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Uw ziekenhuis.

Critically Appraised Topic

Syndromic testing for gastroenteritis: are conventional diagnostic methods ancient history?

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Gastroenteritis

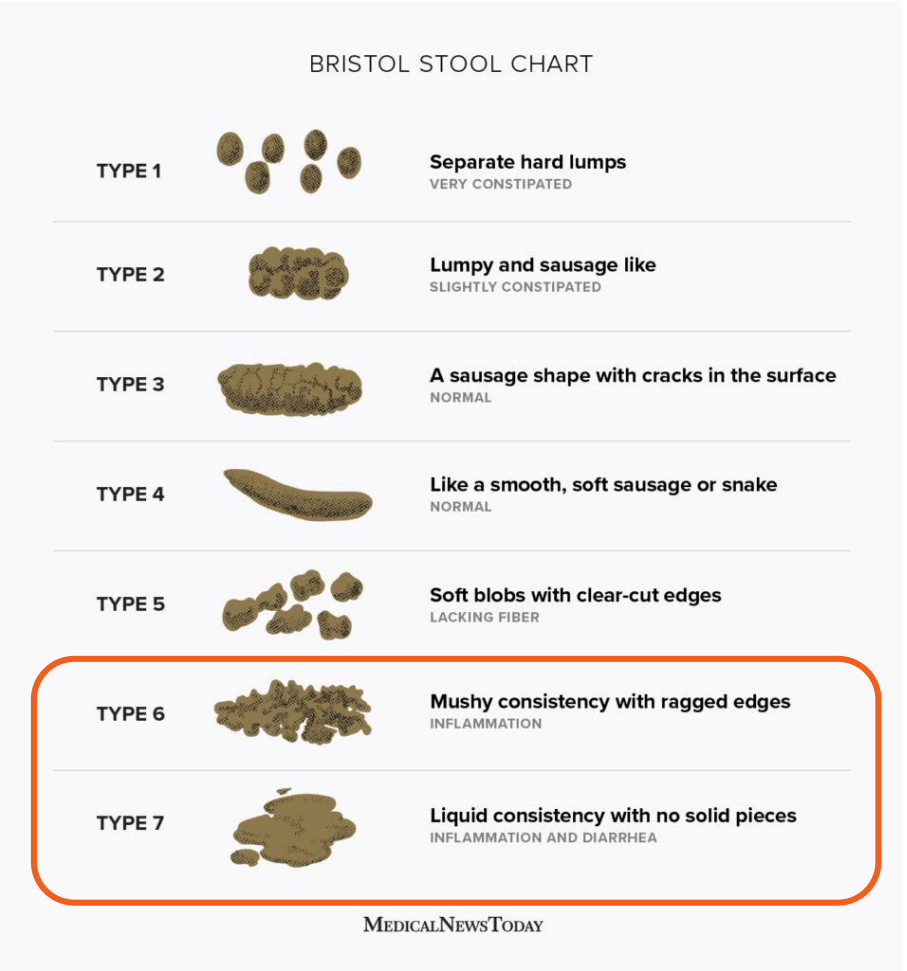
Buikpijn, krampen en diarree (+/- koorts)

10 miljoen gevallen per jaar (België) → 28.000 hospitalisaties

Voedselvergiftigingen of dicht contact met besmette personen
- **Viraal**
- Bacterieel
- Parasitair

Zelf-limiterend

Richtlijnen testen in geval van:
- Aanhoudend (>7 dagen)
- Acuut (<7 dagen)
  - Ernstig
  - Risicofactoren
    - Ouderen
    - Immuungecompromitteerden



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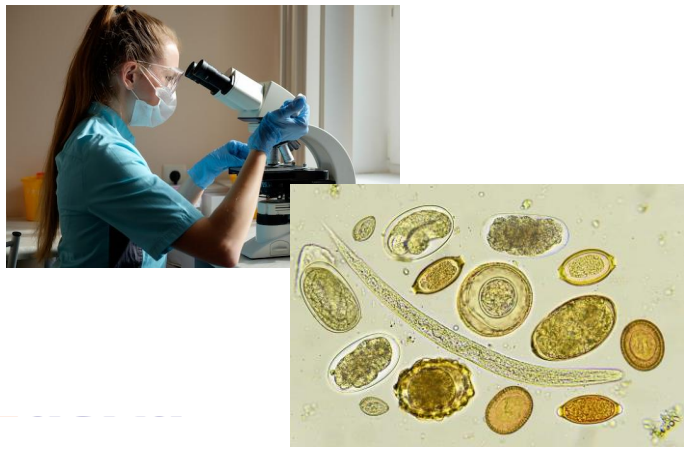
Workflow in AZ Delta

Allplex™
Gastrointestinal Panel Assays

Aeromonas spp.
Campylobacter spp.
Salmonella spp.
Shigella spp./EIEC
Yersinia spp.
Vibrio spp.



C. difficile

Allplex™ GI-Bacteria(II) Assay

- EAEC[1] (*aggR*)
- EPEC[2] (*eaeA*)
- *Escherichia coli* O157 (*E. coli* O157)
- ETEC[3] (*lt/st*)
- Hypervirulent *Clostridium difficile* (CD hyper)
- STEC[4] (*stx1/2*)
- Internal Control (IC)

Allplex™ GI-Bacteria(I) Assay

- *Aeromonas* spp. (Aer)
- *Campylobacter* spp. (Cam)
- *Clostridium difficile* toxin B (CdB)
- *Salmonella* spp. (Sal)
- *Shigella* spp./EIEC[5] (Sh/EI)
- *Vibrio* spp. (Vib)
- *Yersinia enterocolitica* (Yer)
- Internal Control (IC)

Allplex™ GI-Parasite Assay

- *Blastocystis hominis* (BH)
- *Cryptosporidium* spp. (CR)
- *Cyclospora cayetanensis* (CC)
- *Dientamoeba fragilis* (DF)
- *Entamoeba histolytica* (EH)
- *Giardia lamblia* (GL)
- Internal Control (IC)

Allplex™ GI-Virus Assay

- Adenovirus (AdV)
- Astrovirus (AstV)
- Norovirus GI (NoV-GI)
- Norovirus GII (NoV-GII)
- Rotavirus (RotV)
- Sapovirus (SV)
- Internal Control (IC)

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Voor- en nadelen van PCR

Lage detectiegrens

Veel sneller dan klassieke cultuur

Hoge throughput mogelijk

Simultane detectie meerdere pathogenen

Hoge mate van automatisatie mogelijk

Onafhankelijk van uitvoerder

Complexe diagnostiek en interpretatie

Geen oplossing voor antibiogram

Je vindt enkel wat in het panel zit

Aanwezigheid ≠ infectie

Contaminatie mogelijk

Kwalitatieve rapportering

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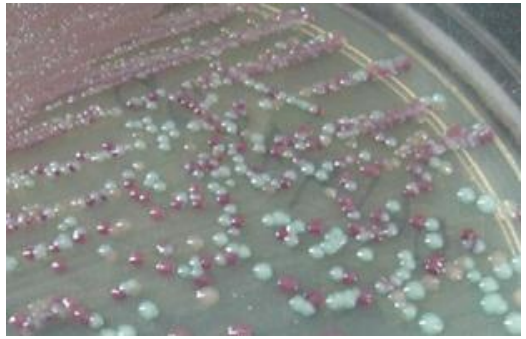
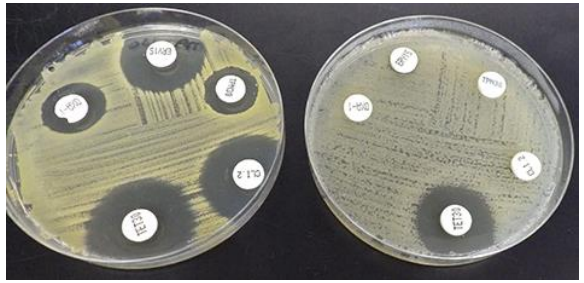
Conventionele testwijze

Dag 1

Dag 2



Dag 3



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Conventionele testwijze

Beperkt aantal pathogenen
Omslachtig
Tijdrovend
Beperkte gevoeligheid
Arbeidsintensief
Klinisch beeld: bacterieel, viraal, parasitair, niet-infectieus?

