

Critically appraised topic: Goodbye culture, hello PCR? Implementation of a molecular diagnostic panel for enteropathogens in routine faeces diagnostics

Dr. Jan Van Elslande – ASO klinische biologie 4^e jaar

Cultuur en/of PCR?



Type 6 Mushy consistency with ragged edges



Type 7 Liquid consistency with no solid pieces



Inhoud

- **Introductie**
- Question 1) literatuur?
- Question 2) voor-nadelen PCR vs cultuur?
- Question 3) hoe beslissen welk panel?
- Question 4) evaluatie data voor-en na implementatie
- Question 5) toekomst?

Acute gastro-enteritis



- Frequent
 - 1.500.000.000 x/jaar
- Impact
 - Ontwikkelingslanden: kindermortaliteit
 - België: morbiditeit en kost
- Etiologie
 - Viraal
 - Bacterieel ← Deze CAT (excl. *C. difficile*)
 - (Parasitair)



Type 1 Separate hard lumps	SEVERE CONSTIPATION
Type 2 Lumpy and sausage like	MILD CONSTIPATION
Type 3 A sausage shape with cracks in the surface	NORMAL
Type 4 Like a smooth, soft sausage or snake	NORMAL
Type 5 Soft blobs with clear-cut edges	LACKING FIBRE
Type 6 Mushy consistency with ragged edges	MILD DIARRHEA
Type 7 Liquid consistency with no solid pieces	SEVERE DIARRHEA

WHO: “≥3x/dag”



Farthing M, Salam MA, Lindberg G, et al. Acute diarrhea in adults and children: A global perspective. *J Clin Gastroenterol* 2013; 47:12–20. Available at: https://journals.lww.com/jcge/Fulltext/2013/01000/Acute_Diarrhea_in_Adults_and_Children__A_Global.7.aspx. Accessed 28 December 2021.

Shane AL, Mody RK, Crump JA, et al. 2017 Infectious Diseases Society of America Clinical Practice Guidelines for the Diagnosis and Management of Infectious Diarrhea. *Clin Infect Dis* 2017; 65:e45–e80. Available at: <https://pubmed.ncbi.nlm.nih.gov/29053792/>. Accessed 28 December 2021.

ESCMID. European Manual of Clinical Microbiology. 2012.

Lal A, Hales S, French N, Baker MG. Seasonality in Human Zoonotic Enteric Diseases: A Systematic Review. *PLoS One* 2012; 7. Available at: [/pmc/articles/PMC3317665/](https://pmc/articles/PMC3317665/). Accessed 23 January 2022.

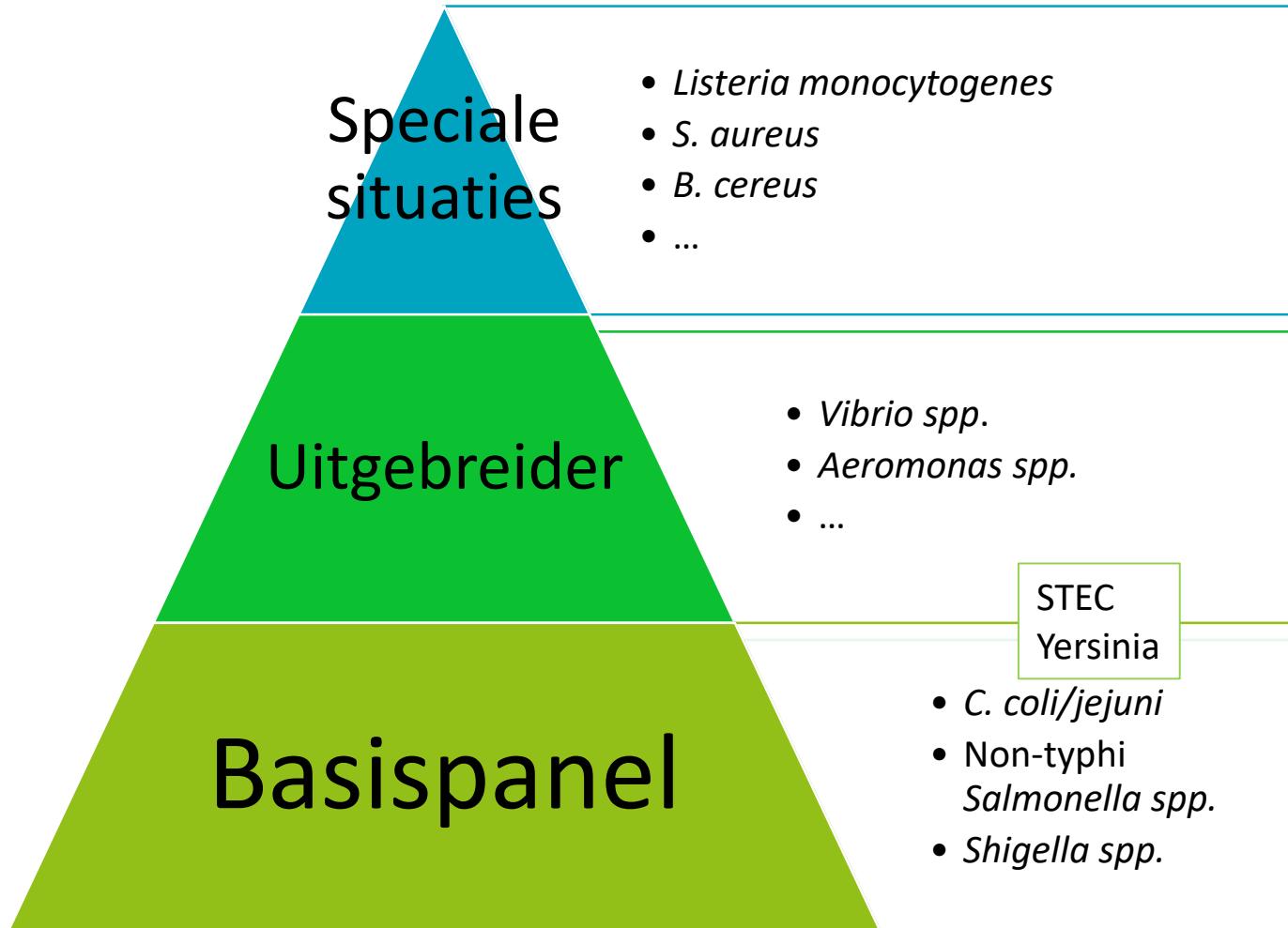
Wetenschappelijk Instituut Volksgezondheid. Voedsel- en watergerelateerde infectieziekten - Epidemiologische surveillance in België. 2017. Available at: <https://epidemio.wiv-isp.be/ID/reports/Voedsel- en watergerelateerde infectieziekten - Epidemiologie - Jaarrapport 2015-2016.pdf>.

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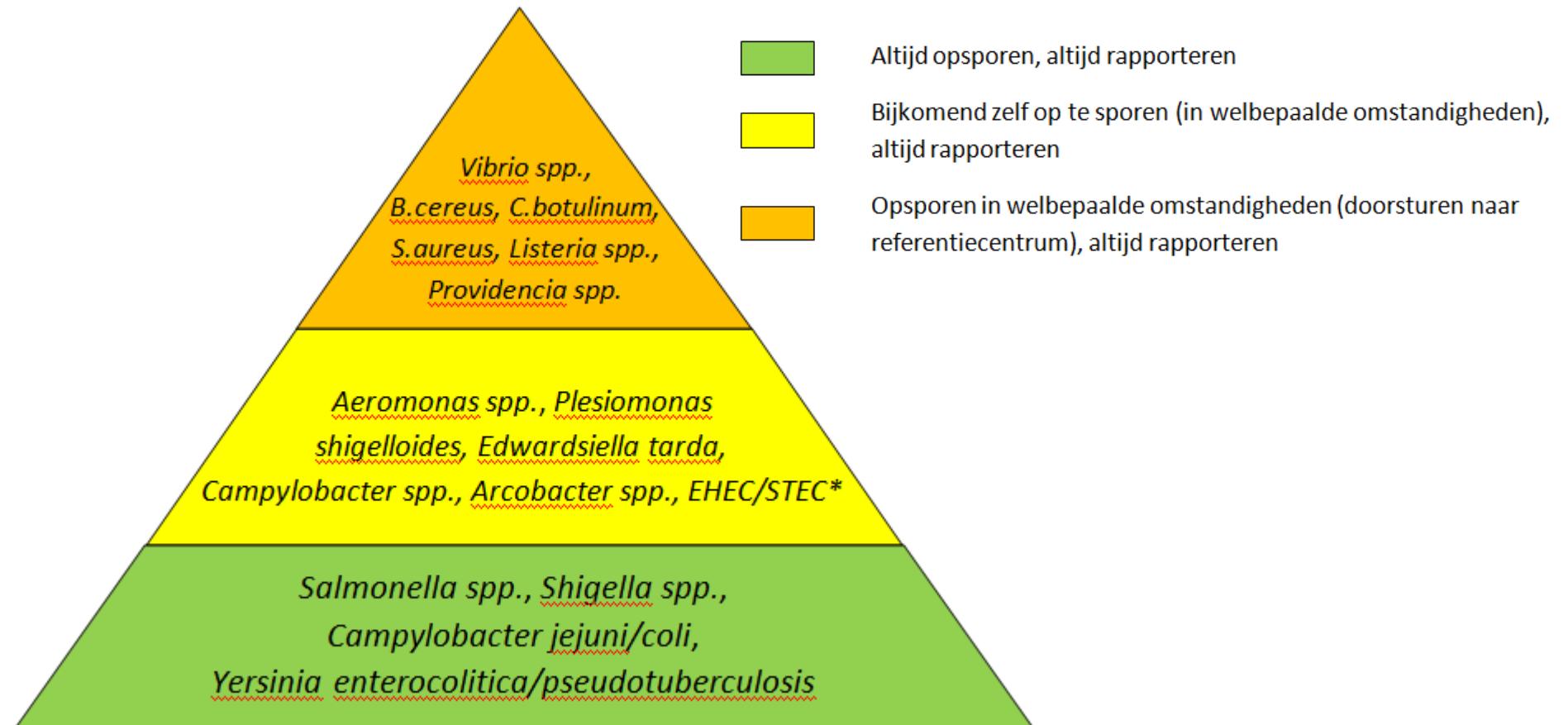
Literatuur aanbevelingen

- **Testindicatie** ~behandelingsindicatie
 - Ernstig/risicofactoren
 - Uitbraak
- Getrapte aanpak
 - “Basis”
 - Uitgebreider
- Onduidelijk
 - 3d gehospitaliseerd?
 - Vaste stoelgang?



RIZIV: "549835 549846 Kweek die tenminste het opzoeken van Salmonella, Shigella, Yersinia en Campylobacter omvat, met identificatie van de kiemen in faeces"

Voorbeeld: BILULU consensus 2017



*Op te sporen bij (anamnese van) bloederige stoelgang of HUS.

