Giardia and *Cryptosporidium* Contamination in Surface Water: From Where, by How and Does Identification Methods Matter?

Real-Time PCR

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Real-Time PCR

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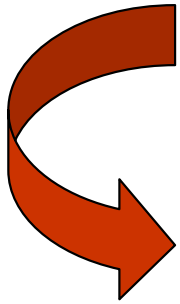
-Detects the accumulation of amplicon during the reaction progresses



"Real Time"

- Measure fluorescent signal as amplification occurs

Real-Time PCR

Fluorescent chemistries :

1. DNA-binding dyes

2. Fluorescent labeled sequence-specific probes

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Real-Time PCR Applications

- **Pathogen detection & Quantification**
- **Viral Quantification**
- **Quantification of Gene Expression**
- **DNA Damage Measurement**

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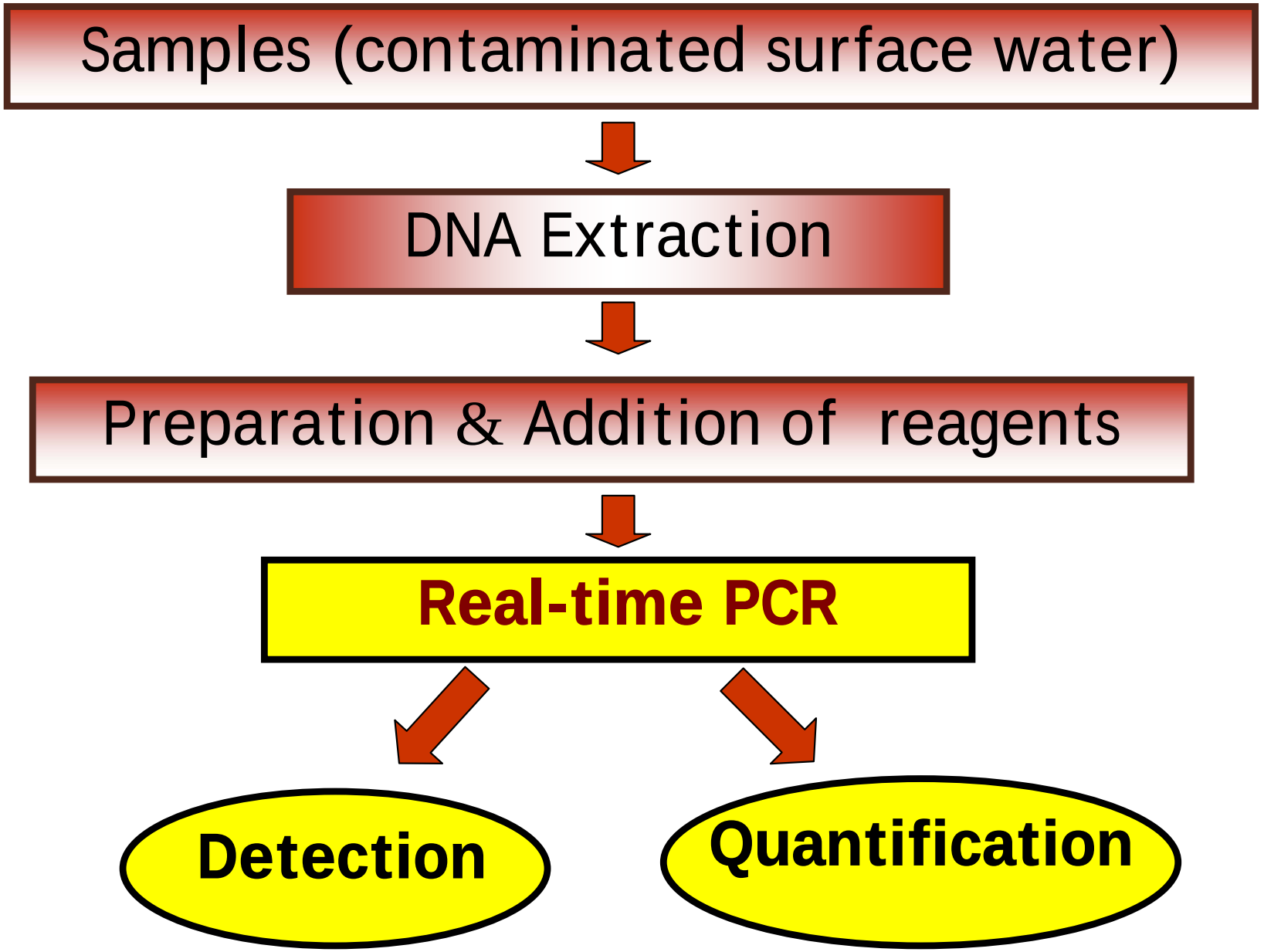
Real-Time PCR for detecting surface water contamination