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VRAAG 1

IN WELKE MATE KOMT HET AANVRAAGPROFIEL, DAT GEBASEERD OP IS KLINISCHE PRESENTATIE, OVEREEN MET DE RESULTATEN VAN EEN MULTIPLEX PCR?

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Literatuur

Aanvraagprofiel

In 25-65% oorzakelijke kiem gemist in geval van 'physician-requested testing'

Conventionele methoden vs. PCR

→ High overall agreement
Moleculaire methoden
Aantal ⇧ (tot 2x meer)
Gemengde infecties ⇧

Microscopie parasieten lage gevoeligheid t.o.v. PCR

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Algemeen

1238 stalen

Conventionele methoden

267 positief (21.6%)

281 pathogenen

Multiplex PCR

511 positief (41.3%)

832 pathogenen

Table 1 An overview of the requested tests (conventional methods) compared to the FTD Gastrointestinal panel and both its positivity rates

Pathogen	Conventional methods		FTD Gastrointestinal Panel	
	No. samples	No. positive	No. samples	No. positive
Bacteria	1222	49 (4.1%)	1238	70 (5.6%)
Campylobacter		29		38
Campylobacter spp.		1		-
Campylobacter coli		2		-
Campylobacter jejuni		26		-
Salmonella spp.		9		10
Shigella spp.		0		0
STEC		7		11
Yersinia spp.		5		11
Viruses	1009	195 (19.5%)	1238	563 (45.5%)
Adenovirus	334	105		187
Astrovirus	42*	-		58
Norovirus	378	75		161
Norovirus GI				34
Norovirus GII				127
Rotavirus	297	15		41
Sapovirus	96*	-		116
Parasites	434**	36 (8.3%)	1238	51 (4.1%)
Cryptosporidium spp.		24		36
Entamoeba histolytica		0		0
Giardia lamblia		12		15
Clostridioides difficile	451	35 (7.8%)	1238	148 (11.9%)

* Amount of times adeno-, noro- and/or rotavirus was requested

**404 requests for Combined Rapid Antigen Test (Cryptosporidium and Giardia lamblia) and 28 for direct microscopic examination

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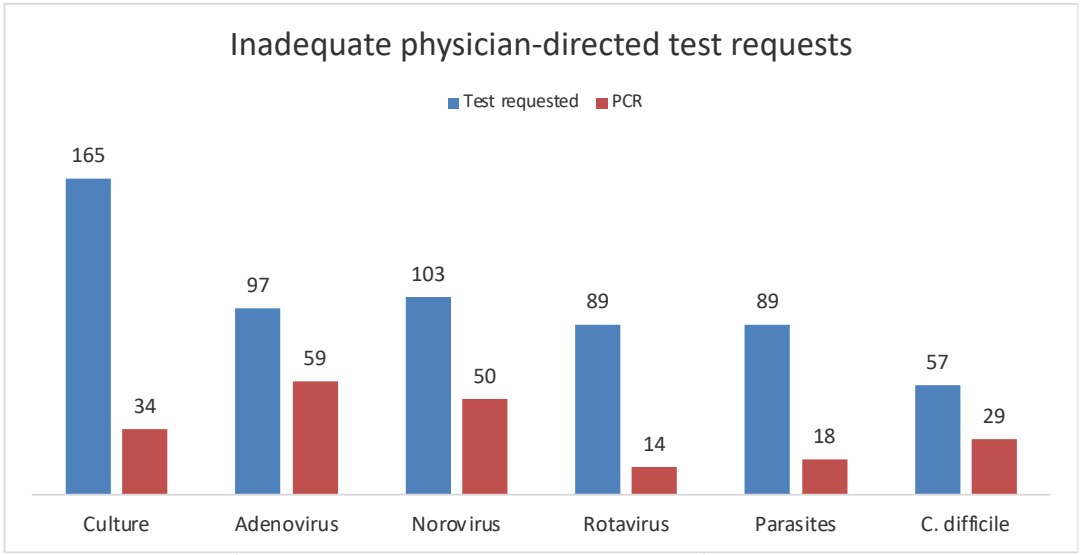
Aanvraagprofiel

Succesvol (36.6%, n=187)

Adequaat (n=22)

Overshooting (**n=165**)
≥ 3 testen



Niet succesvol (32.3%, n=165)

Exclusie 20 stalen *C. difficile* <2 jaar

Profiel matcht niet met PCR (**n=106**)

Profiel matcht gedeeltelijk (n=39)

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Cultuur vs. PCR

Table 3 Stool culture vs. PCR (bacteria)

	FTD positive	Stool culture positive	Stool culture negative
***Campylobacter* spp.**	38	29 (76.0%)	9 (24.0%)
***Salmonella* spp.**	10	9 (90.0%)	1 (10.0%)
***Yersinia* spp.**	11	5 (45.5%)	6 (54.5%)
STEC	11	7* (63.6%)	4 (36.4%)
Total	70	50 (71.4%)	20 (28.6%)

*Seven *E. coli* isolates were obtained by culture. Only 3 of these were confirmed as being a *Shiga toxin-producing E. coli* strain. Two samples were negative for Shiga toxins and for two samples no confirmation was received.

18/20 stalen Ct >30

2/20 stalen Ct 20 – 30

- Yersinia spp.
- STEC

Hoge sensitiviteit
Geen bacteriën geïsoleerd die niet in panel zitten

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Virus antigen vs. PCR

Virale mono-infectie **178** stalen
1. **Norovirus** genotype I en II (n=60)
2. **Adenovirus** (n=44)
3. **Sapovirus** (n=33)

Table 4 Viral aetiology suspected based on clinical presentation when a single viral pathogen was identified by FTD

	Adenovirus (n=44)	Norovi…	Rotavirus (n=12)
Clinical suspicion of specific viral aetiology	19 (43.2%)		5 (41.7%)
No viral suspicion	23 (52.3%)		6 (50.0%)

Geen vals positieve sneltesten

Table 5 Comparison between results acquired by physician-reques… and FTD (viral)

	FTD positive	Rapid antigen test positive	Rapid antigen test negative	Rapid antigen test not requested
Adenovirus	187	107 (56.1%)	17 (9.1%)	63 (34.8%)
Norovirus GI	34	11 (32.4%)	1 (2.9%)	22 (64.7%)
Norovirus GII	127	64 (50.4%)	1 (0.8%)	62 (48.8%)
Norovirus I & II	161	75 (46.6%)	2 (1.2%)	84 (52.2%)
Rotavirus	41	15 (36.6%)	7 (17.1%)*	19 (46.3%)
Total	389	197 (50.6%)	26 (6.7%)	166 (42.7%)

*Rotavirus rapid antigen test was not performed in 5 cases as the patient was older than 2 years, however 2 of them had a Ct value <20 and 3 had a Ct value >30 (FTD).