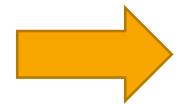
In geval van sterk klinisch vermoeden bijkomend doorsturen naar het referentielaboratorium

Inhoud

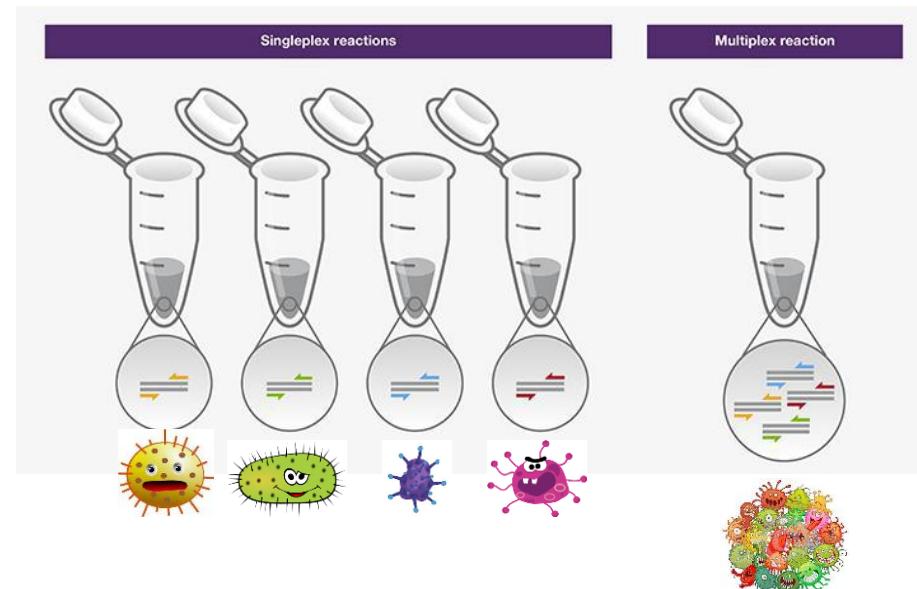
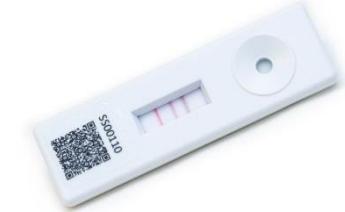
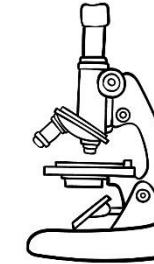
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Diagnostiek

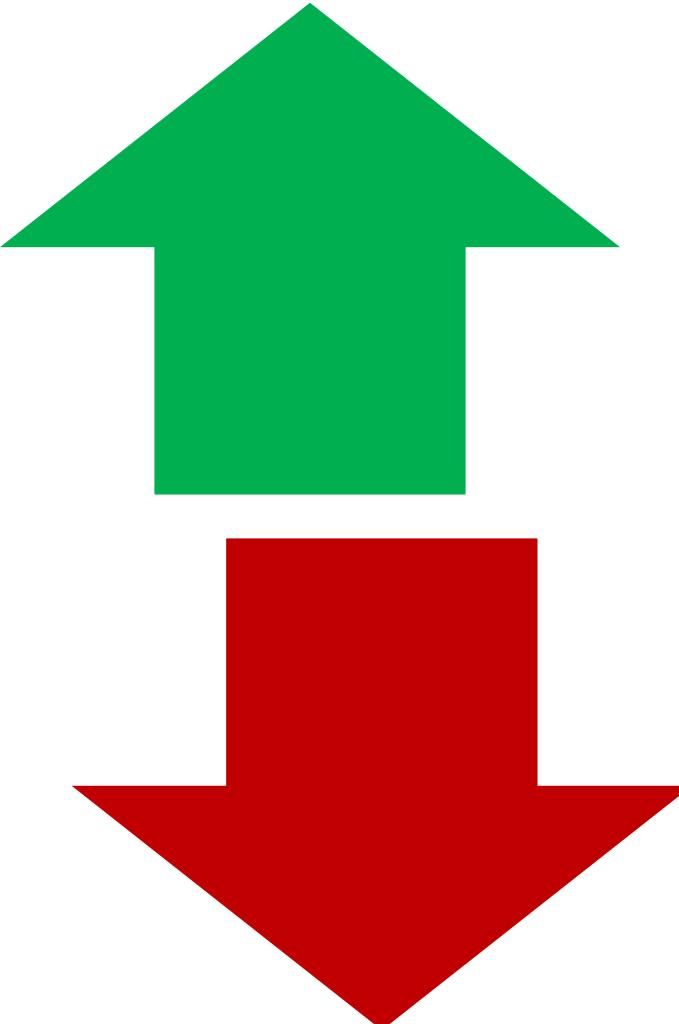
- “Traditioneel”
 - Cultuur
 - Microscopie
 - Sneltesten
- PCR
 - Single-target – multiplex



- Arbeidsintensief
- Lage opbrengst (2-4%)



Literatuur

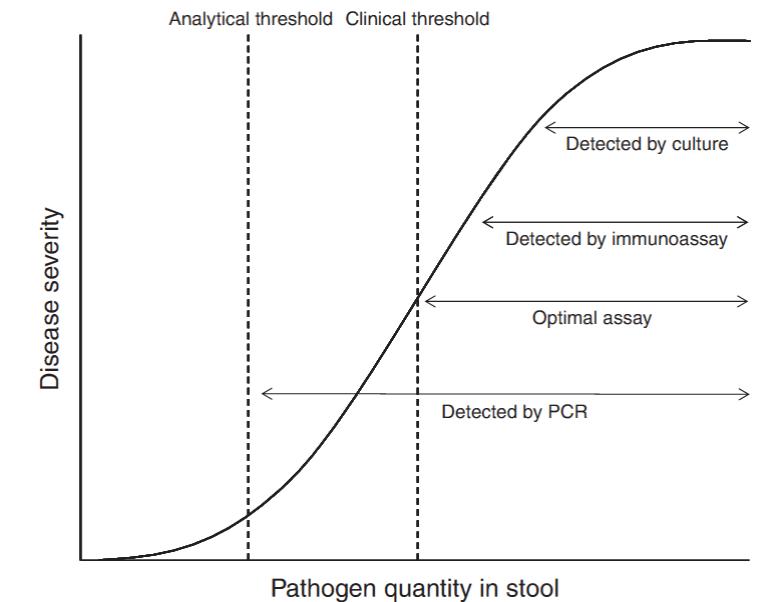


Voordelen PCR

- Kortere turnaround time
- Hogere gevoeligheid
- Minder arbeidsintensief
- Gestandardiseerd

Beperkingen PCR

- Dure toestellen & reagentia
- Limitatieve lijst micro-organismen
- Geen typering van stam
- Geen gevoelighedsbepaling
- Meer interpretatiemoeilijkheden



Review Mucosal Immunology (2013) 6, 876–885; doi:10.1038/mi.2013.50

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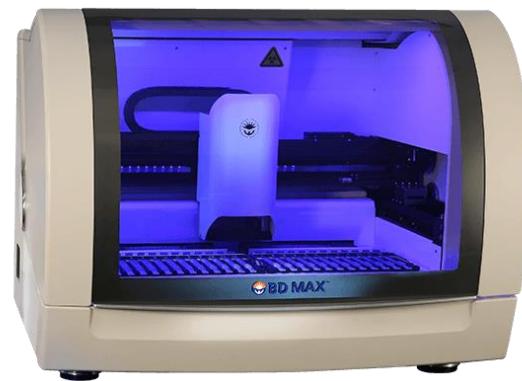
Commercieel beschikbare panels

- CLART Enterobac (Genomica)
- Verigene II (Luminex)
- QIAstat DX GI panel (Qiagen)
- NxTAG GI pathogen panel (Luminex)
- BioFire Filmarray GI panel (Biomérieux)
- Allplex GI panels (Seegene, Accuramed)
- Gastrofinder 2SMART (PathoFinder)
- EntericBio (Serosep, Mediphos)
- AmpliCub (Mikrogen)
- RIDAGENE (R-Biopharm)
- ...

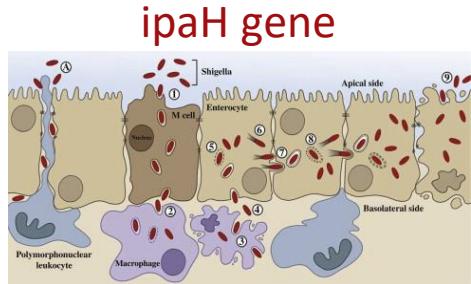


Commerciële PCR panels

- CE-IVD gelabeld
- Verschillen qua
 - # targets
 - Prijs
 - Throughput
 - TAT
 - Workflow
 - Kwant/kwal
- AZ Sint-Lucas: BD Max

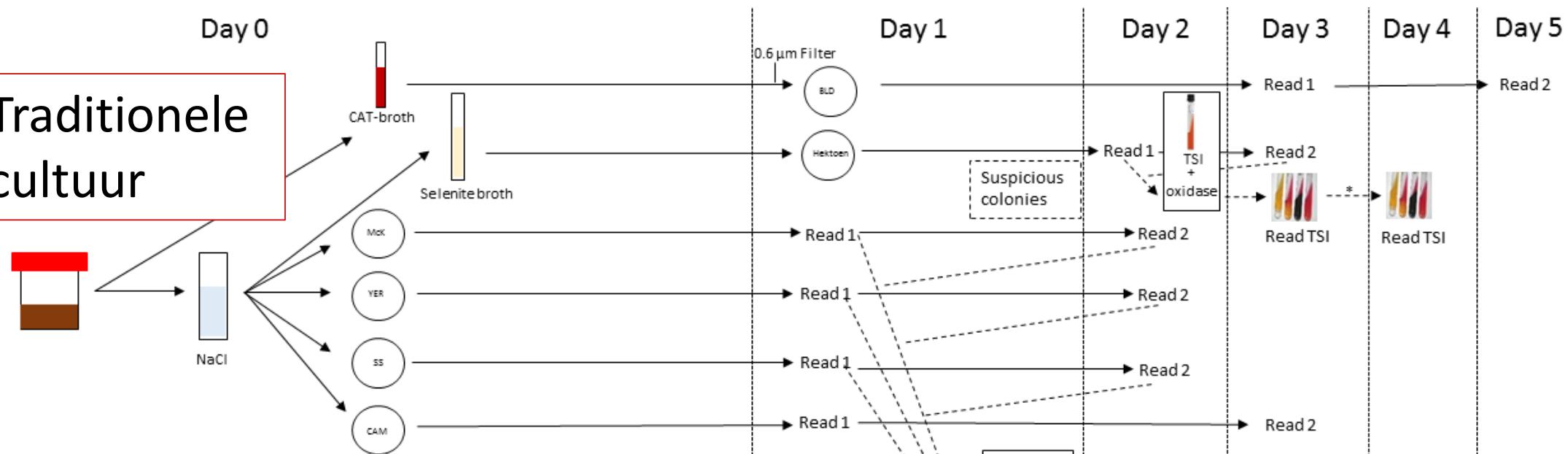


- 2 toestellen present
- Batching: 1-24 stalen
- Routinelab
- Gebruiksvriendelijk
- Ct-waarde



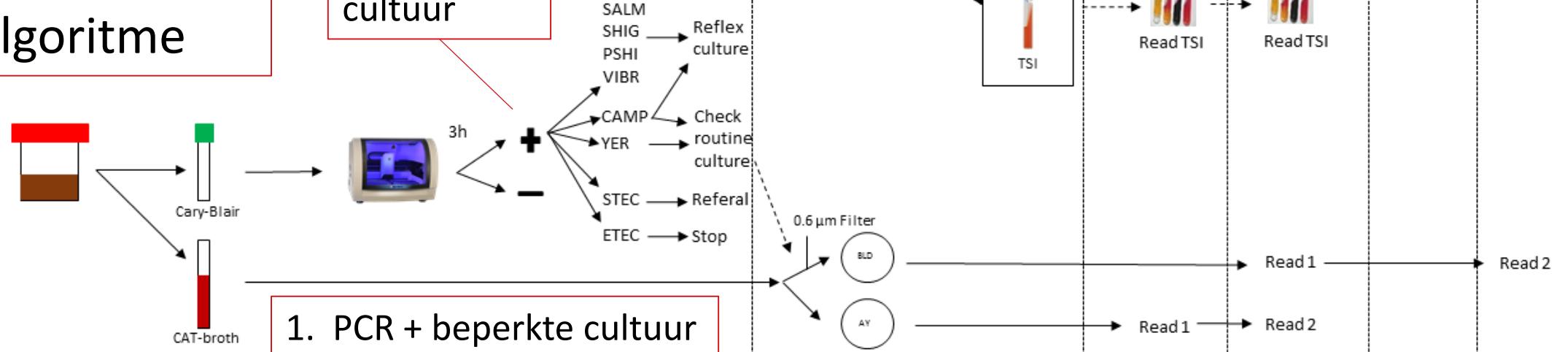
<i>Campylobacter coli/jejuni</i>
<i>Salmonella spp.</i>
<i>Shigella/EIEC</i>
<i>STEC</i>
<i>Yersinia enterocolitica</i>
<i>Vibrio spp.</i>
<i>Plesiomonas shigelloides</i>
<i>ETEC</i>