Focus on:

Giardia duodenalis

Cryptosporidium **spp.**

Real-Time PCR Protocol



Giardia duodenalis

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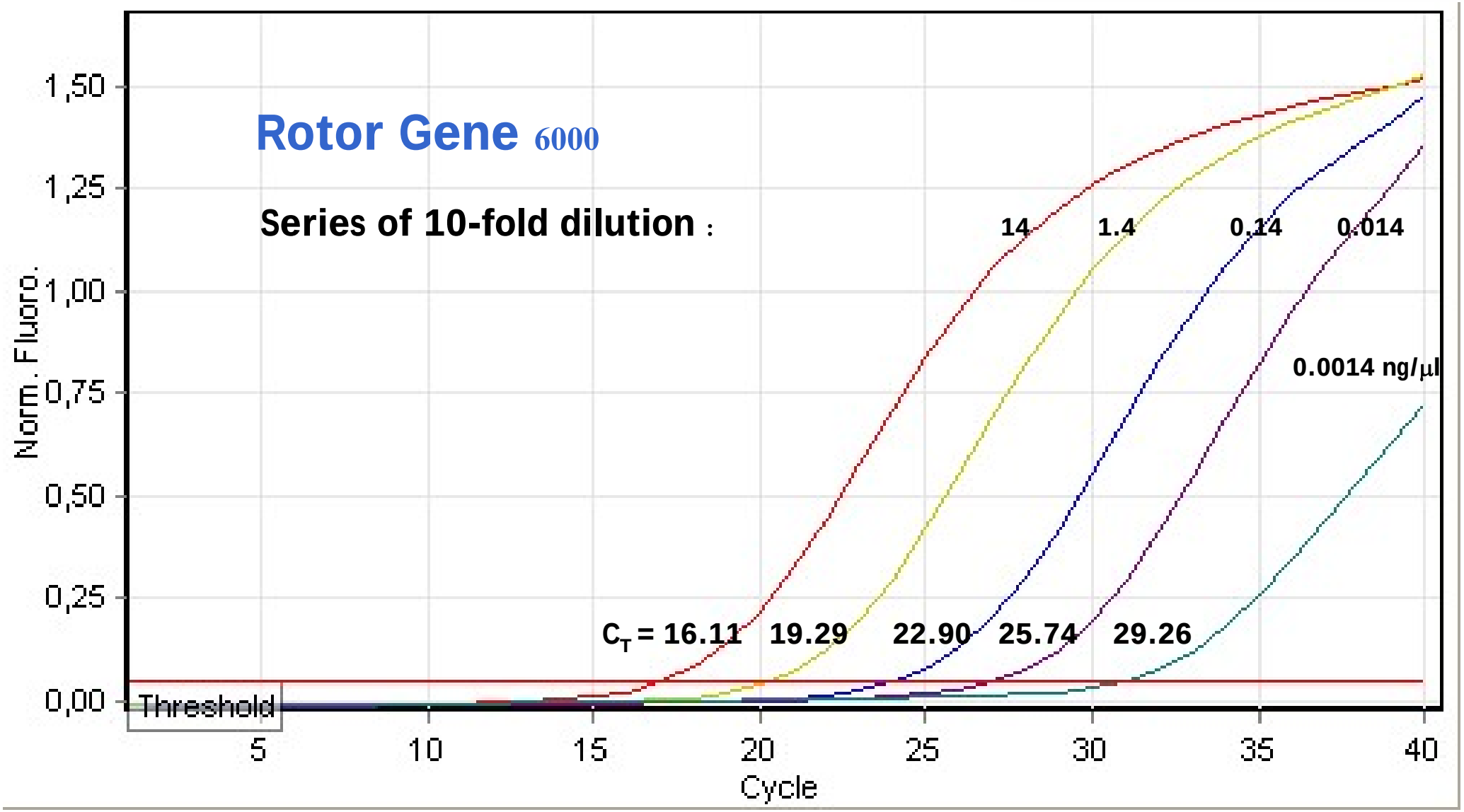
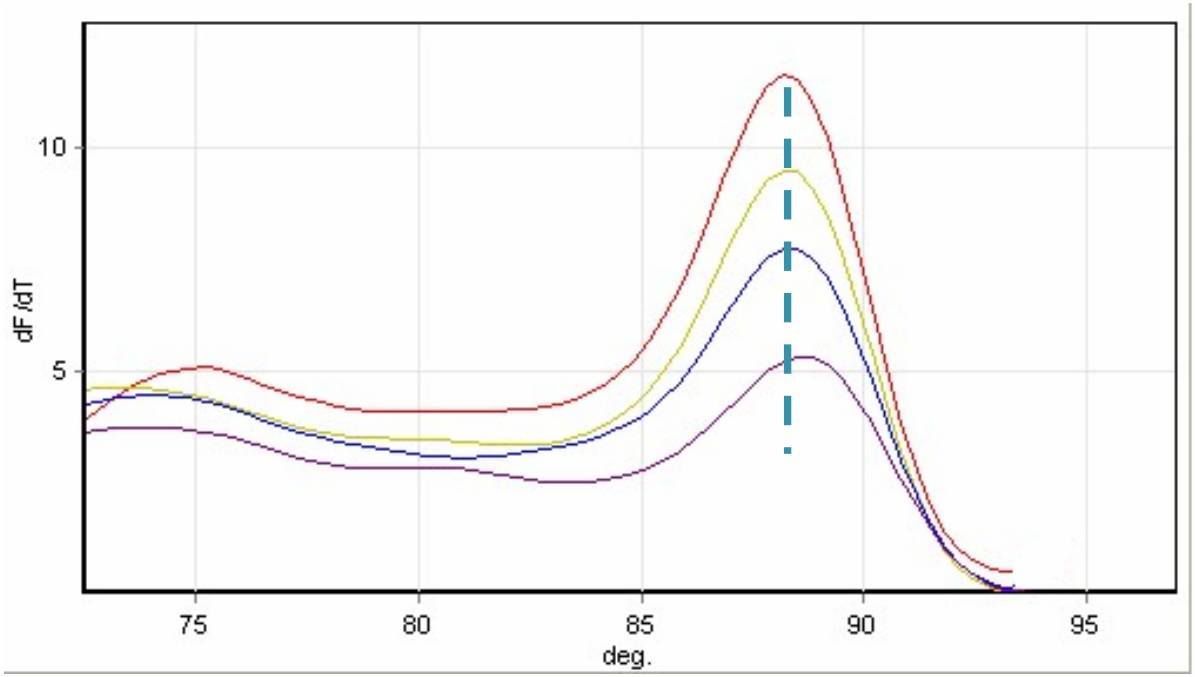