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Aanvraagprofiel

Table 2 Viral ordering profile in case of positive astrovirus or sapovirus by FTD multiplex

	Astrovirus	Sapovirus
All three pathogens	32/58 (55%)	68/116 (59%)
Two pathogens		
Adeno- and norovirus	2/58 (3.4%)	8/116 (6.9%)
Adeno- and rotavirus	3/58 (5.2%)	14/116 (12.1%)
Noro- and rotavirus	1/58 (1.7%)	0
One pathogen		
Adenovirus	3 (5.2%)	2 (1.7%)
Norovirus	1 (1.7%)	4 (3.4%)
Rotavirus	0	0
No viral pathogen request	16/58 (27.6%)	20/116 (17.2%)

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Parasieten sneltest/microscopie

Geen vals positieve sneltesten

Table 6 Comparison of results acquired by physician-requested [illegible] FTD (protozoa)

	FTD positive	Rapid antigen test positive	Rapid antigen test negative	Rapid antigen test not requested
***Cryptosporidium* spp.**	36	24* (66.7%)	0	11 (30.6%)
Giardia lamblia	15	12 (80.0%)	0	3 (20.0%)
Total	51	36 (70.6%)		14 (27.5%)

*One sample was a follow-up sample after therapy initiation. The rapid antigen test was not executed.

Microscopie 2/3 *Cyptosporidium* spp. gemist
(Ct <20 en 20-30)

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Besluit

Aanvraagprofiel

Slechts 36.6% van de aanvragen **succesvol**

↓

88.2% overshooting!

Gericht aanvragen = inadequaat

Klinische presentatie

Conventionele testen niet beschikbaar

→ Onderdiagnosticering

28.6% van bacteriën niet geïsoleerd

42.7% virussen niet aangevraagd

27.5% parasieten niet aangevraagd

40.0% microscopie niet gevonden

Astrovirus en sapovirus

Conventionele methoden hebben geen extra pathogenen opgeleverd in vergelijking met PCR

↓

2 keer meer positieve stalen en pathogenen

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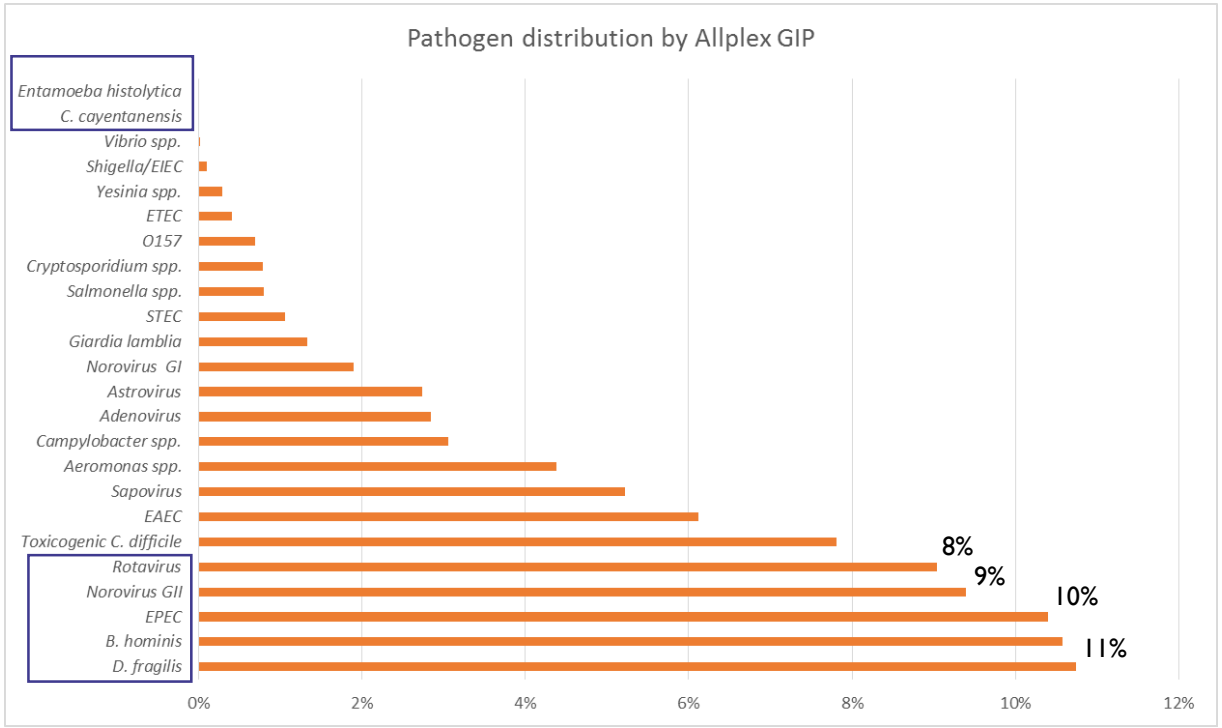
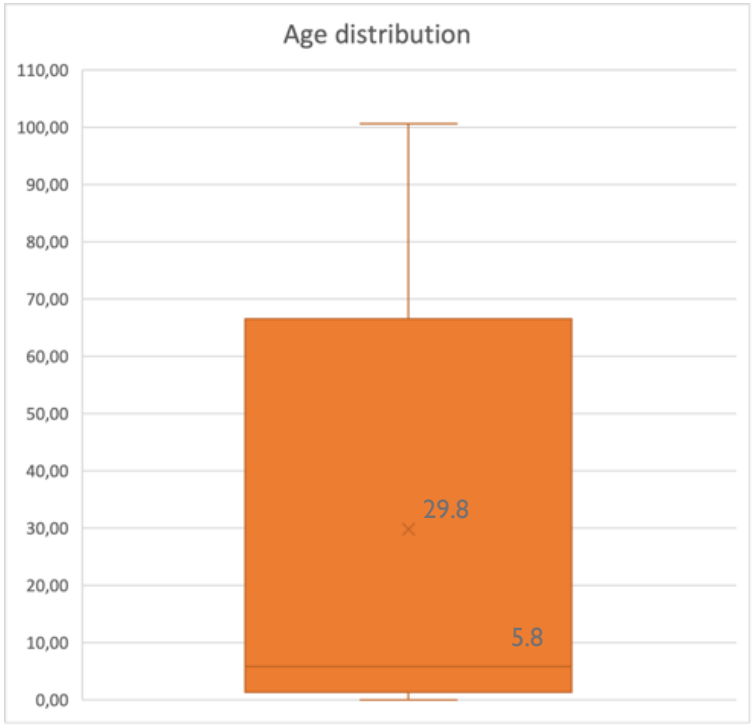
VRAAG 2

HOE KUNNEN DE RESULTATEN GEGENEREERD DOOR SEEGENE SEMI-KWANTITATIEF GERAPPORTEERD WORDEN?