Traditionele cultuur



Nieuw algoritme

2. Reflex cultuur



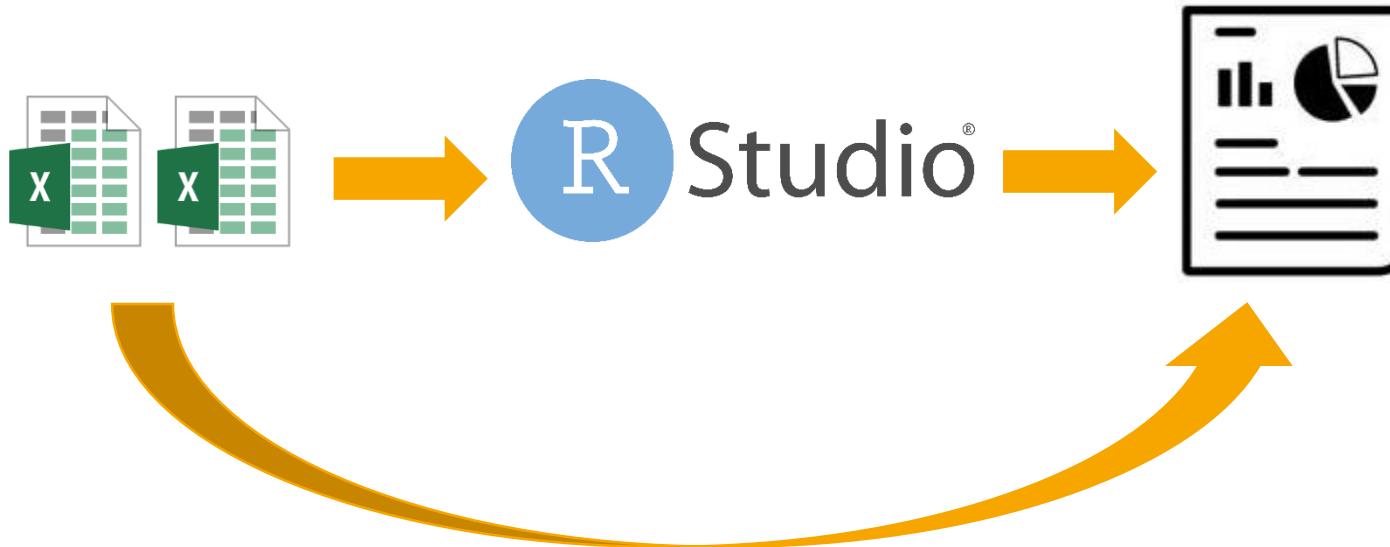
1. PCR + beperkte cultuur

Inhoud

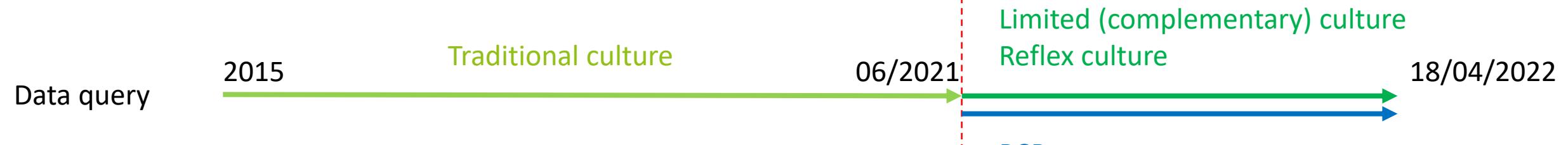
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Methodologie

- Dataquery
- Exclusie
 - Kwaliteitscontroles
- Processing



Tijdslijn



19.556 samples from
13.947 patients

Limited (complementary) culture Reflex culture

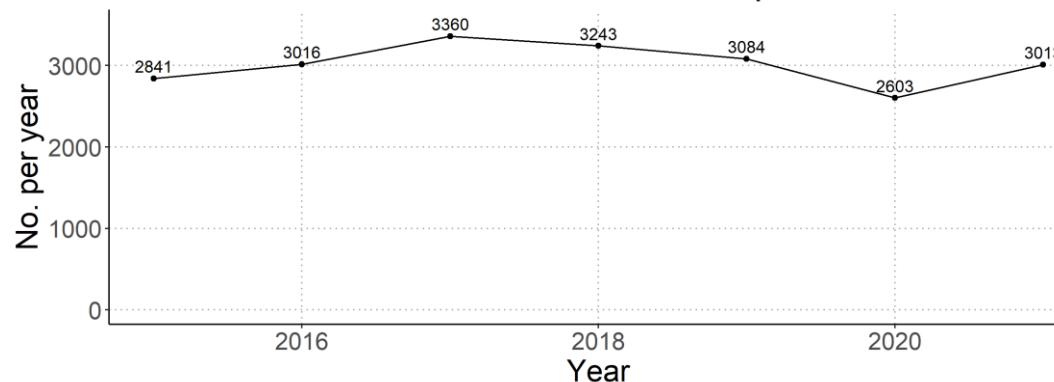
PCR

2.608 samples from
2.242 patients

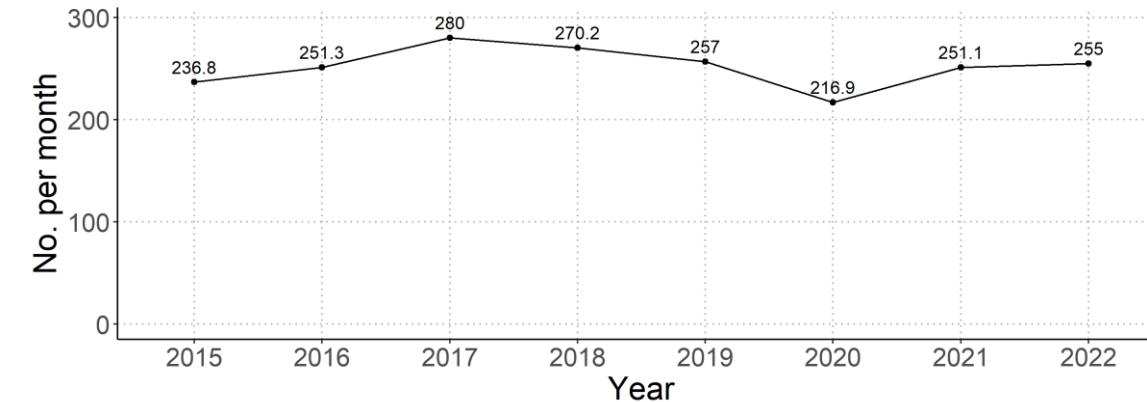
Aantal stalen (gemiddeld)

- +/- 3000 per jaar
- +/- 250 per maand
- +/- 8.3 per dag (incl weekends)

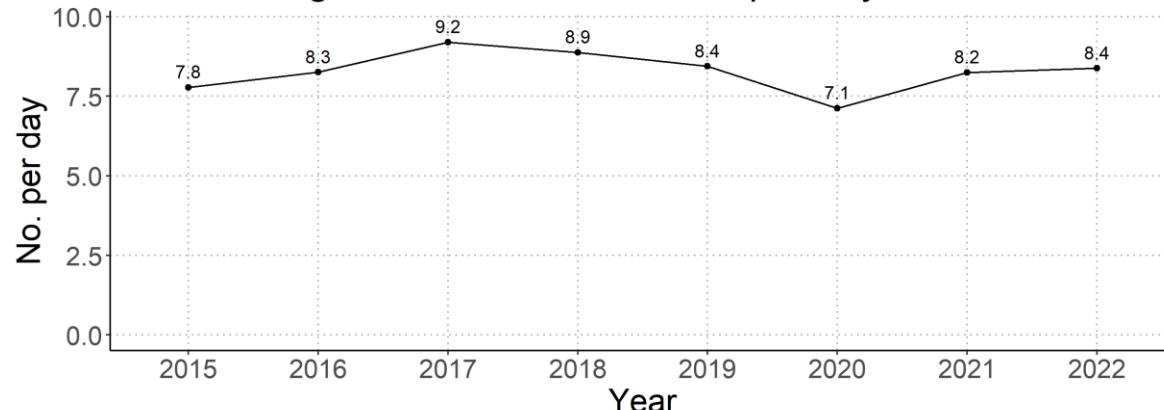
Number of aerobic faeces culture requests 2015-2021



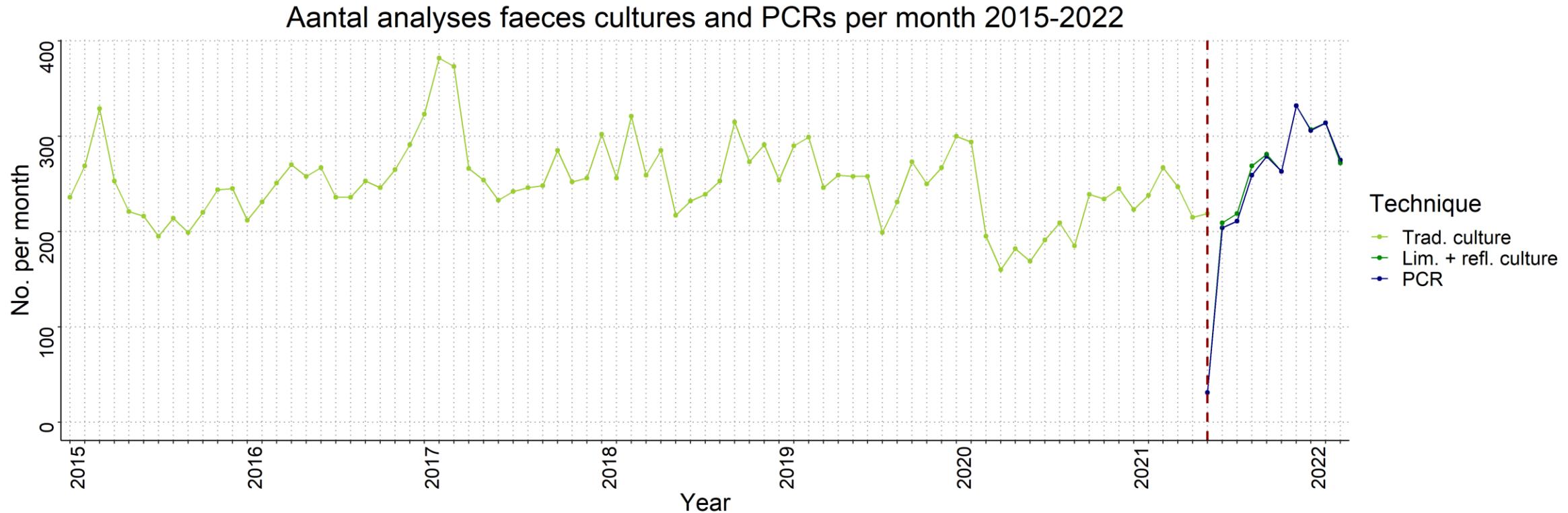
Average no. of faeces cultures per month 2015-2022



