

SKML-parasitologie rondzendingen; tijd voor moleculaire diagnostiek?

Theo Schuurs, moleculair bioloog

Lid namens WMDI / NVMM

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Stichting Kwaliteitsbewaking
Medische Laboratoriumdiagnostiek

Sectie Parasitologie

Huidige rondzendingen SKML-parasitologie:

- 4x per jaar rondzending t.b.v. detectie darm en/of bloedparasieten
- 2 feces en 2 bloedmonsters (gefixeerd materiaal)
- 92 participerende labs (2010)
 - > 79 beide panels
 - > 9 bloed panel
 - > 4 feces panel
- materiaal niet geschikt voor Mdx



Aanbieders kwaliteits-rondzendingen:

	parasieten	gefixeerd
- UK NEQAS	ja	ja
- QCMD	nee	-
- Instand	nee	-
- SKML-par	ja	ja

Momenteel geen rondzendingen
beschikbaar voor Mdx



Doel: panel uitbreiden om kwaliteit moleculaire diagnostiek laboratoria te testen

Voorwaarden:

- pathogeen in feces
 - > geen gevriesdroogd materiaal
 - > geen DNA
 - > niet gefixeerd
- uitdagende loads
- vragenlijst gebruikte methoden



Molecular diagnostics of gastroenteritis in clinical samples; a multicenter quality control study in The Netherlands

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Materials and Methods

Panel composition

Monster nr	Salmonella (Ct)	Campylobacter (Ct)	Giardia (Ct)	Cryptosporidium (Ct)
1	33	ud	30	ud
2	33	27	30	ud
3	27	33	30	ud
4	ud	33	30	ud
5	ud	ud	30	ud
6	30	ud	33	ud
7	30	ud	33	27
8	30	ud	27	33
9	30	ud	ud	33
10	30	ud	ud	ud
11	ud	ud	ud	ud

Nr. of participants: 20

Bacteria only: 3

Parasites only: 8

Both type of pathogens: 11

Results

Performance of participating laboratories (2)

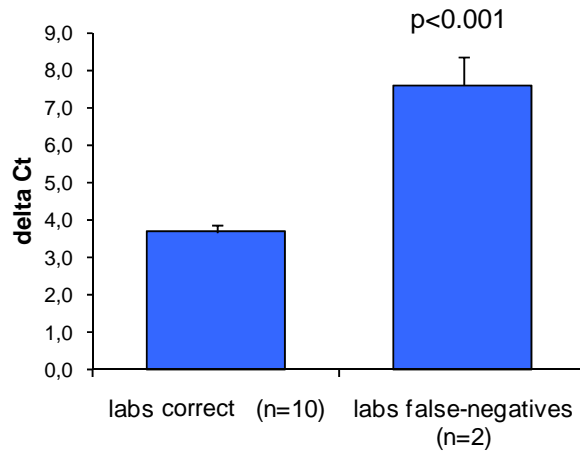
sample nr	Salmonella enterica (12 labs participating)			Campylobacter jejuni (12 labs participating)			Giardia lamblia (17 labs participating)			Cryptosporidium parvum (17 labs participating)		
	expected result	average Ct-values ¹⁾	incorrect result by participant nr.	expected result	average Ct-values ¹⁾	incorrect result by participant nr.	expected result	average Ct-values ¹⁾	incorrect result by participant nr.	expected result	average Ct-values ¹⁾	incorrect result by participant nr.
09-A-13	N			N			N			N		
09-A-20	N			N			N			N		
09-A-08	N			N			P	30,2		N		
09-A-21	N		15	N		15	P	30,4	15	N		15
09-A-07	N			P	37,3	21;25;28;35	P	30,7		N		22
09-A-18	N		20	P	37,2	20; 18;21;25;28;35	P	30,8		N		
09-A-03	P	28,8		N			N		16	P	30,9	
09-A-06	P	28,7		N		20	N		16	P	30,8	
09-A-02	P	33,1		N			N			N		
09-A-16	P	33,3	20	N			N			N		
09-A-01	P	32,5		N			P	36,0	13;14;15;22;26;27;28	N		
09-A-17	P	32,6	18	N			P	35,2	13;14;15;22;26;27;28	N		
09-A-04	P	32,5		N			P	30,7	22	P	28,4	
09-A-10	P	32,4		N			P	30,9		P	28,8	
09-A-11	P	31,9		N			P	33,8	22;28	P	29,0	
09-A-14	P	32,2		N			P	33,6	22;28	P	28,8	
09-A-05	P	35,7	18;20	N			P	30,9		N		
09-A-09	P	35,2	18;20	N			P	30,9		N		
09-A-15	P	30,6		P	36,6	21;28	P	30,3		N		
09-A-19	P	30,6		P	36,8	21;25;28;35	P	30,4		N		
09-A-12	P	35,5	18;20	P	27,5		P	30,6		N		
09-A-22	P	35,4	18;20	P	27,8	35	P	30,9		N		
total samples incorrect			12 (4,5%)			19 (7,2%)			22 (5,9%)			2 (0,53%)
false negatives			10			16			19			
false positives						1						1
result not interpretable			2			2			3			1

