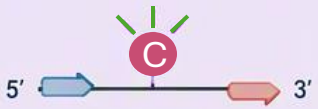
Compatible with real-time qPCR system with up to 4 detection channels

Faecal samples only



Synthetic plasmid (spiked into extracted DNA)

Cy5 Internal Amplification Control (IAC)



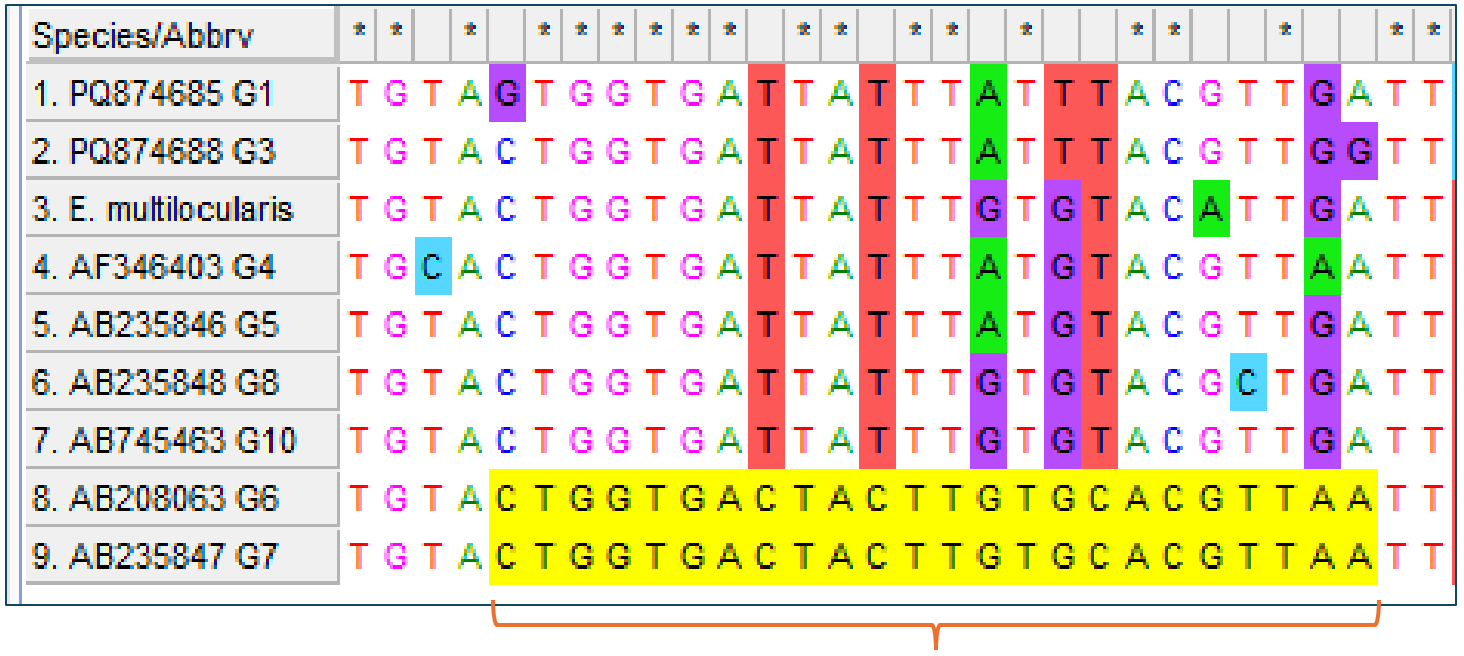
- Amplification = no inhibition
- No Cy5 signal = possible inhibition

1. PROBES DESIGN: Identification of species-specific probe regions

Criteria:

- mitochondrial targets
- Tm >70°C (MGB probes)
- 2-6 diagnostic SNPs polymorphisms preferentially located in the central region
- compatibility with multiplexing

Example:



E. canadensis G6-G7 probe

target	mismatches
Eg ss (G1-G3)	6-8
Em	5
E. equinus G4	4
E. ortleppi G5	5
Ecnd (G8-G10)	4-5