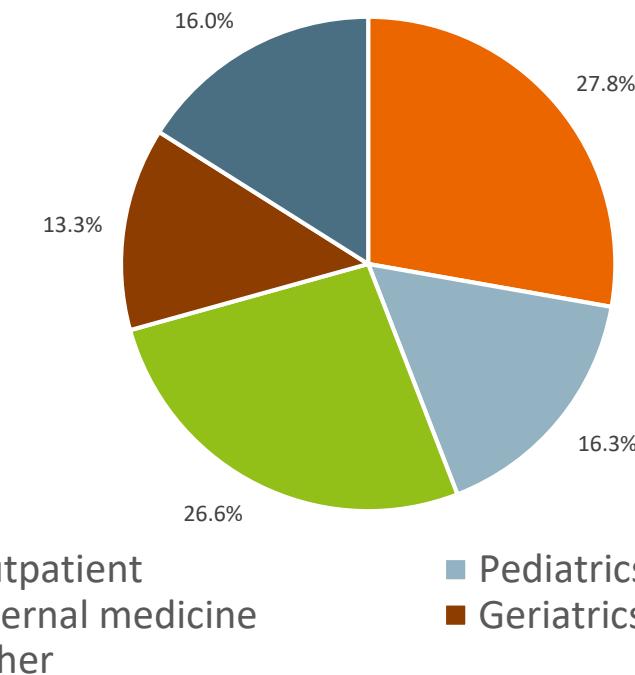
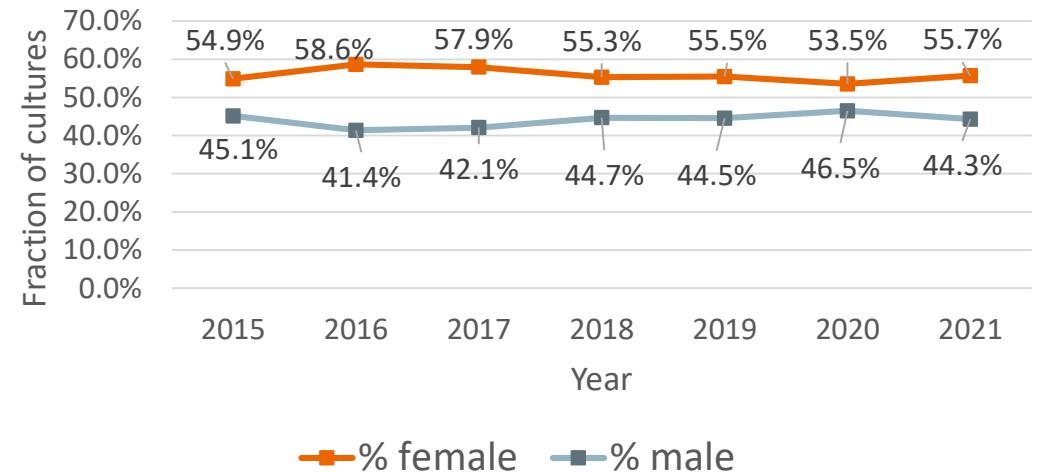
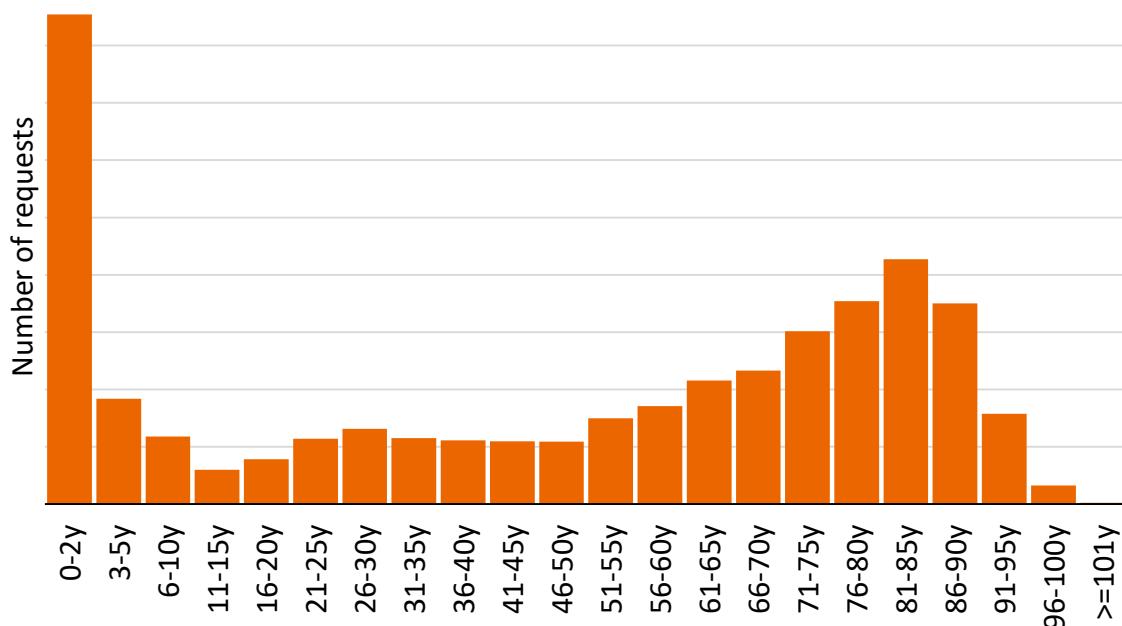
Average no. of faeces cultures per day 2015-2022



Aantal stalen per maand



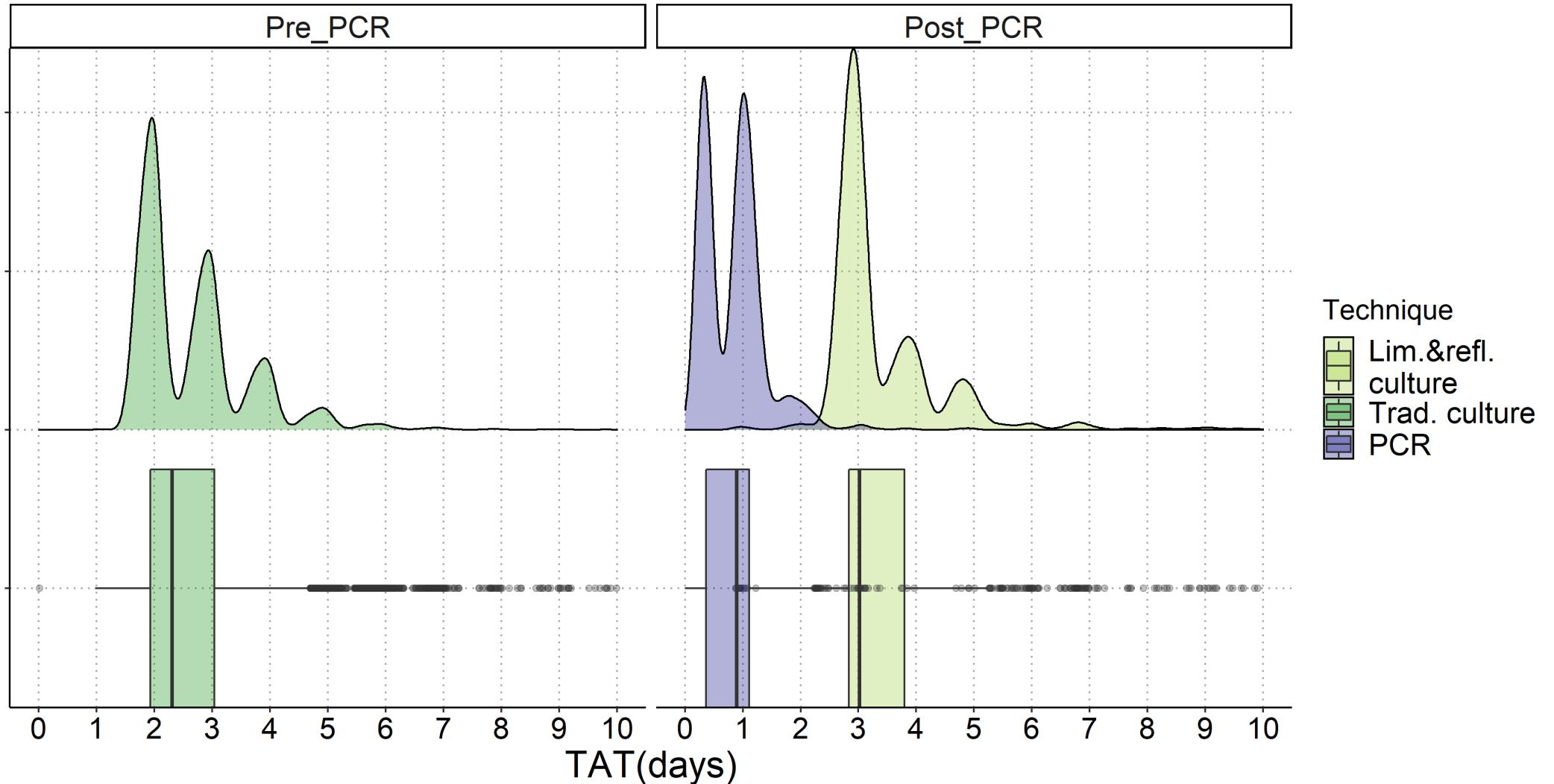
Demografie



TAT impact

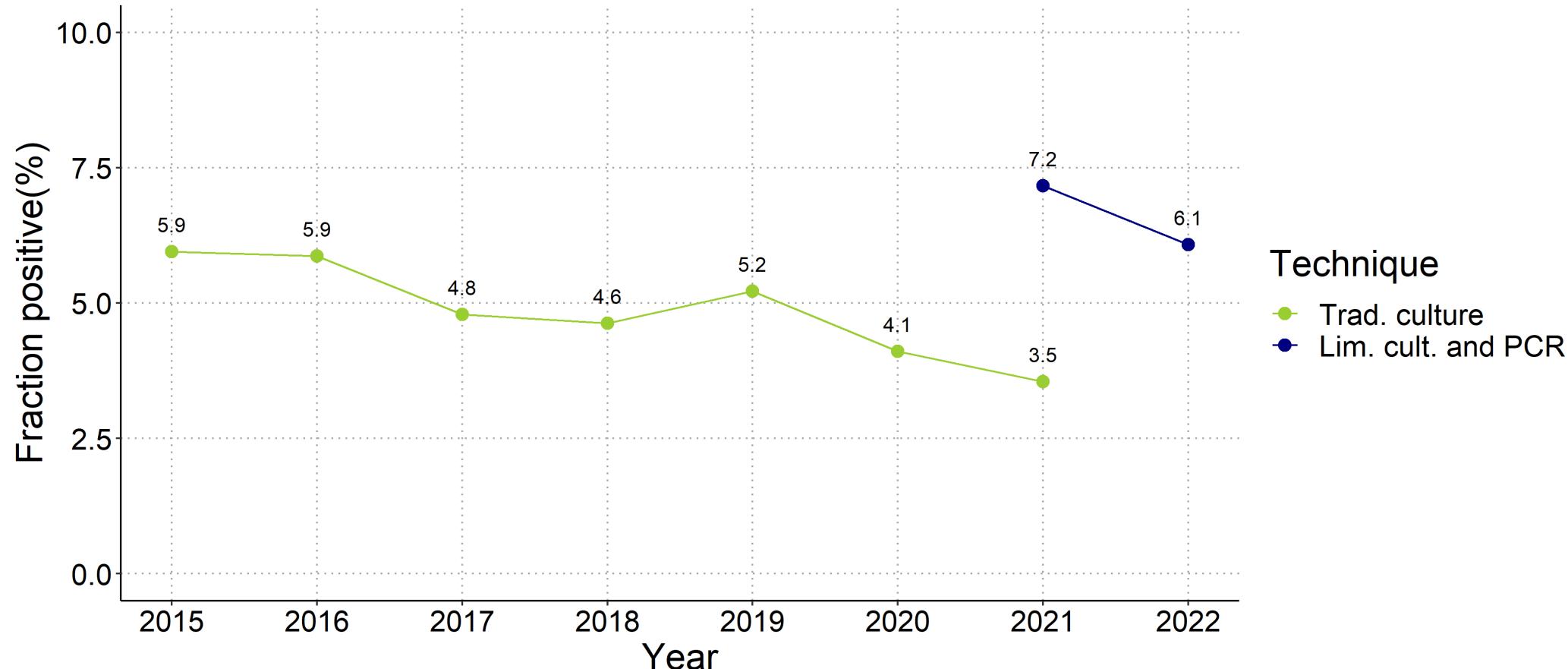
	P25	Median (P50)	P75	P90	
Trad. Culture (n=19556)	1.9 days	 1 1 0	2.3 days	3.0 days	4.0 days
PCR (n=2608)	0.4 days	 1	0.9 days	1.1 days	1.4 days
TAT limited/reflex culture (n=2608)	2.8		3.0 days	3.8 days	4.8 days