Algemeen

8442 stalen → **4527** (53.6%) positief
7676 pathogenen





Algemeen

Mono-infectie 54.9%

Blastocystis hominis (n=448)
Dientamoeba fragilis (n=302)
Rotavirus (n=261)

Co-infectie 45.1%

Enteropathogene *E. coli* (n=638)
Norovirus genotype II (n=571)
Dientamoeba fragilis (n=594)

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Table 9 Total number of Seegene Allplex Gastrointestinal panel detections by age group, pathogens and co-detection

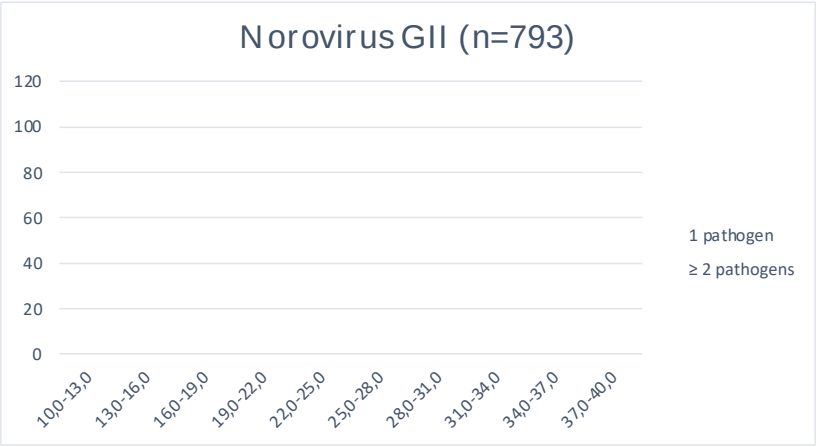
Pathogen	No (pos. ratio)	No. associated with co-infections (%)	Total no./no. associated with co-infections					
			< 1 year (n=748)	1-5 years (n=1529)	6-12 years (n=274)	13-20 years (n=109)	21-60 years (n=577)	> 60 years (n=1290)
Bacteria	**3067 (36.6%)**							
Aeromonas spp.	470 (5.6%)	331 (**70.4%**)	136/109	126/109	15/13	8/4	50/29	135/67
Campylobacter spp.	258 (3.1%)	152 (65.5%)	19/16	54/47	13/9	19/10	65/27	88/46
Clostridioides difficile	658* (7.8%)	431 (65.5%)	270/206	184/161	0/0	2/1	45/19	156/42
E. coli								
EAEC	517 (6.1%)	393 (**76.0%**)	111/96	280/241	7/5	5/3	38/24	76/27
EPEC	878 (10.4%)	638 (**72.7%**)	159/137	342/295	33/24	10/8	115/73	219/111
ETEC	35 (0.4%)	21 (60.0%)	3/3	8/8	2/2	0/0	8/5	14/4
STEC	90 (1.1%)	70 (77.8%)	8/8	16/14	5/5	5/3	16/12	40/31
O157**	59 (0.7%)	52 (88.1%)	10/8	12/12	2/2	3/3	9/7	24/22
EIEC/*Shigella* spp.	9 (0.1%)	5 (55.6%)	0/0	1/0	0/0	2/1	6/4	0/0
Salmonella spp.	68 (0.8%)	31 (45.6%)	11/5	19/10	12/5	5/2	6/5	15/4
Yersinia spp.	25 (0.3%)	14 (56.0%)	1/1	7/4	0/0	0/0	3/2	14/8
Vibrio spp.	2 (0.0%)	2 (100%)	0/0	1/1	0/0	0/0	1/1	0/0
Viruses	**2628 (31.1%)**							
Adenovirus	240 (2.8%)	194 (**80.8%**)	86/68	129/107	4/4	3/3	8/7	10/7
Astrovirus	231 (2.7%)	172 (74.5%)	65/50	137/110	4/0	1/1	13/9	11/5
Norovirus genotype I	160 (1.9%)	127 (**79.4%**)	26/25	86/77	5/5	3/2	9/7	31/11
Norovirus genotype II	793 (9.4%)	571 (**72.0%**)	220/173	403/306	20/15	10/6	69/36	71/35
Rotavirus	763 (9.0%)	497 (65.1%)	161/105	335/284	2/2	25/23	55/31	165/57
Sapovirus	441 (5.2%)	292 (66.2%)	140/100	268/179	9/6	1/0	15/5	8/3
Parasites	**1979 (23.4%)**							
Blastocystis hominis	893 (10.6%)	438 (49.0%)	4/4	101/92	89/68	48/31	185/96	466/154
Cyclospora cayentanensis	0 (0.0%)							
Cryptosporidium spp.	67 (0.8%)	44 (65.7%)	3/2	38/28	8/5	3/1	13/7	2/1
Dientamoeba fragilis	907 (10.7%)	594 (65.5%)	18/10	449/331	176/107	53/34	101/57	110/66
Entamoeba histolytica	0 (0.0%)							
Giardia lamblia	112 (1.3%)	77 (68.8%)	3/3	48/43	9/7	2/1	24/11	26/12

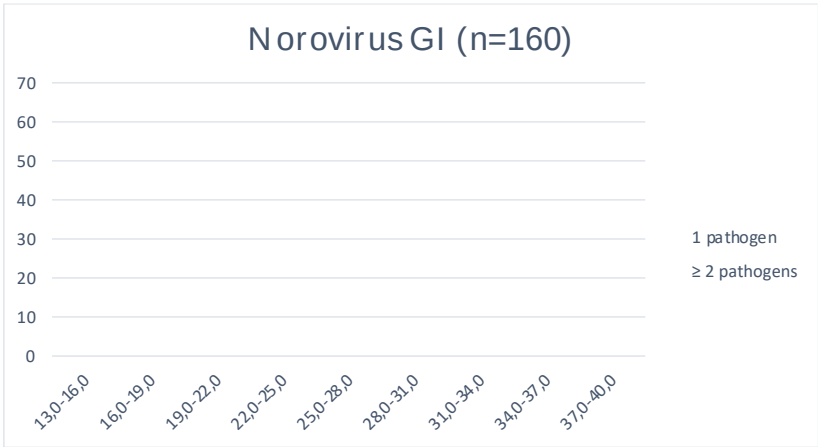
Semi-kwantitatieve rapportering

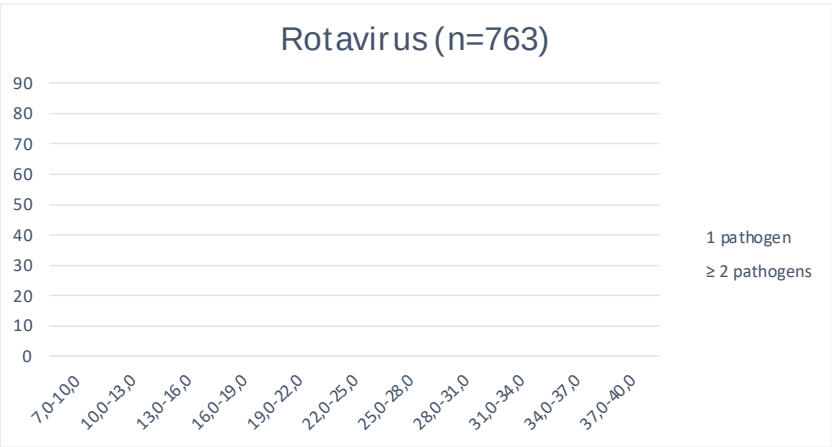
Distributie van Ct waarden voor **elk viraal pathogeen** → semi-kwantitatieve rapportering