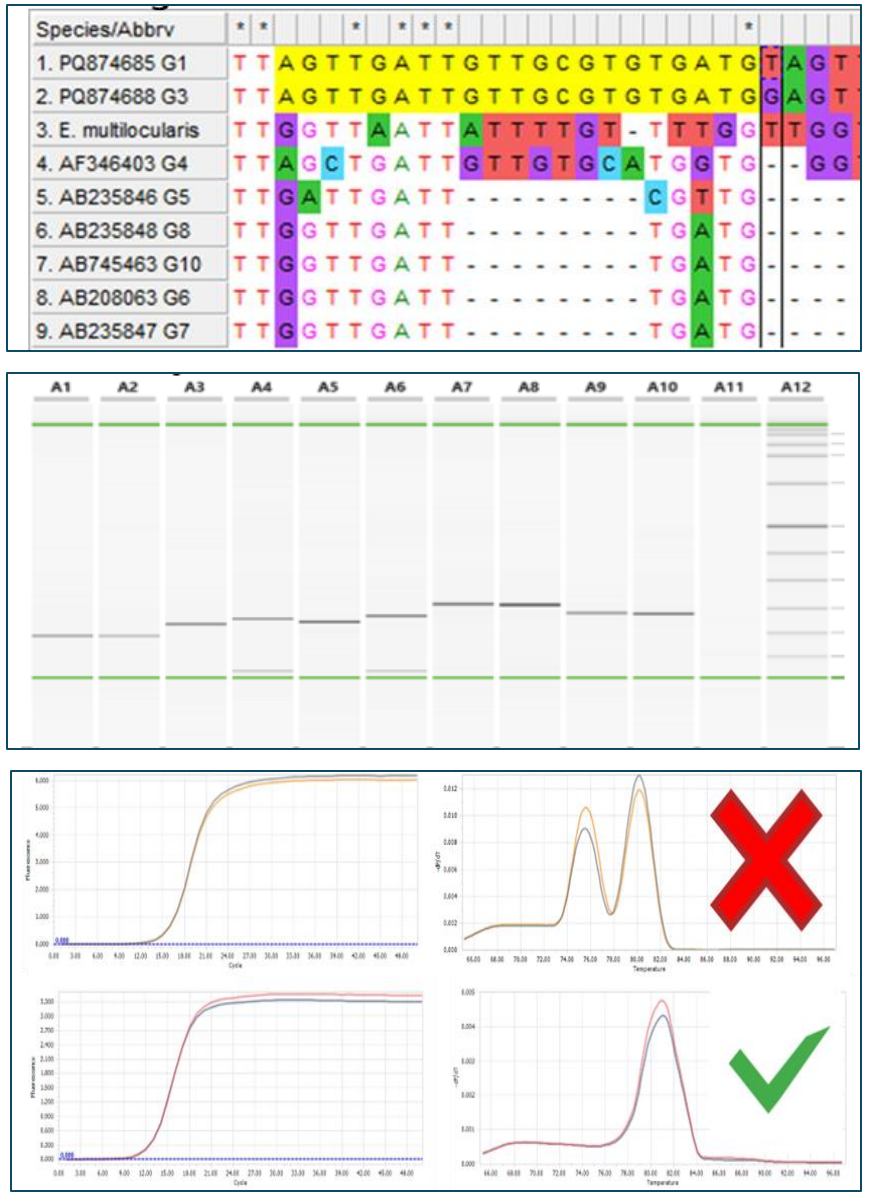
2. PRIMER SELECTION

Criteria:

- Tm ≥60°C
- amplicon 70-150 bp
- probe >10 bp from primers
- species-specific mismatches
  (preferably close to 3'; preferably insertions/deletions)
- progressive elimination of poorly performing primer pairs