TAT impact



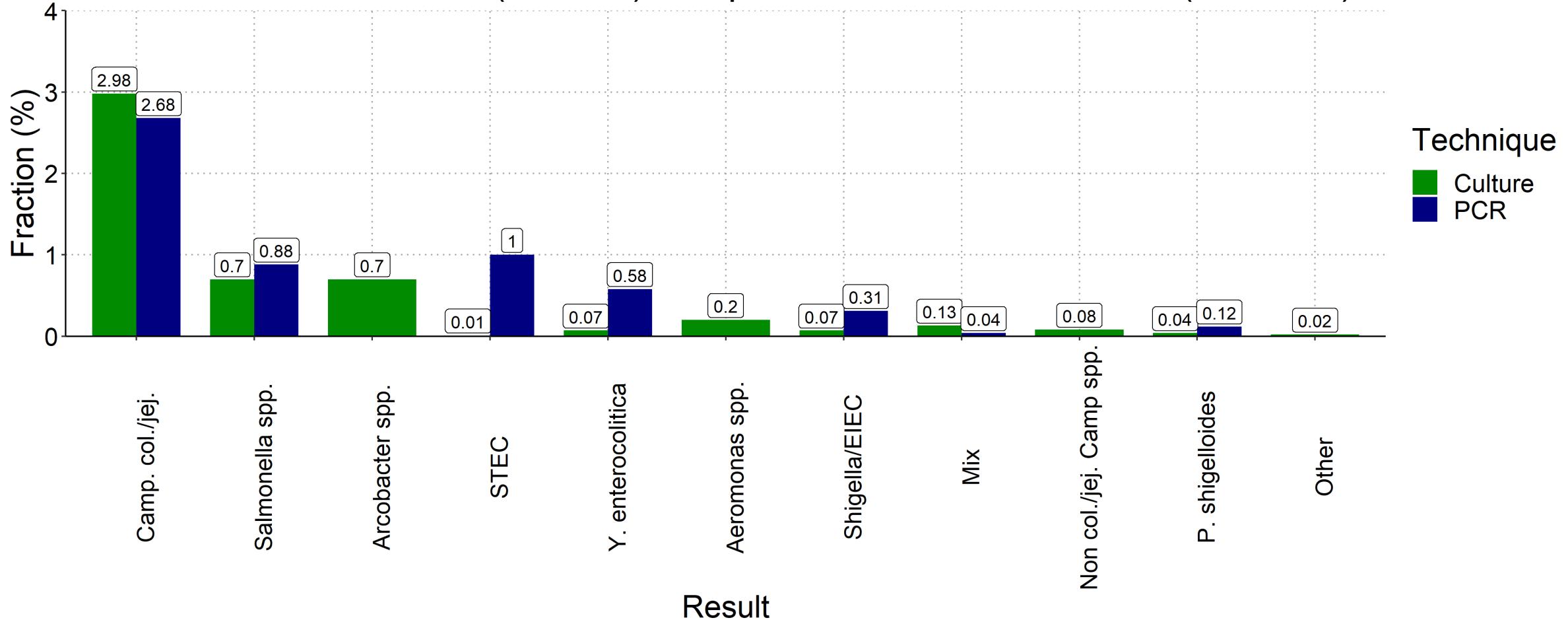
Diagnostische opbrengst - totaal

Diagnostic yield traditional culture (n=19556)
and PCR panels (n=2624) 2015-2022



Diagnostische opbrengst 2015-2022

Positive results of PCR (n=2608) and pre-PCR traditional culture (n=19556)

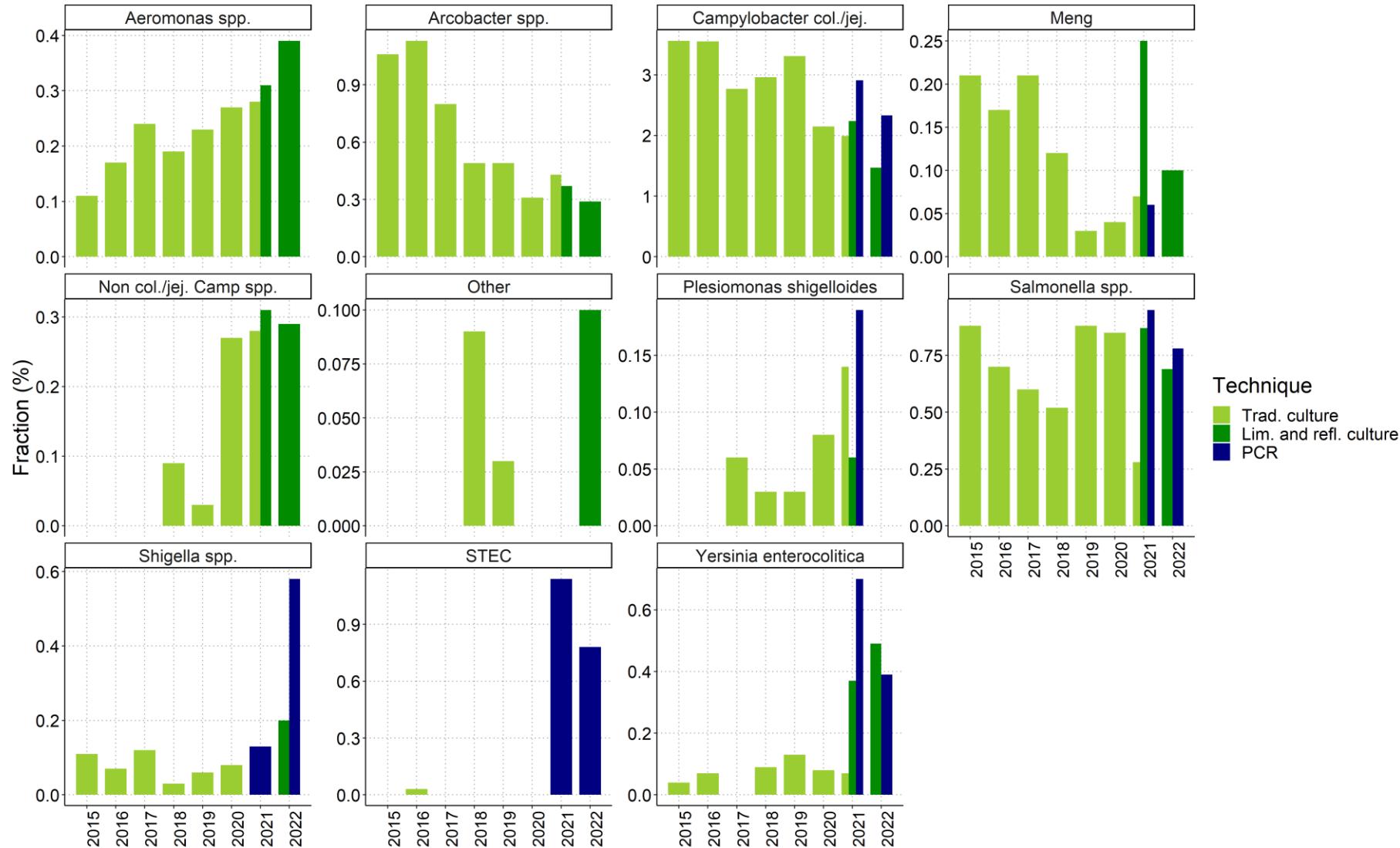


Diagnostische opbrengst over 7 jaar, per kiem per jaar

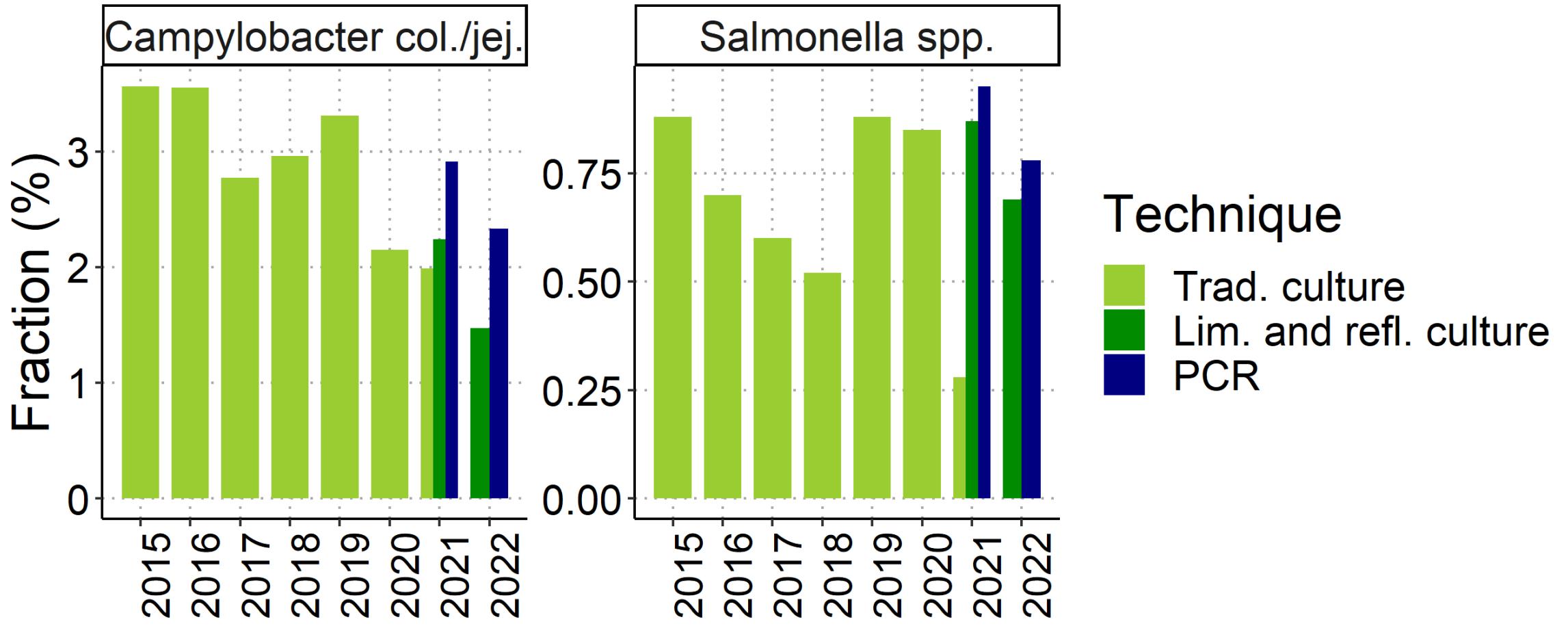
01/01/2015- 18/04/2022 (n=24.788)		Period	Negative	Campylobacter col./jej.	Salmonella spp.	Arcobacter spp.	Aeromonas spp.	Yersinia enterocolitica	STEC	Shigella/EIEC spp.	Non col./jej. Camp	P. shigell oides	Mixed	Other	Grand Total
Trad. culture			2672												2841
	2015		(94,1%)	101 (3,6%)	25 (0,9%)	30 (1,1%)	3 (0,1%)	1 (0%)		3 (0,1%)			6 (0,2%)		(100%)
			2839												3016
	2016		(94,1%)	107 (3,5%)	21 (0,7%)	34 (1,1%)	5 (0,2%)	2 (0,1%)	1 (0%)	2 (0,1%)			5 (0,2%)		(100%)
			3199										2		3360
	2017		(95,2%)	93 (2,8%)	20 (0,6%)	27 (0,8%)	8 (0,2%)			4 (0,1%)		(0,1%)	7 (0,2%)		(100%)
			3093												3243
	2018		(95,4%)	96 (3%)	17 (0,5%)	16 (0,5%)	6 (0,2%)	3 (0,1%)		1 (0%)	3 (0,1%)	1 (0%)	4 (0,1%)	3 (0,1%)	(100%)
			2923												3084
	2019		(94,8%)	102 (3,3%)	27 (0,9%)	15 (0,5%)	7 (0,2%)	4 (0,1%)		2 (0,1%)	1 (0%)	1 (0%)	1 (0%)	1 (0%)	(100%)
PCR			2496										2		2603
	2020		(95,9%)	56 (2,2%)	22 (0,8%)	8 (0,3%)	7 (0,3%)	2 (0,1%)		2 (0,1%)	7 (0,3%)	(0,1%)	1 (0%)		(100%)
Lim. and refl. culture		2021 (jan- june)	1359 (96,5%)	28 (2%)	4 (0,3%)	6 (0,4%)	4 (0,3%)	1 (0,1%)			4 (0,3%)	(0,1%)	1 (0,1%)		1409 (100%)
	2021 (july- dec)	1527 (95,2%)		36 (2,2%)	14 (0,9%)	6 (0,4%)	5 (0,3%)	6 (0,4%)			5 (0,3%)	(0,1%)	4 (0,2%)		1604 (100%)
PCR		2021 (july- dec)	1483 (93,9%)		46 (2,9%)	15 (0,9%)			11 (0,7%)	18 (1,1%)	2 (0,1%)	(0,2%)	1 (0,1%)		1579 (100%)
	2022 (jan- apr)	979 (96%)		15 (1,5%)	7 (0,7%)	3 (0,3%)	4 (0,4%)	5 (0,5%)		2 (0,2%)	3 (0,3%)		1 (0,1%)	1 (0,1%)	1020 (100%)
PCR		2022 (jan- apr)	979 (95,1%)		24 (2,3%)	8 (0,8%)			4 (0,4%)	8 (0,8%)	6 (0,6%)				1029 (100%)
			23549										12		24788
Grand Total			(95%)	704 (2,8%)	180 (0,7%)	145 (0,6%)	49 (0,2%)	39 (0,2%)	27 (0,1%)	24 (0,1%)	23 (0,1%)	(0%)	31 (0,1%)	5 (0%)	(100%)