¹⁾ based on Ct-values of labs without false negatives;

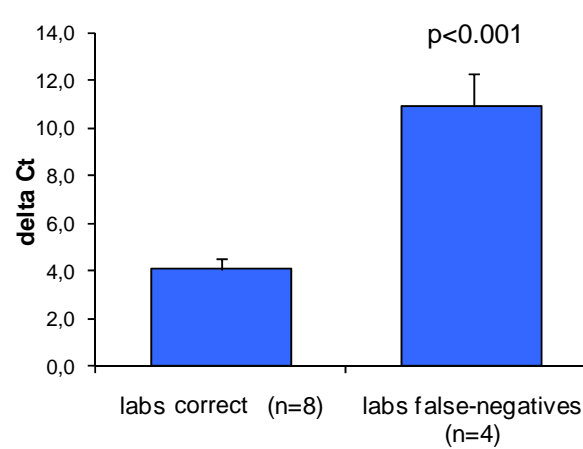
Results

Average delta Ct-values

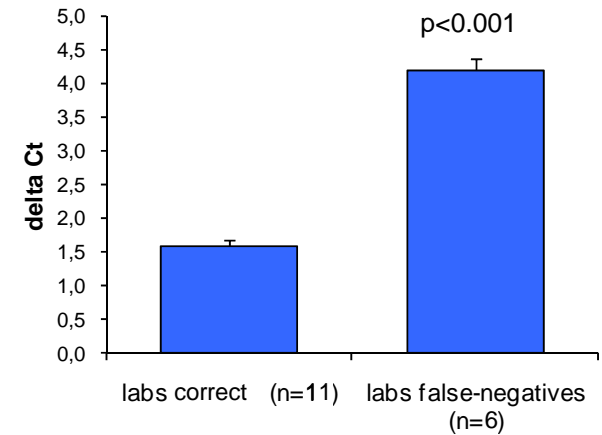
Salmonella (n=12)



Campylobacter (n=12)



Giardia (n=17)



Results

Overview on applied materials and methods for bacterial targets

		Labs false negatives (n=6)	Labs correct (n=6)	
Routine use		2 (33%)	3 (50%)	
Pre-treatment	STAR buffer (Roche)	1 (17%)	3 (50%)	
	Nucl.Lys.Bfr. (BioMerieux)	4 (66%)	2 (33%)	
	Lys.Bfr. + protK (Roche)	1 (17%)	-	
	DXL. Bfr. (Qiagen)	-	1 (17%)	
DNA Extraction Platform	MagnaPure LC (Roche)	2	1	
	EasyMag (BioMerieux)	4	3	
	Biorobot (Qiagen)		1	
	Corbet		1	
PCR platform	ABI 7500	4	4	
	LC 480	2	1	
	Rotorgene	-	1	
Fraction of sample in extraction		33%	18%	n.s (p=0.065)
Total extraction volume		758 µl	883 µl	n.s.
Fraction of sample in PCR (average)		2.3%	1.4%	n.s.