Cut-off Ct waarde van 35

1. Viraal hoge Ct waarden in geval van co-infectie
2. Observatie en confirmatie van vals positieven (Ct >35)
3. Technisch aspect







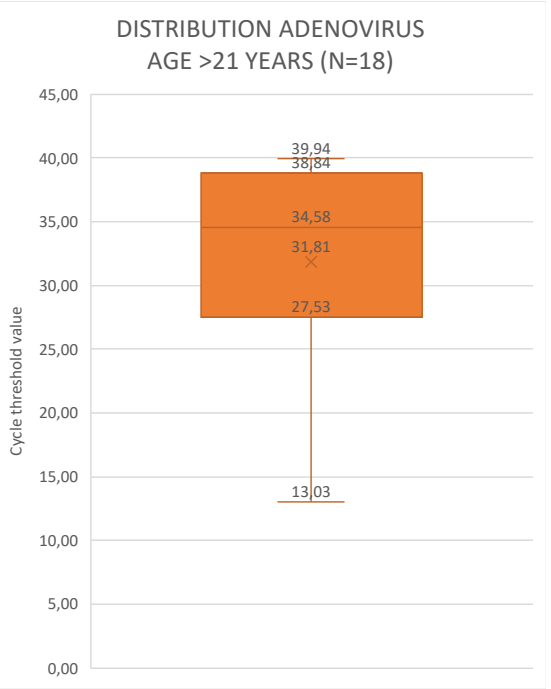
Cut-off waarde (Ct 35)

1. Hoge Ct waarden in geval van co-infectie

Table 10 Median Ct values of different viruses in both single and co-infections and in different patient populations

	Median Ct value single infection	Median Ct value co-infection	Median Ct value ≤ 5 years	Median Ct value >21 years
Adenovirus	16.89	19.71	**16.45**	**34.58**
Astrovirus	18.47	18.62	17.63	21.21
Norovirus				
Genotype I	**25.45**	**36.67**	36.04	29.47
Genotype II	**21.96**	**26.00**	24.35	28.21
Rotavirus	16.43	15.18	15.33	16.75
Sapovirus	24.46	25.63	24.90	28.01

Significant hogere Ct waarde in co-infecties



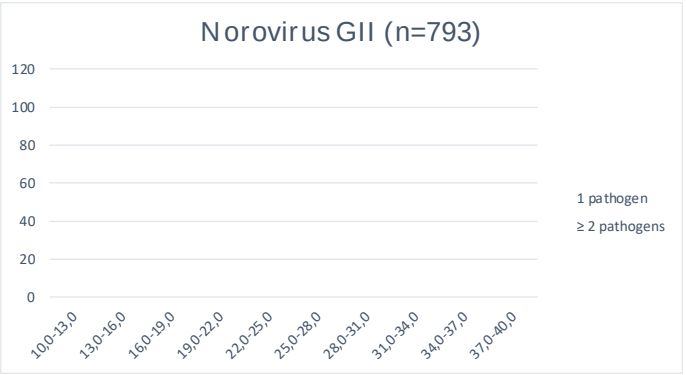
n=18

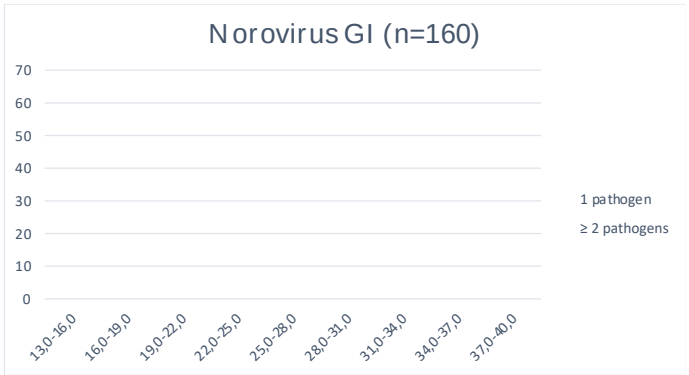
14/18 co-infectie

11/14 met pathogeen Ct <30

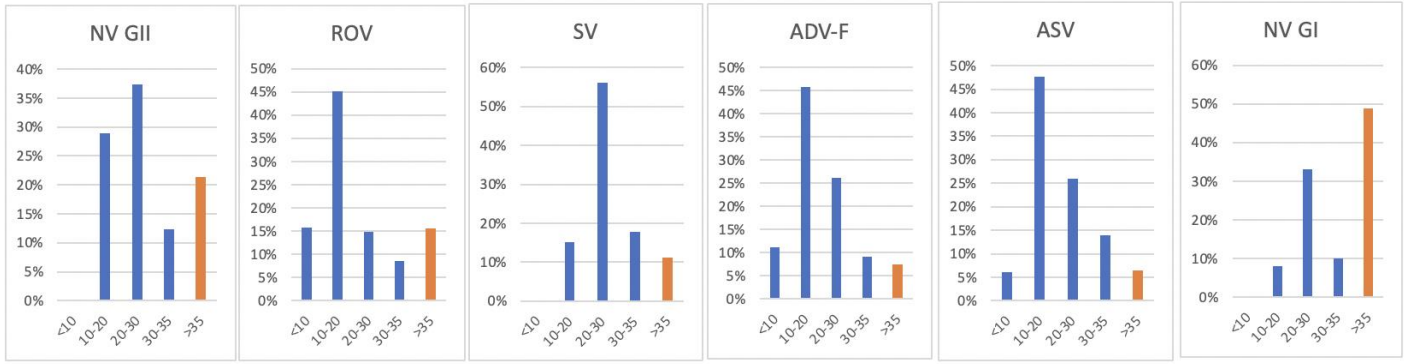
4/18 mono-infectie

1/4 Ct <35 (13.68)