Giardia duodenalis

Melting Peak



One peak

Giardia duodenalis

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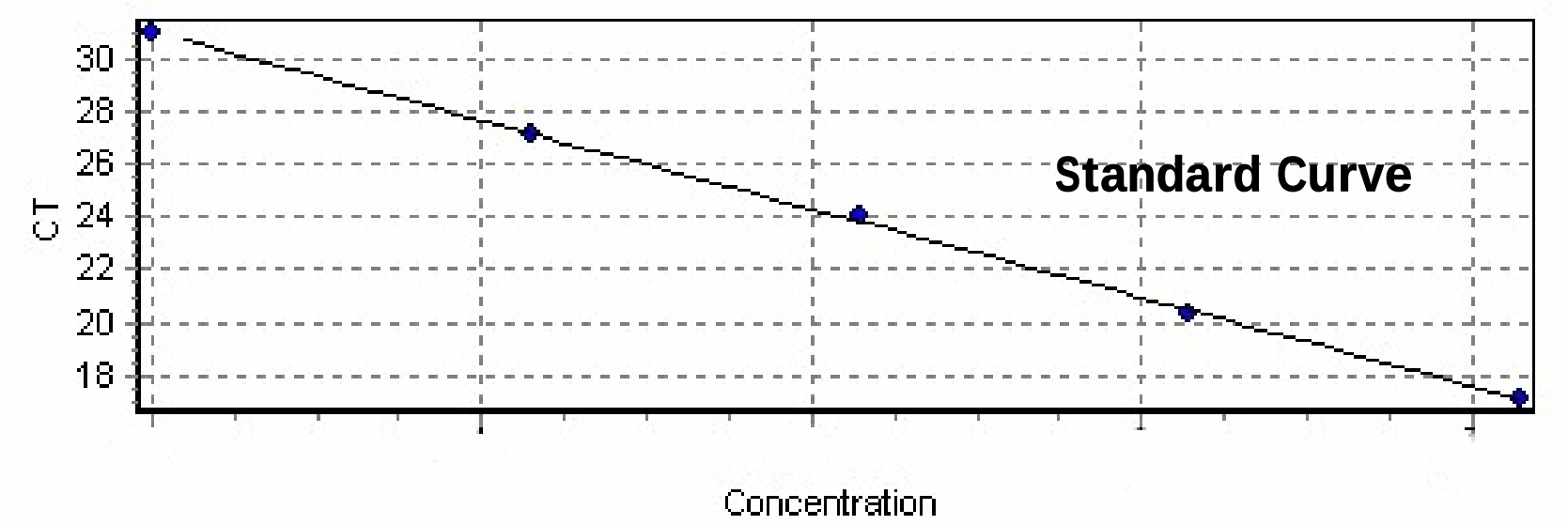
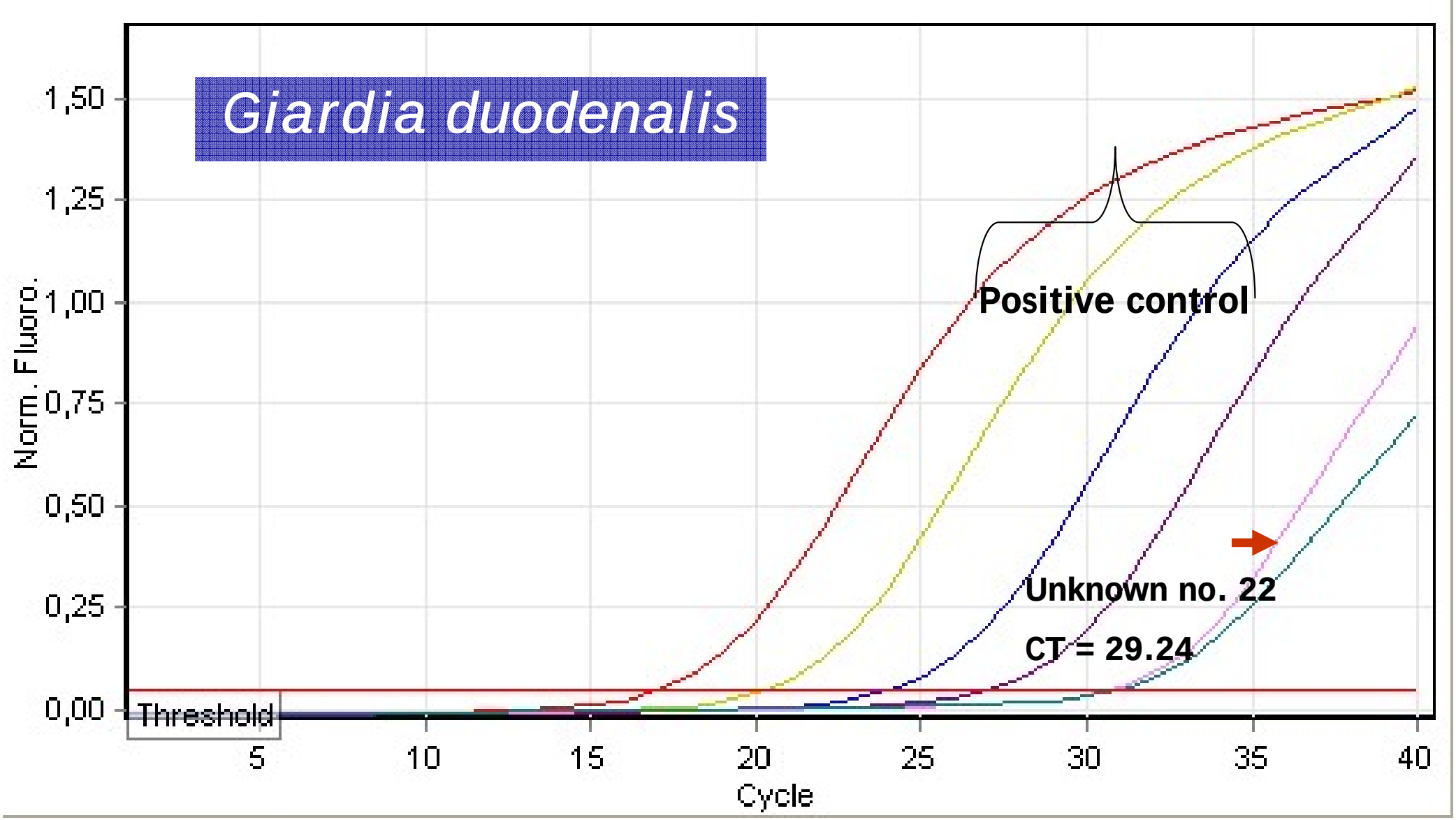
y = mx + b, or CT = m (log quantity) + b ; m = slope, b = y-intercept