Per jaar per kiem: overzicht

Fraction positive of pre-PCR traditional culture (n=19556), PCR (n= 2608) and post-PCR limited/reflex culture (n=2624)



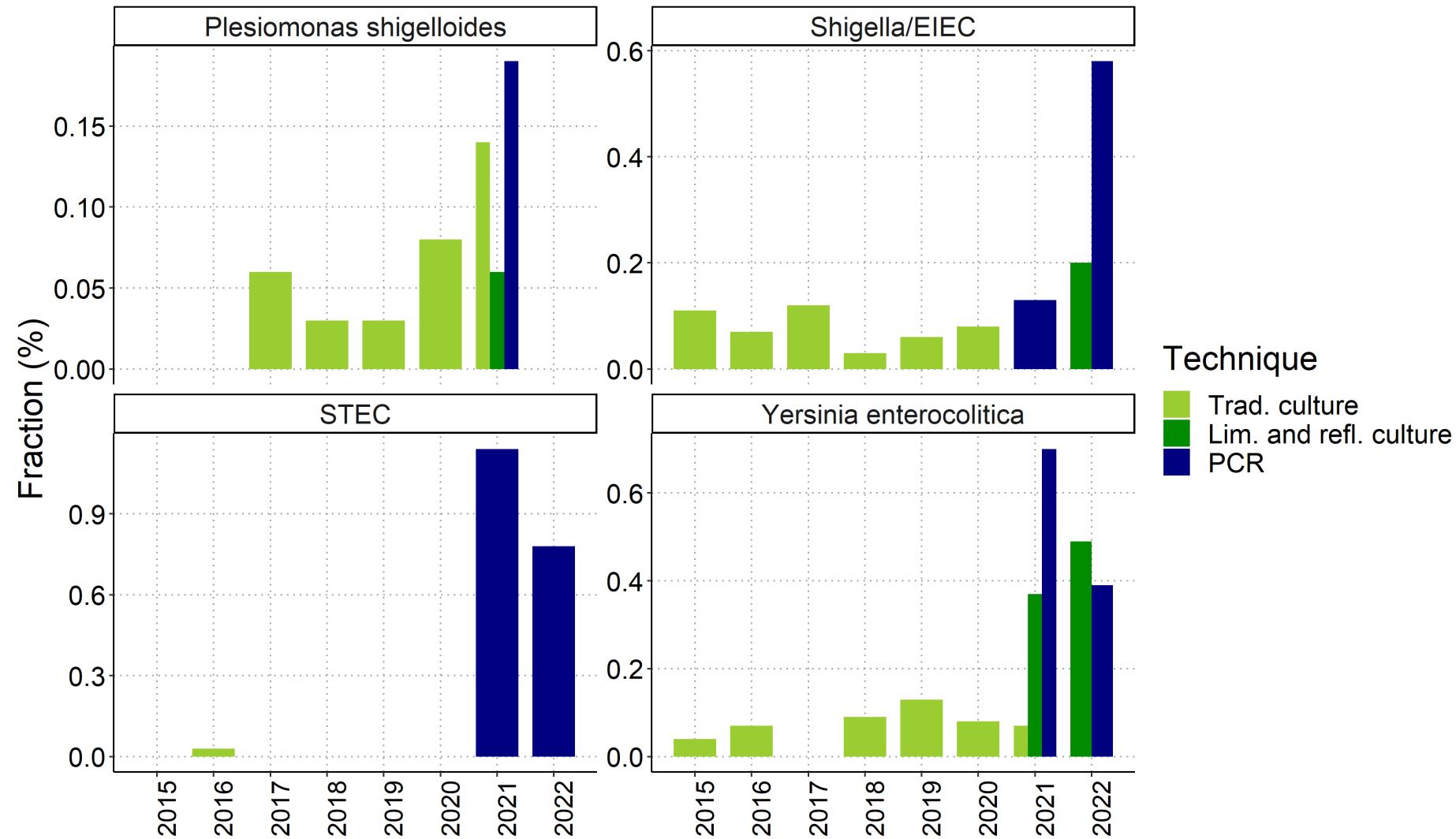
Per jaar per kiem: ongeveer gelijk



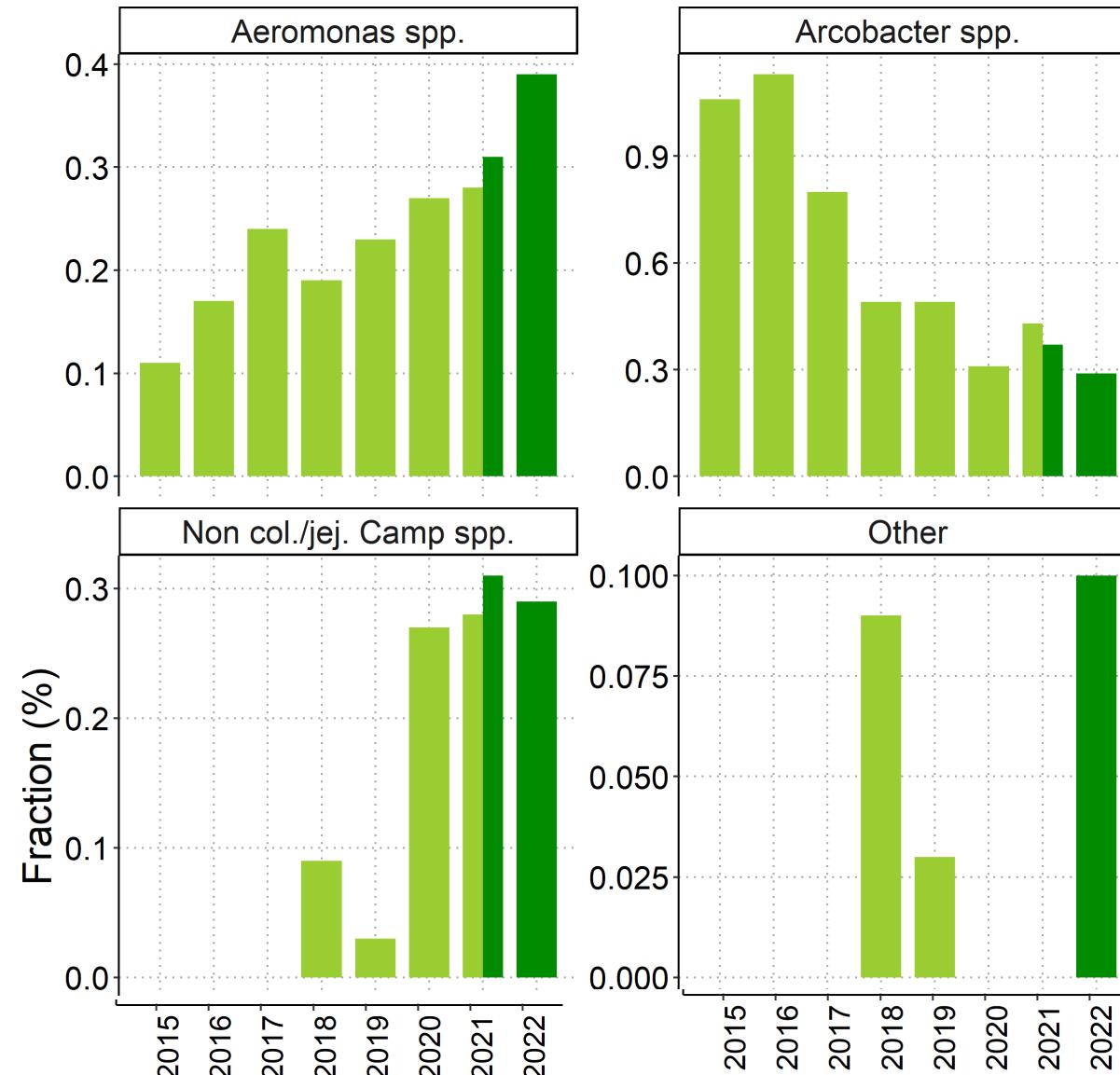
Technique

- Trad. culture
- Lim. and refl. culture
- PCR

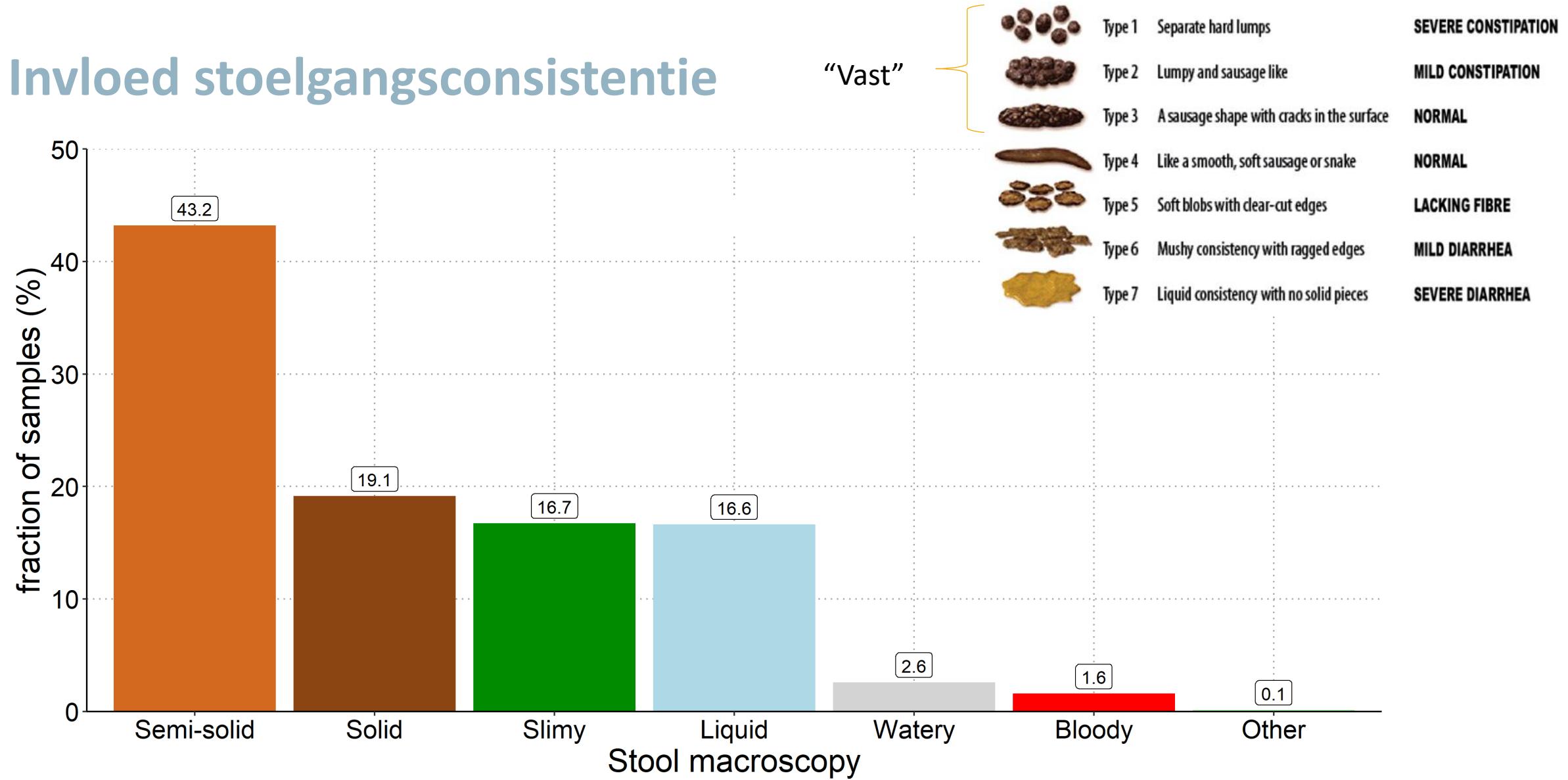
Per jaar per kiem: stijgers