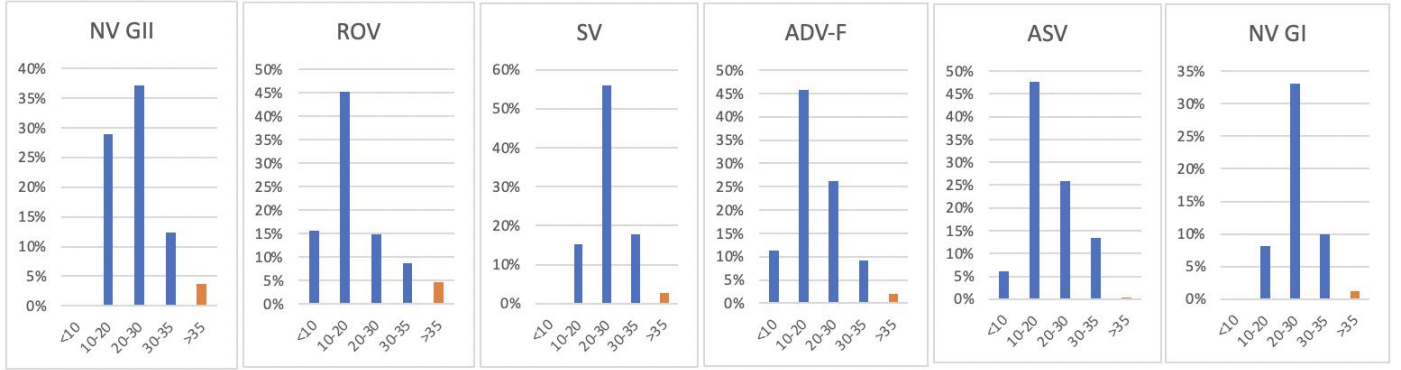




Co-infectie met een pathogeen met Ct waarde <35



	NV GII		ROV		SV		ADV-F		ASV		NV GI	
<10	0	0.0%	120	15.7%	0	0,0%	27	11.3%	14	6.1%	0	0.0%
10-20	229	28.9%	345	45.2%	67	15.2%	110	45.8%	110	47.6%	13	8.1%
20-30	296	37.3%	113	14.8%	247	56.0%	63	26.3%	60	25.9%	53	33.1%
30-35	98	12.4%	66	8.7%	78	17.7%	22	9.2%	32	13.8%	16	10.0%
>35	170	21.4%	119	15.6%	49	11.2%	18	7.5%	15	6.5%	78	48.8%
No other pathogen	29	**3.7%**	36	**4.72%**	12	**2.7%**	5	**2.1%**	1	**0.4%**	2	**1.3%**



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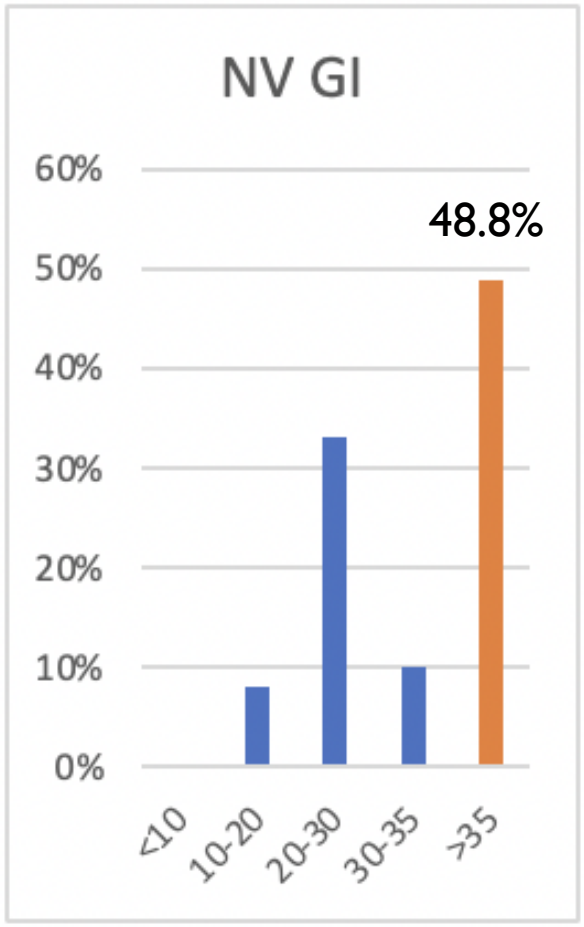
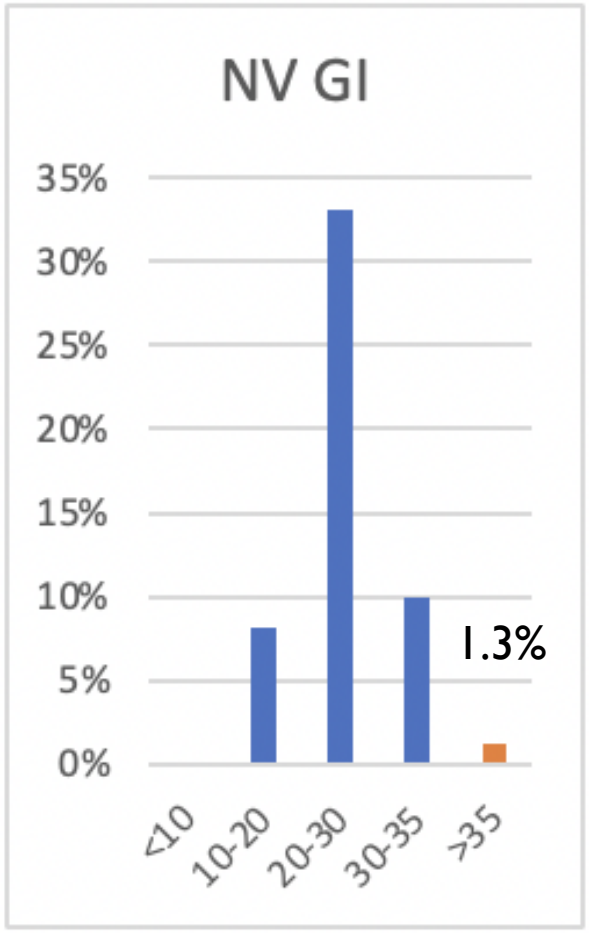