y = -3.276 x + 29.692; r = 0.999, E= 102%


Giardia duodenalis
Positive control
Unknown no. 22
CT = 29.24
Threshold
Norm. Fluoro.
1,50
1,25
1,00
0,75
0,50
0,25
0,00
5
10
15
20
25
30
35
40
Cycle

Giardia duodenalis

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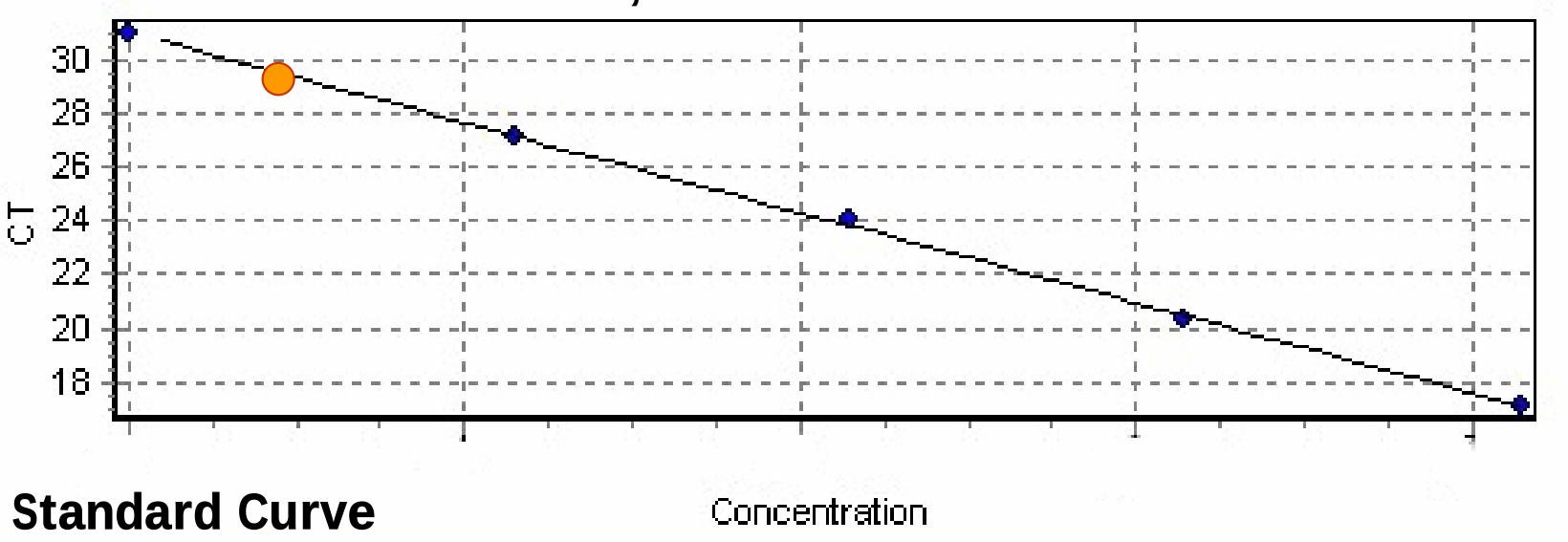
Unknown no. 22; CT = 29.24