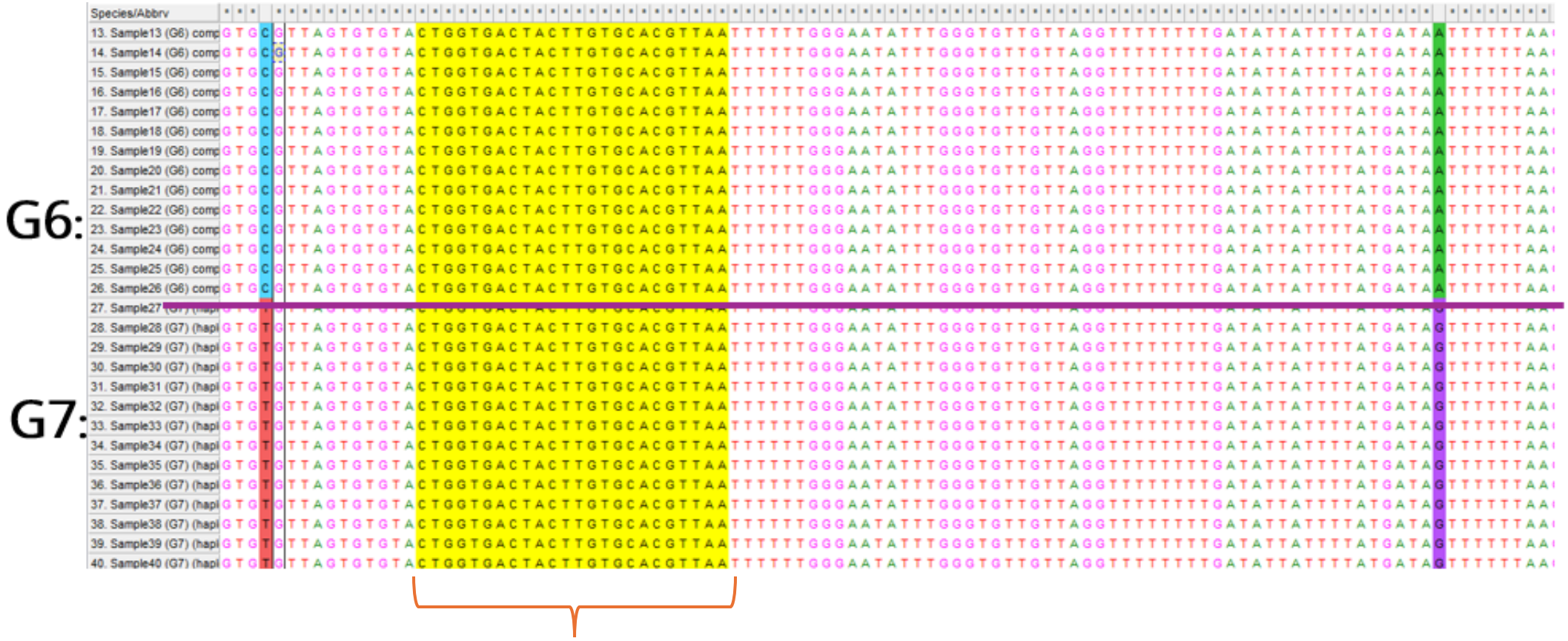
1. DESIGN
2. AMPLIFICATION (CONVENTIONAL PCR)
3. PRIMER MIX TESTED WITH SYBR GREEN
   (MELTING PEAK EVALUATION)

E. granulosus s.s. forward primer



3. IN SILICO VERIFICATION OF REPRESENTATIVE DATASETS (for probes and primer pairs)

Example:



E. canadensis G6-G7 probe

4. FINALISING EXPERIMENTAL SET-UP:

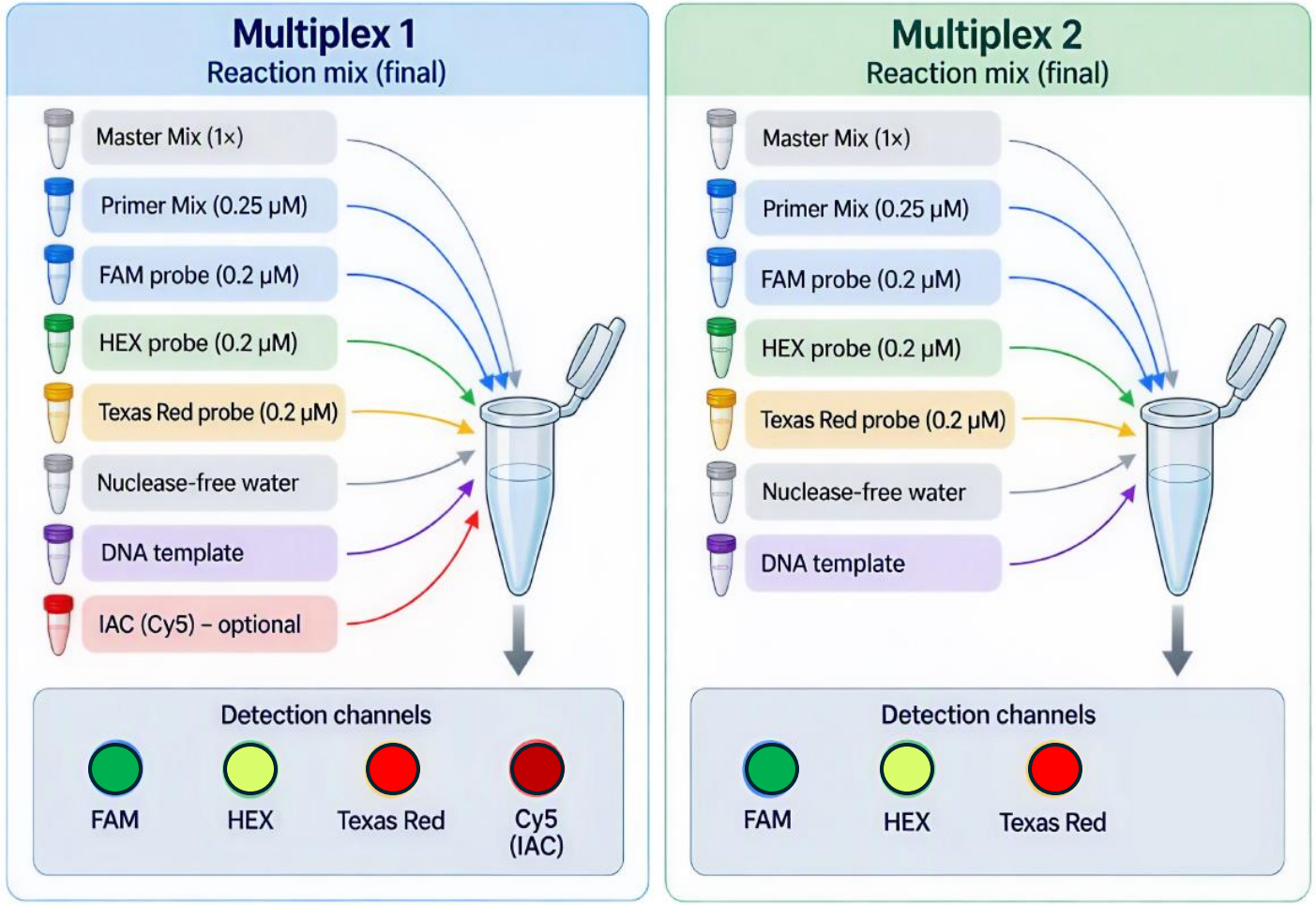
definition of final mixture and amplification conditions

E. multilocularis

E. granulosus s.s.

E. canadensis G6-G7

Internal control (optional)