Niet in PCR panel

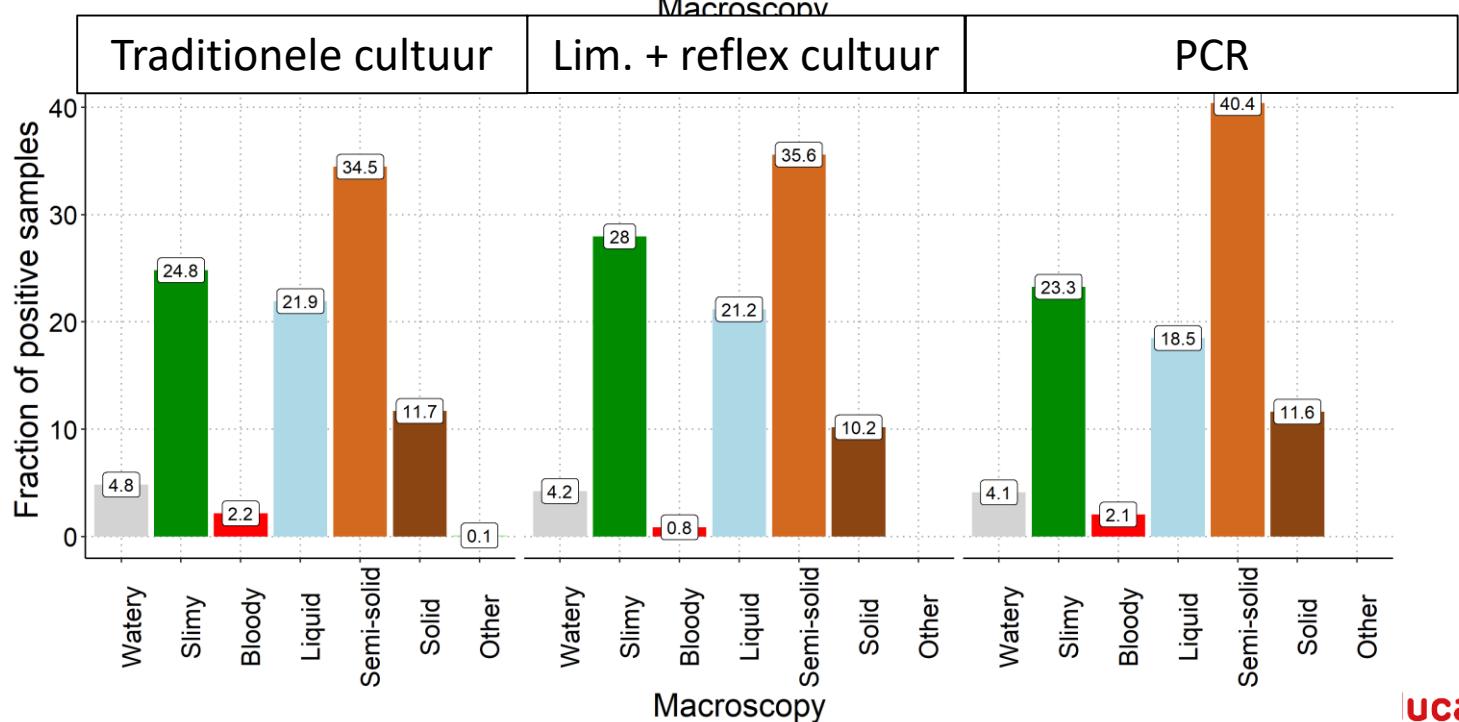
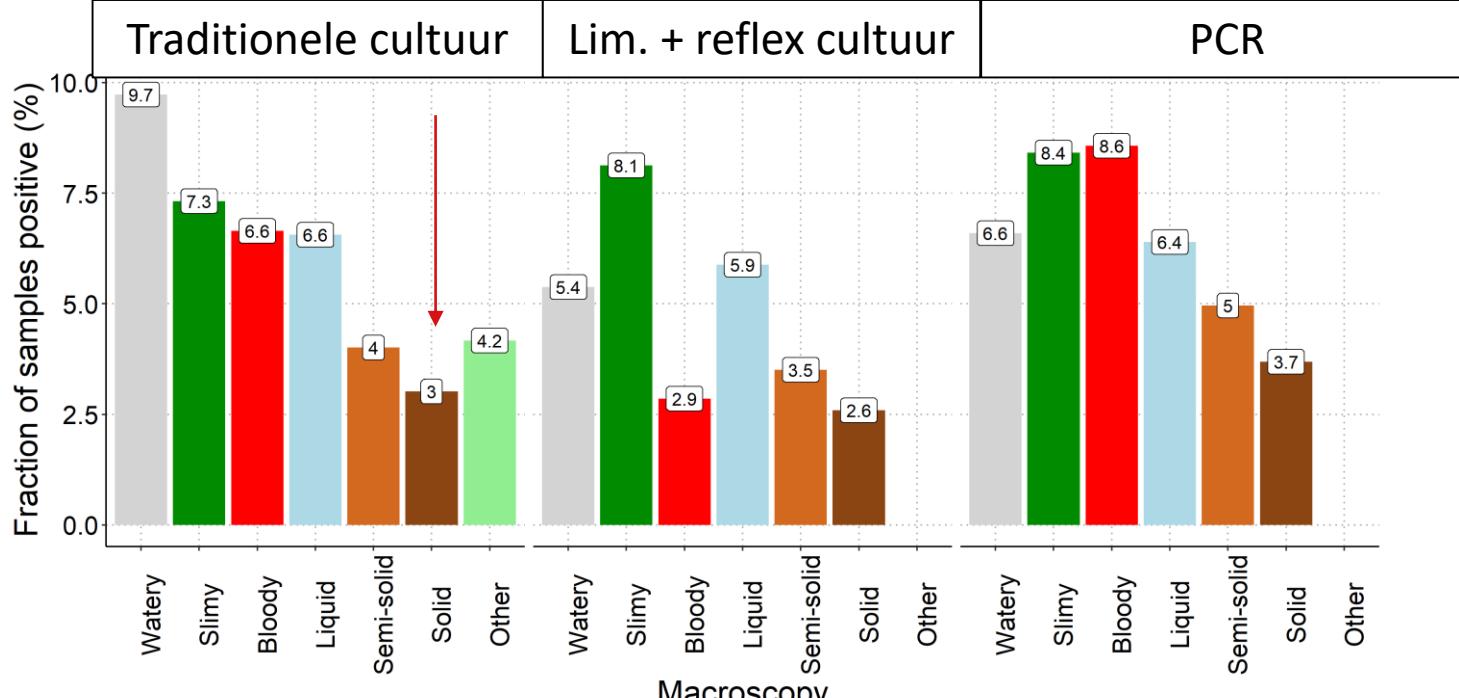


Invloed stoelgangsconsistentie



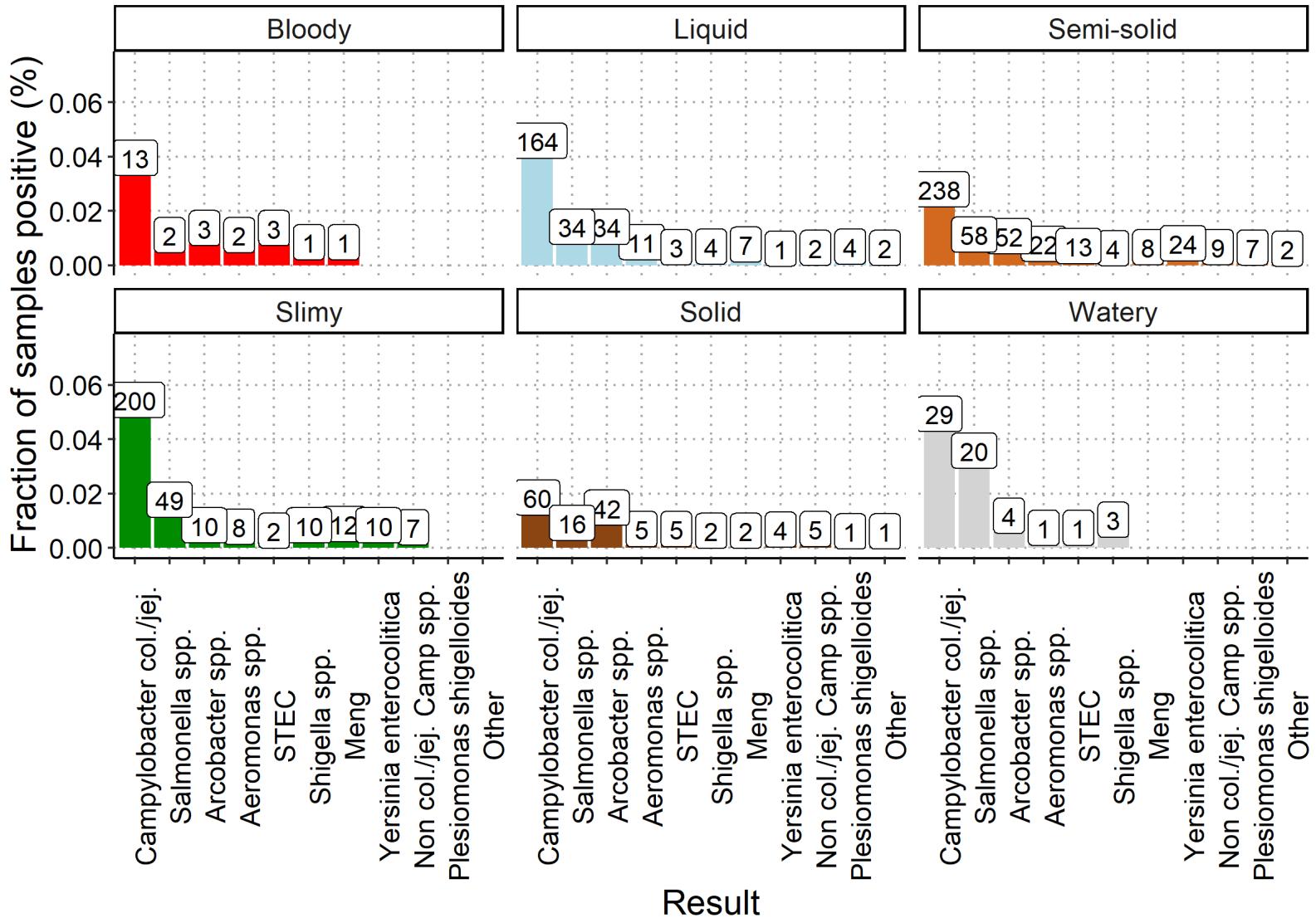
Stoelgangsconsistentie

- Relatie met positiviteitsgraad
 - Vloeibaar/bloederig/slijmerig > halvast > vast
- Vaste stoelgangsstalen
 - 19.1% van de stalen
 - Positiviteit **+/- 3%**
 - **Maar** wel **+/- 12%** positieve resultaten!