Results

Overview on applied materials and methods for parasite targets (1)

		Labs missing Giardia (n=6)	Labs correct (n=11)	
Routine use		2 (33%)	7 (64%)	
Pre-treatment	STAR buffer (Roche)	-	5 (45%)	
	Nucl.Lys.Bfr. (BioMerieux)	3 (50%)	5 (45%)	
	PVPP (Sigma)	2 (33%)	1 (9%)	
	DXL. Bfr. (Qiagen)	1 (17%)	-	
DNA Extraction Platform	MagnaPure LC (Roche)	2 (33%)	3 (27%)	
	EasyMag (BioMerieux)	3 (50%)	6 (55%)	
	Biorobot (Qiagen)	-	1 (9%)	
	Qiagen DNA mini	-	1 (9%)	
	Corbet	1 (9%)	-	
PCR platform	ABI 7500	2 (33%)	9 (81%)	
	LC 480	1 (16%)	1 (9%)	
	LC II	1 (16%)	-	
	Rotorgene	1 (16%)	-	
	Biorad CFX	1 (16%)	-	
Fraction of sample in extraction		50%	27%	n.s. (p=0.19)
Total extraction volume		767 µl	845 µl	n.s.
Fraction of sample in PCR (average)		3.5%	2.1%	n.s.

Results

Overview on applied materials and methods for parasite targets (2)

	Labs false negatives (n=6)	Labs correct (n=11)
Pre-treatment methods		
100°C PVPP; ATL protK 56°C; spin down	1	
suspend LB; 10 min 95°C; spin-down	1	
suspend LB; 30 min 80°C; 30 min 95°C; spin-down	1	
suspend LB; vortex; -20°C; 100°C; spin-down	1	
suspend LB; vortex; 95°C; spin-down; freeze	1	
100°C PVPP	1	1
unknown		1
suspend LB; vortex; spin- down		2
suspend LB; vortex; spin- down; freeze		3
suspend LB; vortex; freeze; spin-down		4

Results

DNA panel

	mean Ct-value	missed by participant
S. enterica #1	27,42	
S. enterica #2	30,99	
S. enterica #3	34,63	
S. enterica #4	38,06	11
C. jejuni #1	26,92	
C. jejuni #2	30,14	
C. jejuni #3	33,60	
C. jejuni #4	37,75	
G. lamblia #1	27,63	
G. lamblia #2	31,20	
G. lamblia #3	34,55	14
G. lamblia #4	37,37	14, 20, 22, 25, 26, 29

Results

Faeces panel combined with DNA panel

laboratory nr.	Salmonella enterica (12 labs participating)			Campylobacter jejuni (12 labs participating)			Giardia lamblia (17 labs participating)		
	faeces panel	DNA panel	likely problem	faeces panel	DNA panel	likely problem	faeces panel	DNA panel	likely problem
20			DNA isolation						
25						DNA isolation			
35						DNA isolation			
12									
13									DNA isolation
14									PCR
16									
19									
22									PCR
27									PCR
29									
11									
15									PCR**
17									
18			DNA isolation						
21						DNA isolation*			
23									
24									
26									PCR
28						DNA isolation			PCR***
nr. of problems	DNA isolation		2	DNA isolation*		4	DNA isolation		1
	PCR		-	PCR		-	PCR		6

* alternative: multiplex problem

** contradicting results

*** uncertain

Recommendations

Bacterial targets: > Optimize amount of sample input
> Optimize DNA isolation

Parasite (Giardia) target: > avoid excessive heating during sample pre-treatment but instead mix vigorously
> Optimize PCR reaction

Preliminare resultaten eerste SKML moleculaire rondzending (pilot-fase):

Monster: Giardia positief (Ct 23-28)
Dientamoebae positief (Ct 31-34)

Deelnemende laboratoria : 10 (tot 9 juni)

Resultaat:

Giardia **P**: 10 (Ct 25-35)

Dientamoebae **P**: 8 (Ct 31-34)

Blastocystis hominis **P**: 2 (Ct 30)



Samenvattend:

- Vanaf voorjaar 2011 gestart met pilot
- Daarna: aan bestaand panel, 2 extra monsters voor Mdx.
- Wensen/behoefte vanuit het veld mede bepalend voor inrichten Mdx-panel