E. canadensis G8-G10

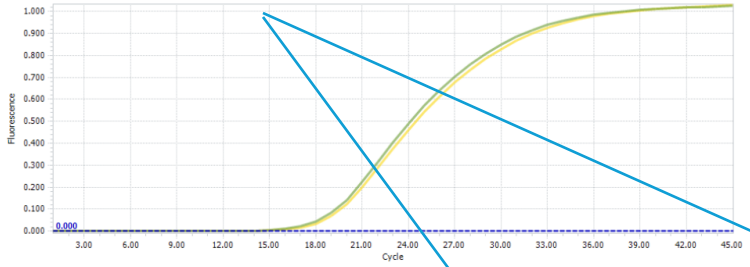
E. equinus

E. ortleppi

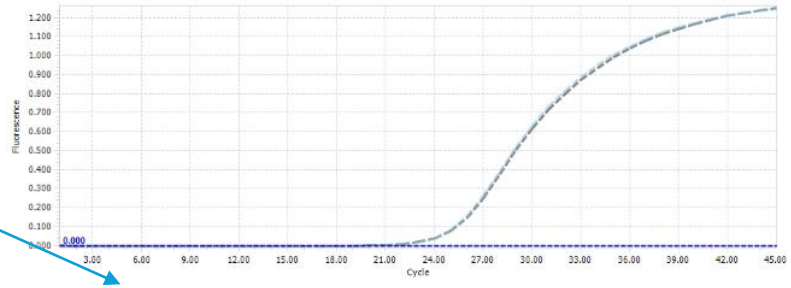
5. TEST ON REFERENCE DNA

MIX 1

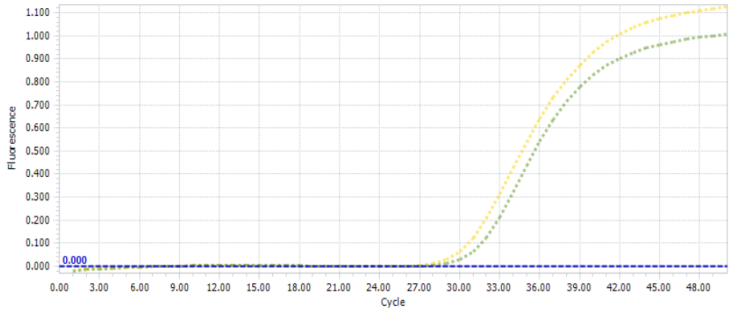
FAM – *E. multilocularis*



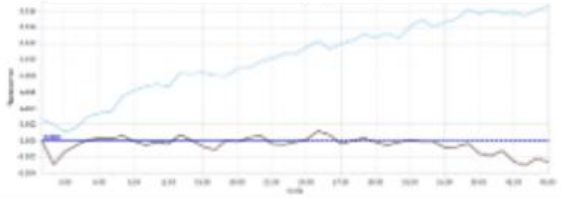
HEX – *Egss* G1-G3



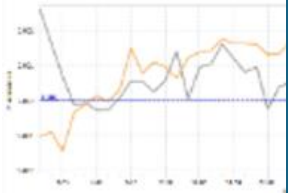
T-RED – *Ecnd* G6-G7



FAM with *Egss* G1-G3

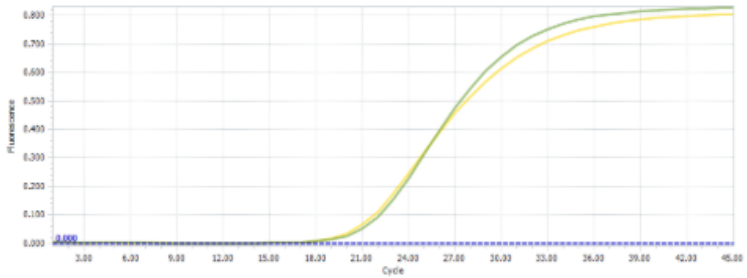


FAM with *Ecnd* G6-G7

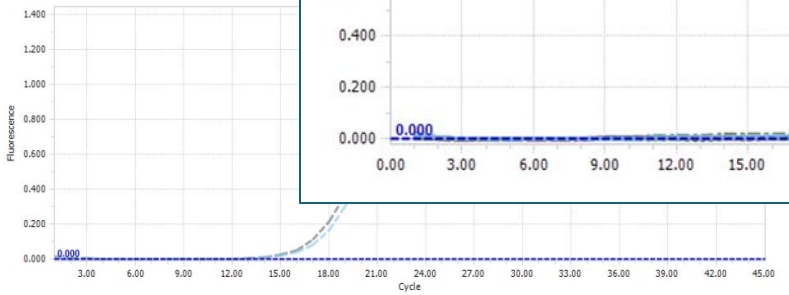