Exclusie virale pathogenen met co-detectie met pathogeen Ct waarde <35



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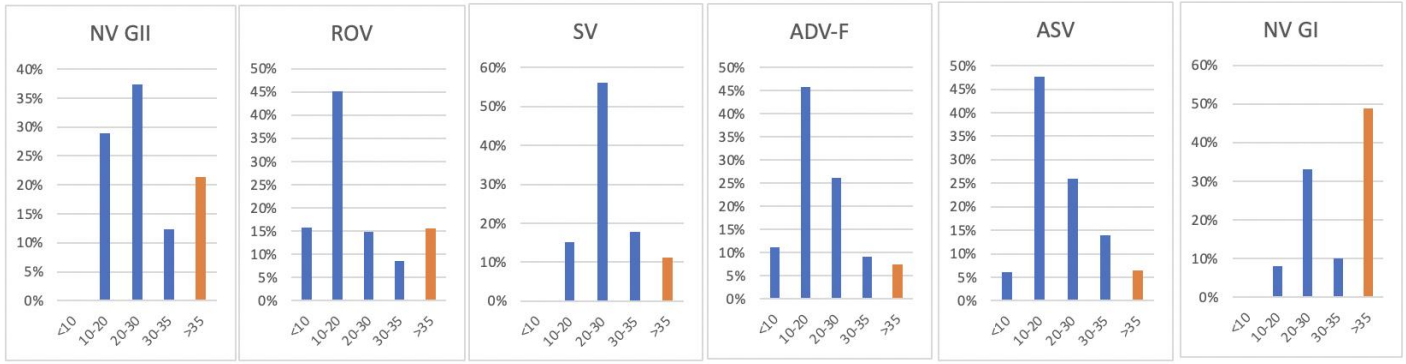
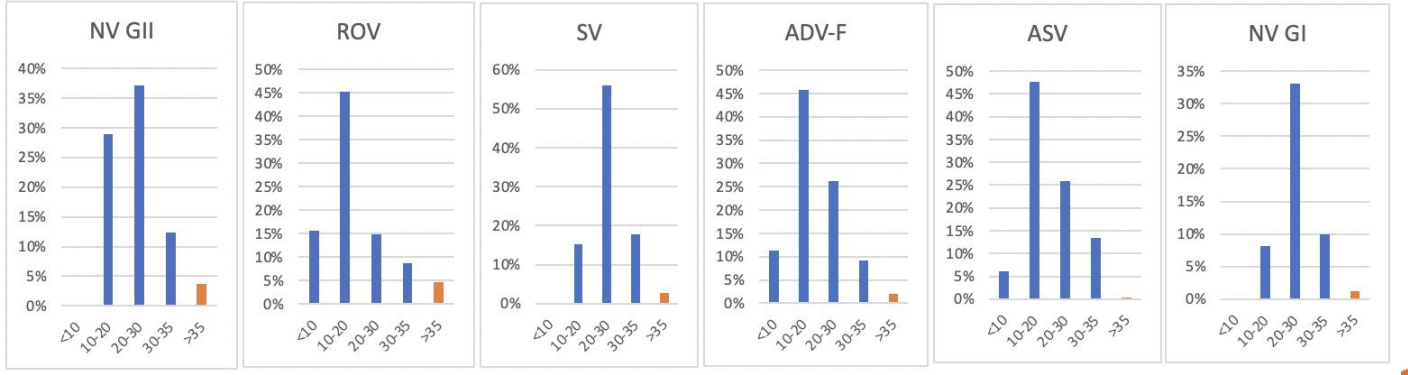
Co-infectie met een pathogeen met Ct waarde <35



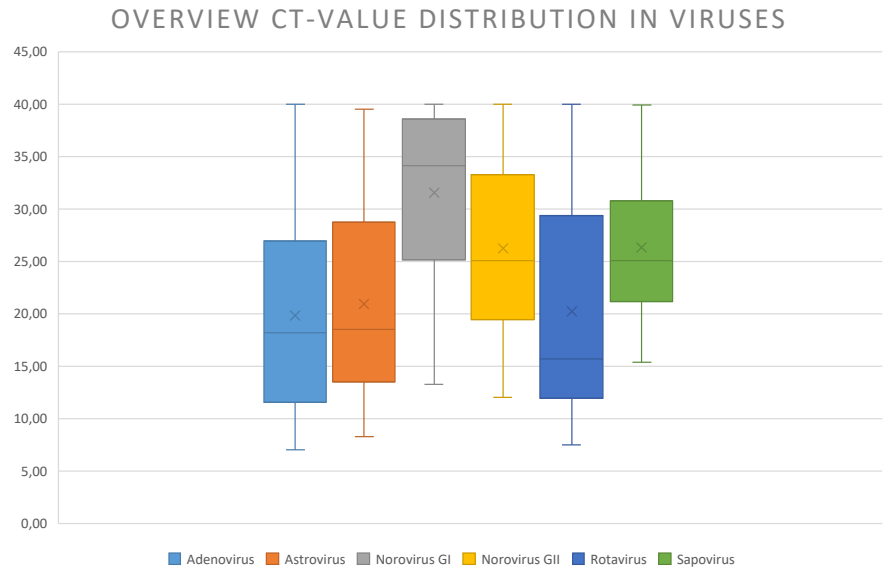
	NV GII		ROV		SV		ADV-F		ASV		NV GI	
<10	0	0.0%	120	15.7%	0	0,0%	27	11.3%	14	6.1%	0	0.0%
10-20	229	28.9%	345	45.2%	67	15.2%	110	45.8%	110	47.6%	13	8.1%
20-30	296	37.3%	113	14.8%	247	56.0%	63	26.3%	60	25.9%	53	33.1%
30-35	98	12.4%	66	8.7%	78	17.7%	22	9.2%	32	13.8%	16	10.0%
>35	170	21.4%	119	15.6%	49	11.2%	18	7.5%	15	6.5%	78	48.8%
No other pathogen	29	**3.7%**	36	**4.72%**	12	**2.7%**	5	**2.1%**	1	**0.4%**	2	**1.3%**



Bepalen van cut-offs

	25th percentile	50th percentile	75th percentile
Norovirus GII	18.31	22.14	27.52
Norovirus GI	21.03	25.50	29.24
Adenovirus	11.12	16.34	23.84
Astrovirus	13.39	17.12	27.71
Rotavirus	10.94	14.36	21.26
Sapovirus	20.91	24.25	23.84

	Very strong positive	Strong positive	Positive	Weak positive
Norovirus GII	<18	≥18 en <22	≥22 en <28	≥28 en < 35
Norovirus GI	<21	≥21 en <26	≥26 en <29	≥29 en <35
Adenovirus	<11	≥11 en <16	≥16 en <29	≥29 en <35
Astrovirus	<14	≥14 en <17	≥17 en <28	≥28 en <35
Rotavirus	<11	≥11 en <14	≥14 en <21	≥21 en <35
Sapovirus	<21	≥21 en <24	≥24 en <29	≥29 en <35



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Cut-off waarde (Ct 35)