Standard Curve

$$y = -3.276\ x + 29.692$$

$$29.24 = -3.276\ \log x + 29.692$$

$$x = 1.34\ \text{pg}/\mu\text{l}$$

[unknown no.22] = 1.34 pg/μl

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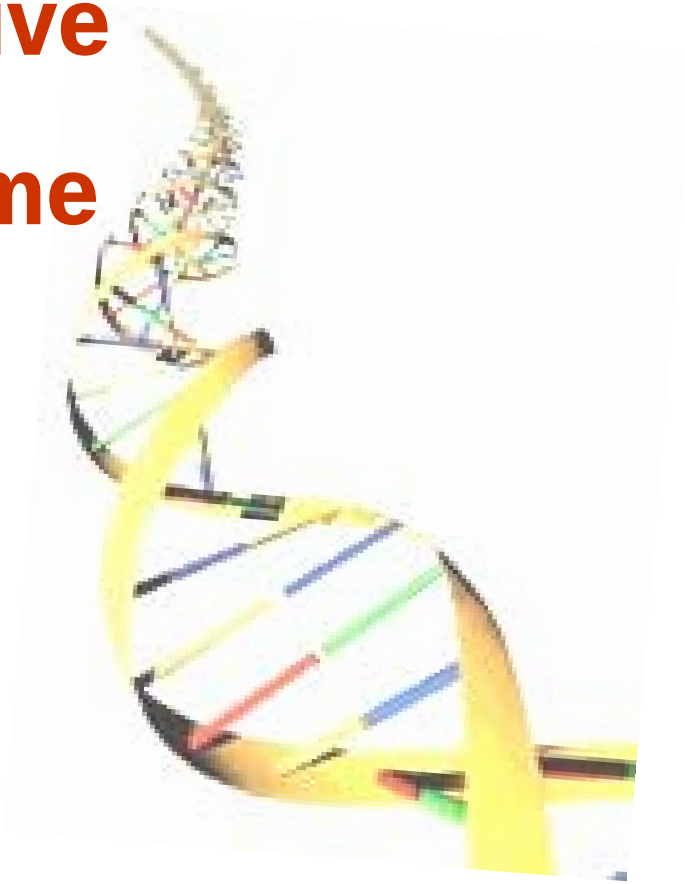
Advantages of Real-Time PCR

- High sensitivity
- Qualitative & Quantitative
- Reduced experiment time
- Reduced contamination



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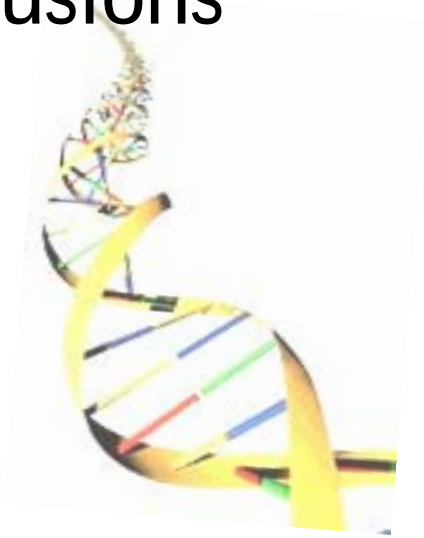
Limitation of Real-Time PCR

- Expensive & requires specialized instrumentation

- Not inherent in the technology but rather resides in human error: improper assay development, incorrect data analysis, or unwarranted conclusions




Thank You