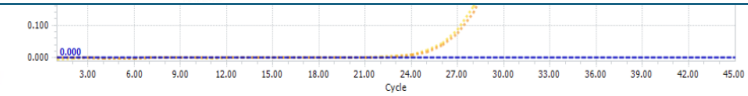
MIX 2

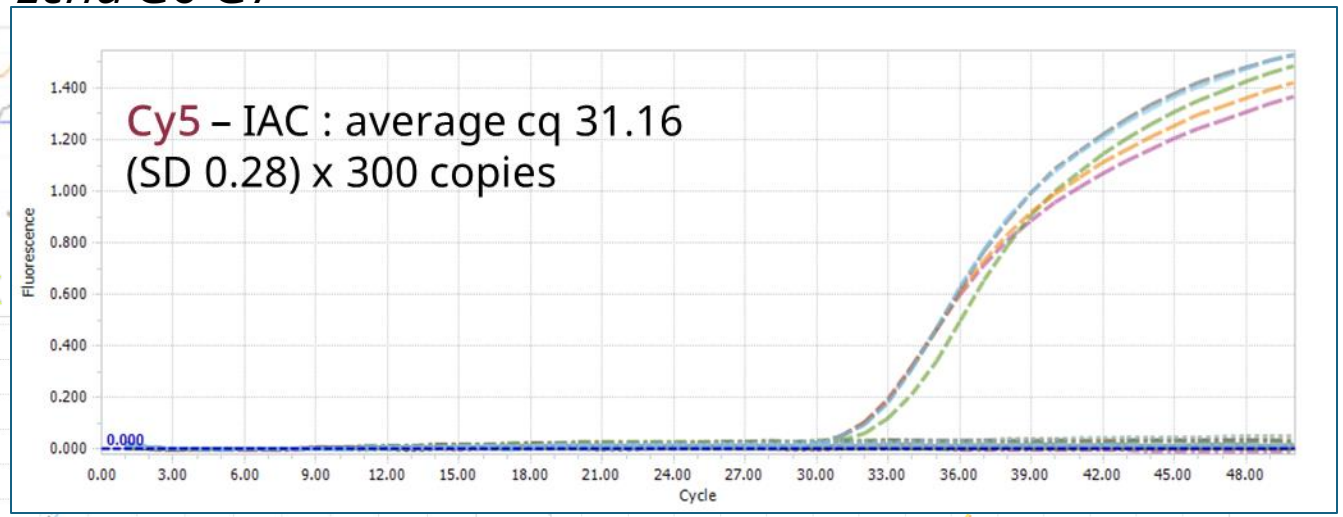
FAM – *Ecnd* G8-G10



HEX







6. SPECIFICITY ASSESSMENT

1

Fluorophore specificity

Each target species was tested against all assay channels





2

Non-target parasite DNA

-*Taenia solium* DNA

-*Taenia saginata* DNA

-*Taenia multiceps* DNA

Other parasites?



3

Human DNA

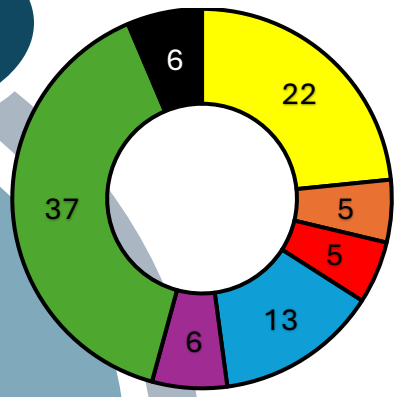
DNA extracted from whole human blood

Other hosts?