2. Observatie en confirmatie van vals positieve resultaten

Well	Name	Type	FAM	C(t)	FAM	C(t)	HEX	C(t)	Cal Red 610	C(t)	Cal Red 610	C(t)	Quasar 670	C(t)	Quasar 670	C(t)	HEX	C(t)	Auto Interpretation
A01	2051611801	SAMPLE	ASV	C(t)	NVG2	C(t)	ADV-F	C(t)	SV	C(t)	NVG1	C(t)	ROV	C(t)		C(t)	IC	C(t)	"ADV-F,NVG1,ROV"
			-	N/A	-	N/A	+	"39,97"	-	N/A	+	"39,89"	+	"8,09"			+	"26,89"	
A06	2051611801		Sh/El	C(t)	Cam	C(t)	Yer	C(t)	Vib	C(t)	CdB	C(t)	Aer	C(t)	Sal	C(t)	IC	C(t)	
			-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	"31,16"	
B02	2051981301	SAMPLE	ASV	C(t)	NVG2	C(t)	ADV-F	C(t)	SV	C(t)	NVG1	C(t)	ROV	C(t)		C(t)	IC	C(t)	"NVG2,SV,NVG1,ROV"
			-	N/A	+	"39,52"	-	N/A	+	"30,98"	+	"39,51"	+	"9,51"			+	"26,75"	
B07	2051981301		Sh/El	C(t)	Cam	C(t)	Yer	C(t)	Vib	C(t)	CdB	C(t)	Aer	C(t)	Sal	C(t)	IC	C(t)	
			-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	"31,47"	
C01	2051794701	SAMPLE	ASV	C(t)	NVG2	C(t)	ADV-F	C(t)	SV	C(t)	NVG1	C(t)	ROV	C(t)		C(t)	IC	C(t)	"SV,NVG1,CdB"
			-	N/A	-	N/A	-	N/A	+	"20,99"	+	"37,75"	-	N/A			+	"28,27"	
C06	2051794701		Sh/El	C(t)	Cam	C(t)	Yer	C(t)	Vib	C(t)	CdB	C(t)	Aer	C(t)	Sal	C(t)	IC	C(t)	
			-	N/A	-	N/A	-	N/A	-	N/A	+	"30,58"	-	N/A	-	N/A	+	"30,59"	
D02	2052037301	SAMPLE	ASV	C(t)	NVG2	C(t)	ADV-F	C(t)	SV	C(t)	NVG1	C(t)	ROV	C(t)		C(t)	IC	C(t)	"NVG2,ROV,CdB"
			-	N/A	+	"35,43"	-	N/A	-	N/A	-	N/A	+	"14,22"			+	"26,71"	
D07	2052037301		Sh/El	C(t)	Cam	C(t)	Yer	C(t)	Vib	C(t)	CdB	C(t)	Aer	C(t)	Sal	C(t)	IC	C(t)	
			-	N/A	-	N/A	-	N/A	-	N/A	+	"40,90"	-	N/A	-	N/A	+	"30,86"	
G03	2052129501	SAMPLE	ASV	C(t)	NVG2	C(t)	ADV-F	C(t)	SV	C(t)	NVG1	C(t)	ROV	C(t)		C(t)	IC	C(t)	"NVG2,NVG1,ROV"
			-	N/A	+	"37,77"	-	N/A	-	N/A	+	"17,02"	+	"38,97"			+	"27,30"	
G08	2052129501		Sh/El	C(t)	Cam	C(t)	Yer	C(t)	Vib	C(t)	CdB	C(t)	Aer	C(t)	Sal	C(t)	IC	C(t)	
			-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	"32,06"	

Niet gedetecteerd bij herhaling met GI-TAC assay

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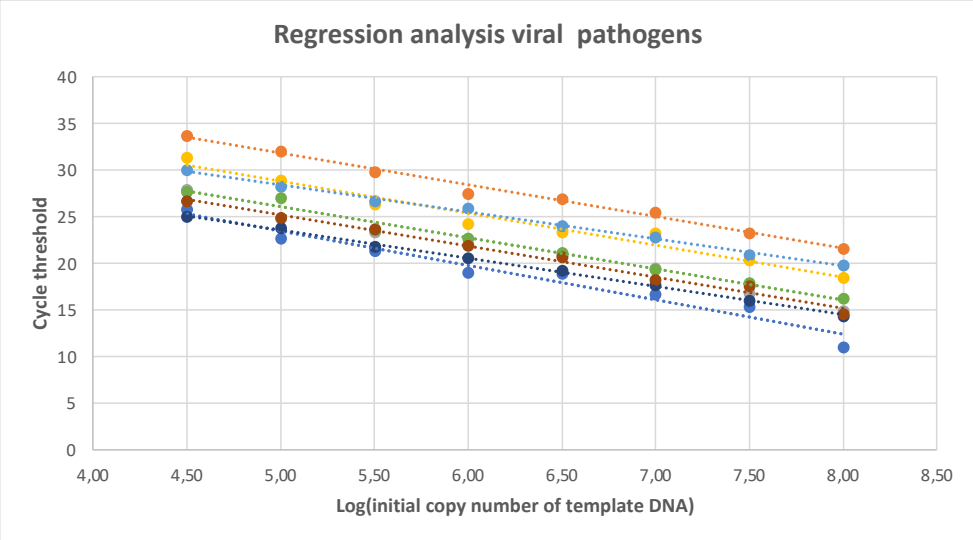
Virale co-infecties

8 stalen met sterk positief signaal voor één virus

0.5 log dilutie (=1.66 Ct ⇧)

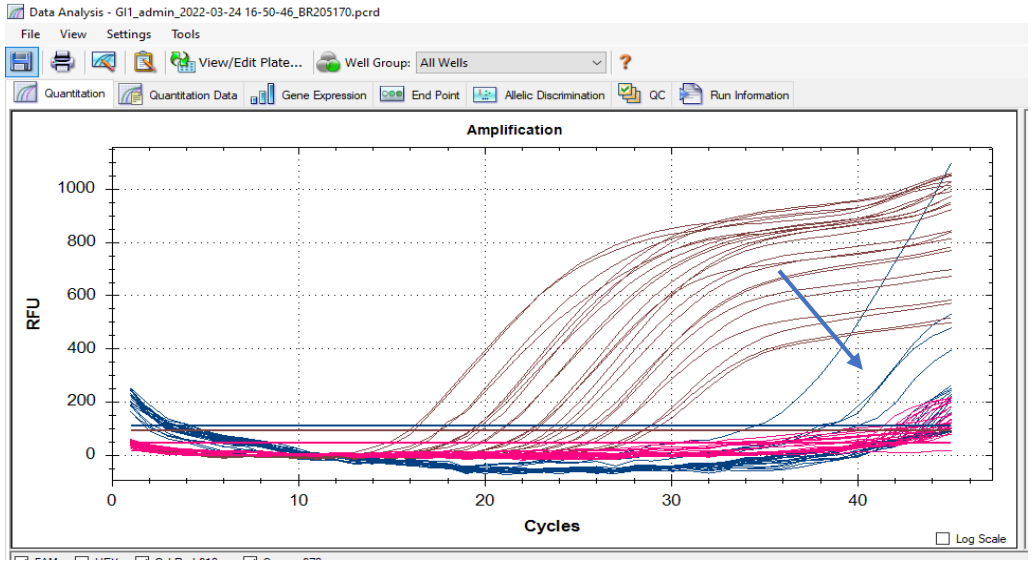


Efficiëntie van 87% tot 114%
Rico's van -3.67 tot -2.89

Extra positieve signalen

Astrovirus → norovirus GII (Ct 39.65)
→ rotavirus (Ct 38.81)
Astrovirus → sapovirus (Ct 37.91)

Rotavirus (5e dilutie) → norovirus GII (Ct 38.38)



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Cut-off waarde (Ct 35)

3. <u>Technisch aspect</u>

Minimale detectie van 100 kopie/ml staal → Ct tussen 35-40

↓

2,5 kopieën/25µl

Besluit

Cut-off Ct waarde 35 → rapportering co-infecties ⇩

Semi-kwantitatieve cut-offs zijn **verschillend per pathogeen**

PCR is niet foutloos!

→ **Geen causaal verband** aantonen
Hoge Ct in combinatie met lage Ct → Exclusie van oorzakelijke kiem met hoge waarschijnlijkheid

Ct is hoog of laag in een populatie met gastroenteritis
Ct als **proxy voor probabiliteit** (dus waarschijnlijk/minder waarschijnlijk als causatieve kiem) in **co-infecties**

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To do

1. Bepalen van semi-kwantitatieve cut-off waarden voor parasieten en bacteriën
2. Dezelfde analyse doen met andere multiplex panels om ook daar semi-kwantitatief rapporteren mogelijk te maken
3. Positiviteitsratio's en Ct waarden vergelijken met een asymptomatische groep om een cut-off te kunnen stellen tussen klinisch relevant en klinisch irrelevant

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Vragen?

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