7. TESTED SAMPLES

TARGETS

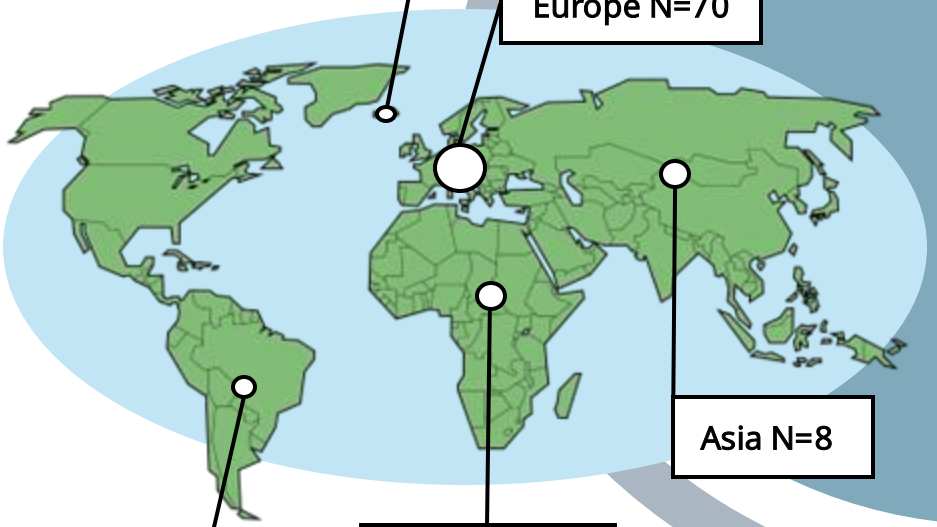


- *E. granulosus s.s.* (G1-G3)
- *E. equinus* (G4)
- *E. ortleppi* (G5)
- *E. canadensis* (G6-G7)
- *E. canadensis* (G8-G10)
- *E. multilocularis*
- *Non-target species*

GEOGRAPHIC ORIGIN / SAMPLING AREA

Circumpolar N=1

Europe N=70



Asia N=8

Africa N=10

South America N=1

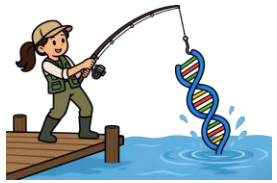
N = 94 SAMPLES TESTED

FYI:

Norwegian Veterinary Institute (NVI)

SOP Magnetic capture of *Echinococcus multilocularis* DNA from red fox (*Vulpes vulpes*) faecal samples: DNA extraction with manual washing steps.

qPCR was performed also on DNA extracted with this SOP (targeted DNA extraction -> NAD1 fishing probe)
It worked (LOD not defined).

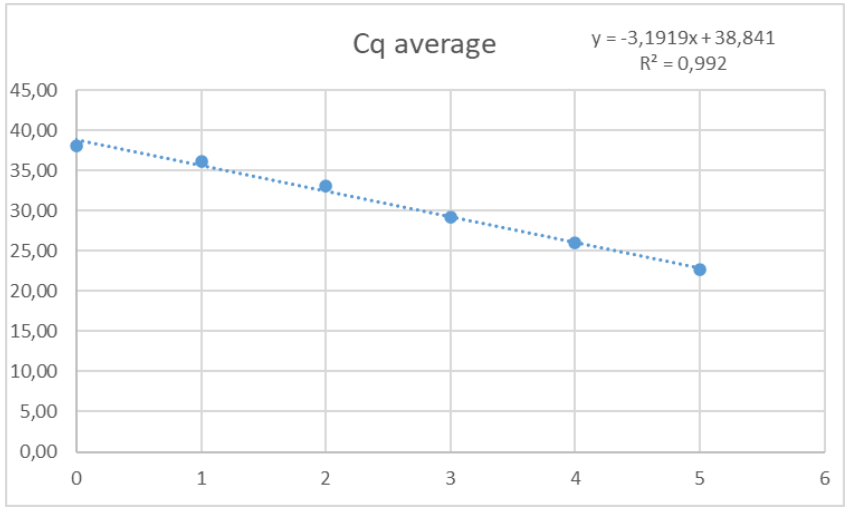


N = 13

DNA M

8. EFFICIENCY (preliminary results)

Eg ss



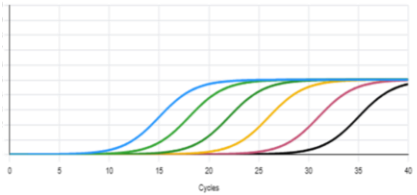
Testing serial dilutions, in triplicates, of syntetic target:

Copies	log10(copies)	Cq average
100000	5	22,63
10000	4	25,95
1000	3	29,22
100	2	33,13
10	1	36,19
1	0	38,05

Slope: -3,19
R^2:0,9912
Efficiency: 105%

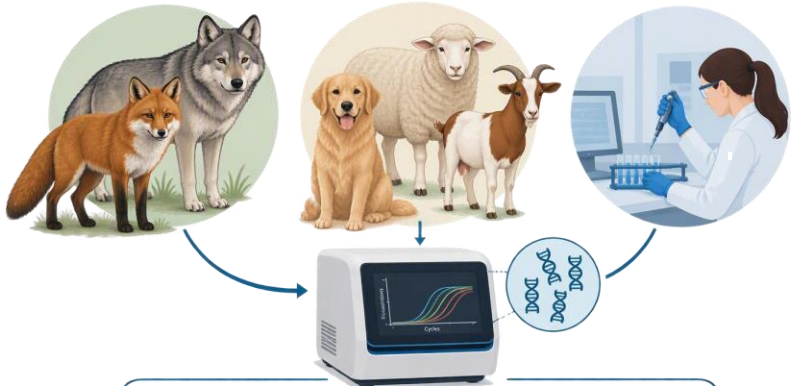
Next steps:

- Testing the remaining targets
- Determining the limit of detection (LOD) in terms of both copy number and DNA concentration (g/L)
- Testing synthetic targets and DNA extracted from parasite material

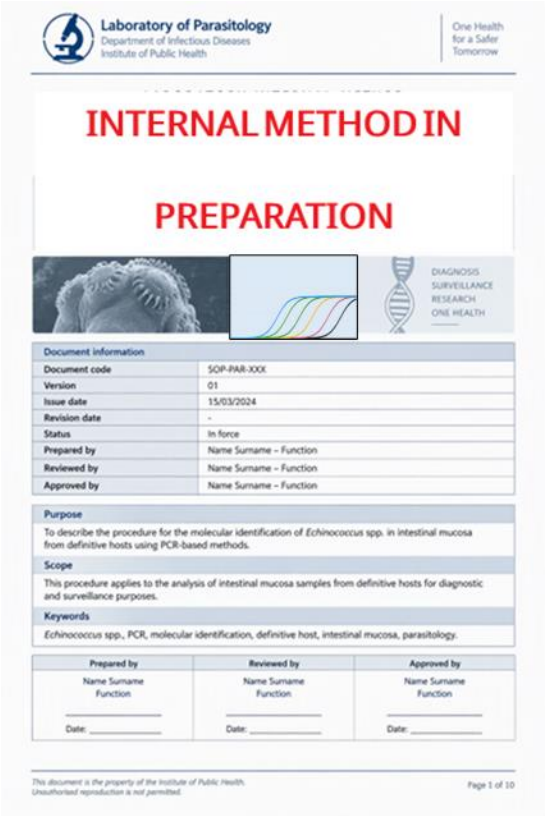


9. Applications

Deliverable: a multiplex, single step assay for **molecular detection** and **typing** of main *Echinococcus* species/genotypes.



- Diagnosis of human and animal cystic and alveolar echinococcosis
- Species and genotype identification
- Molecular epidemiology studies



Laboratory of Parasitology
Department of Infectious Diseases
Institute of Public Health

One Health for a Safer Tomorrow

INTERNAL METHOD IN

PREPARATION

DIAGNOSIS
SURVEILLANCE
RESEARCH
ONE HEALTH

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Prepared by	Name Surname – Function
Reviewed by	Name Surname – Function
Approved by	Name Surname – Function

Purpose

To describe the procedure for the molecular identification of *Echinococcus* spp. in intestinal mucosa from definitive hosts using PCR-based methods.

Scope

This procedure applies to the analysis of intestinal mucosa samples from definitive hosts for diagnostic and surveillance purposes.

Keywords

Echinococcus spp., PCR, molecular identification, definitive host, intestinal mucosa, parasitology.

Prepared by	Reviewed by	Approved by
Name Surname Function Date:	Name Surname Function Date:	Name Surname Function Date:

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Thanks for your attention

Questions or suggestions?

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