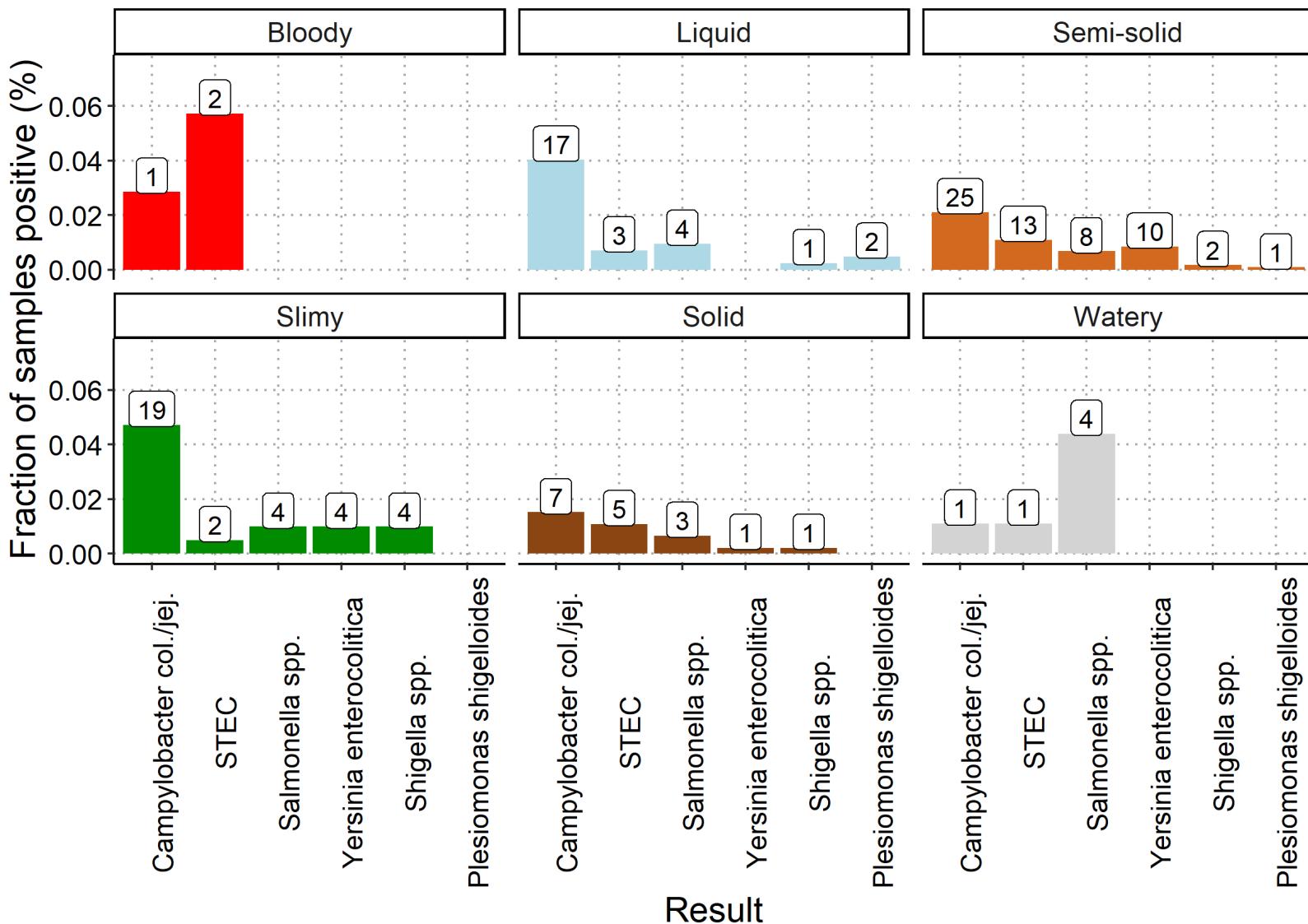


Welke kiemen in vaste stoelgang?

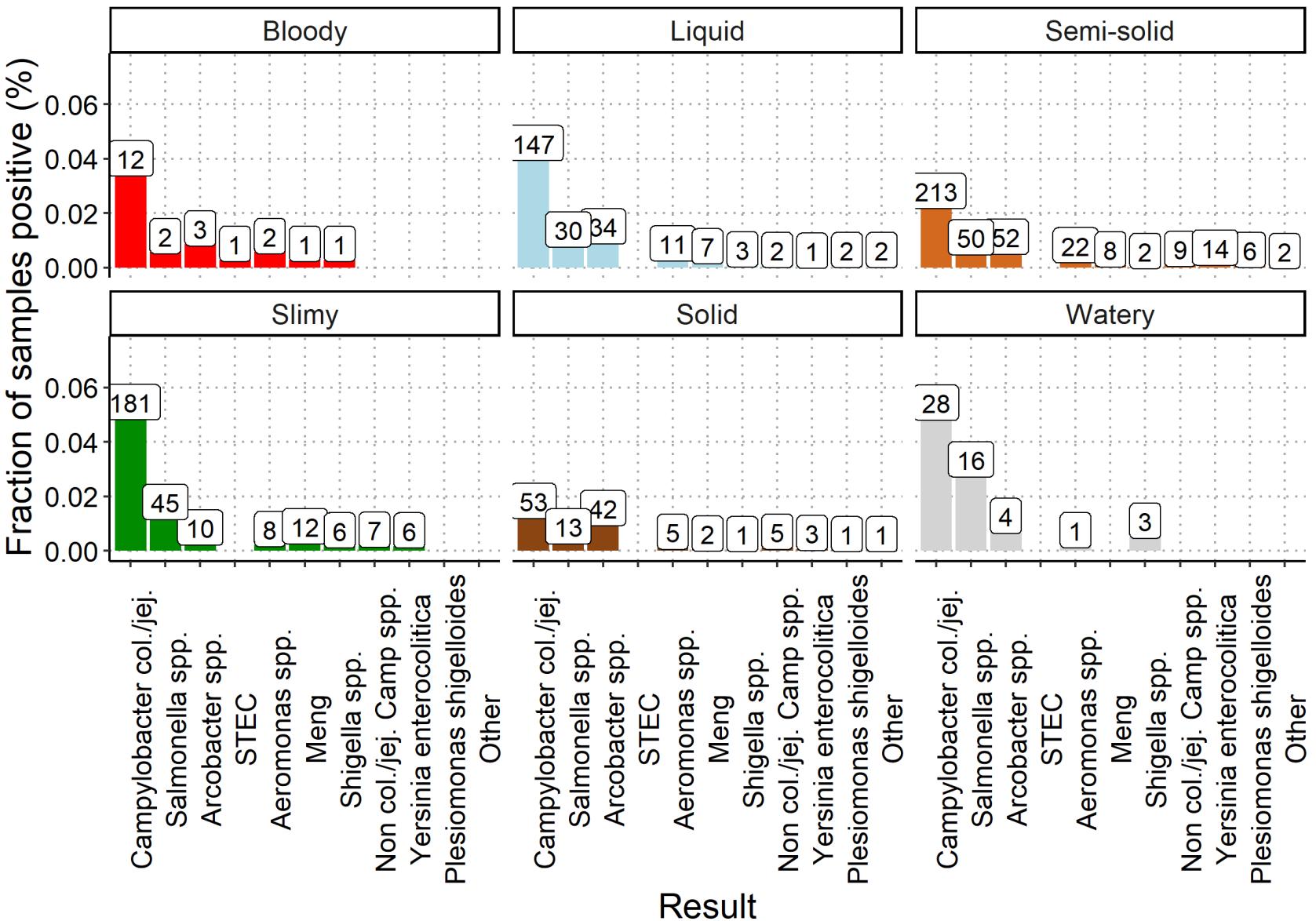
Positive results (culture & PCR) in relation to stool macroscopy



Positive PCR results in relation to stool macroscopy



Positive culture results in relation to stool macroscopy



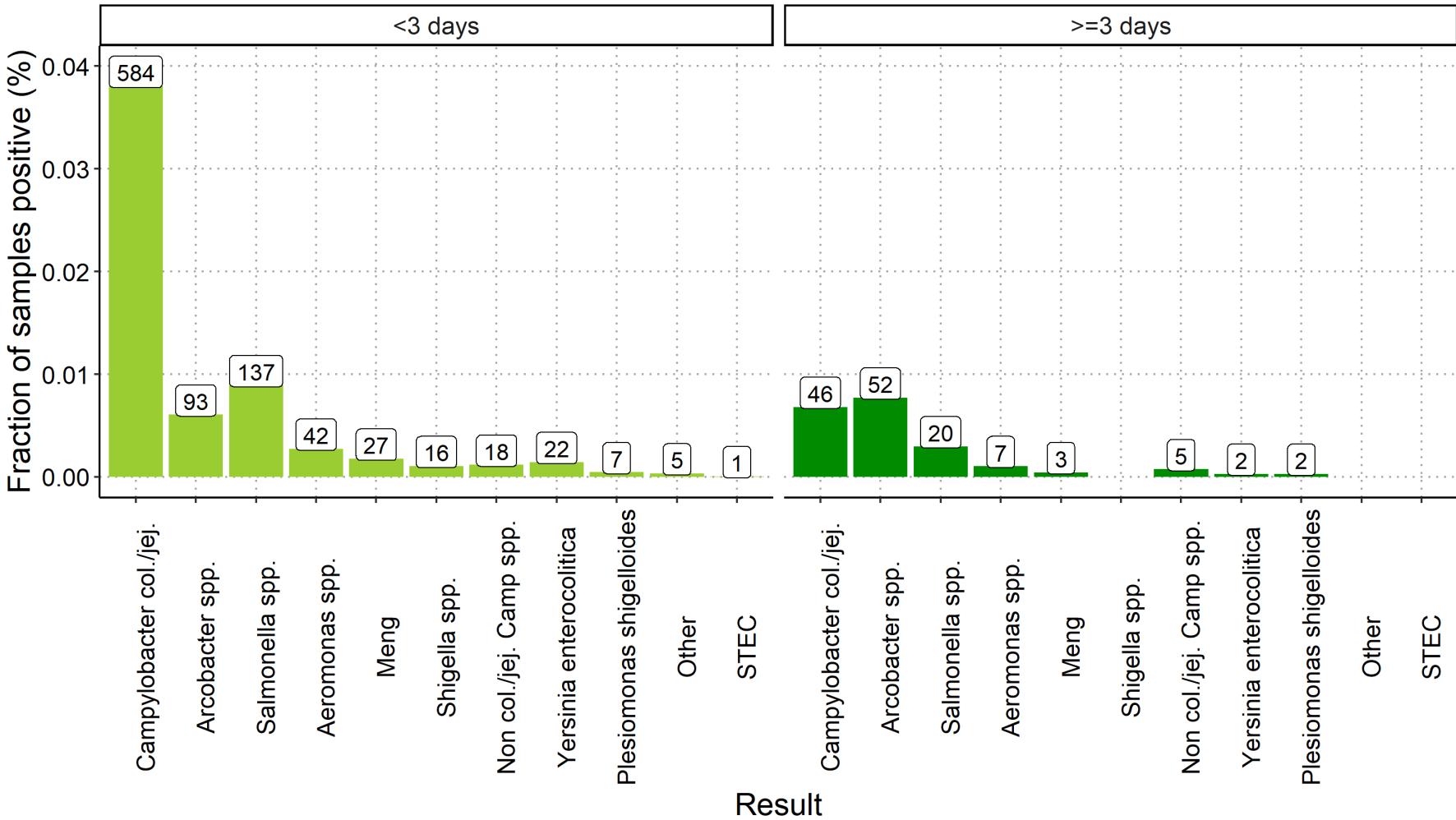
Invloed tijd gehospitaliseerd

- >3d opgenomen (30% stalen)
 - Lagere positiviteitsratio
 - Cultuur: 2.0% vs 6.2%
 - PCR: 1.8 vs 6.8%
 - Maar belangrijk deel van de positieve resultaten
 - Cultuur: 12.6%
 - PCR: 8.3%



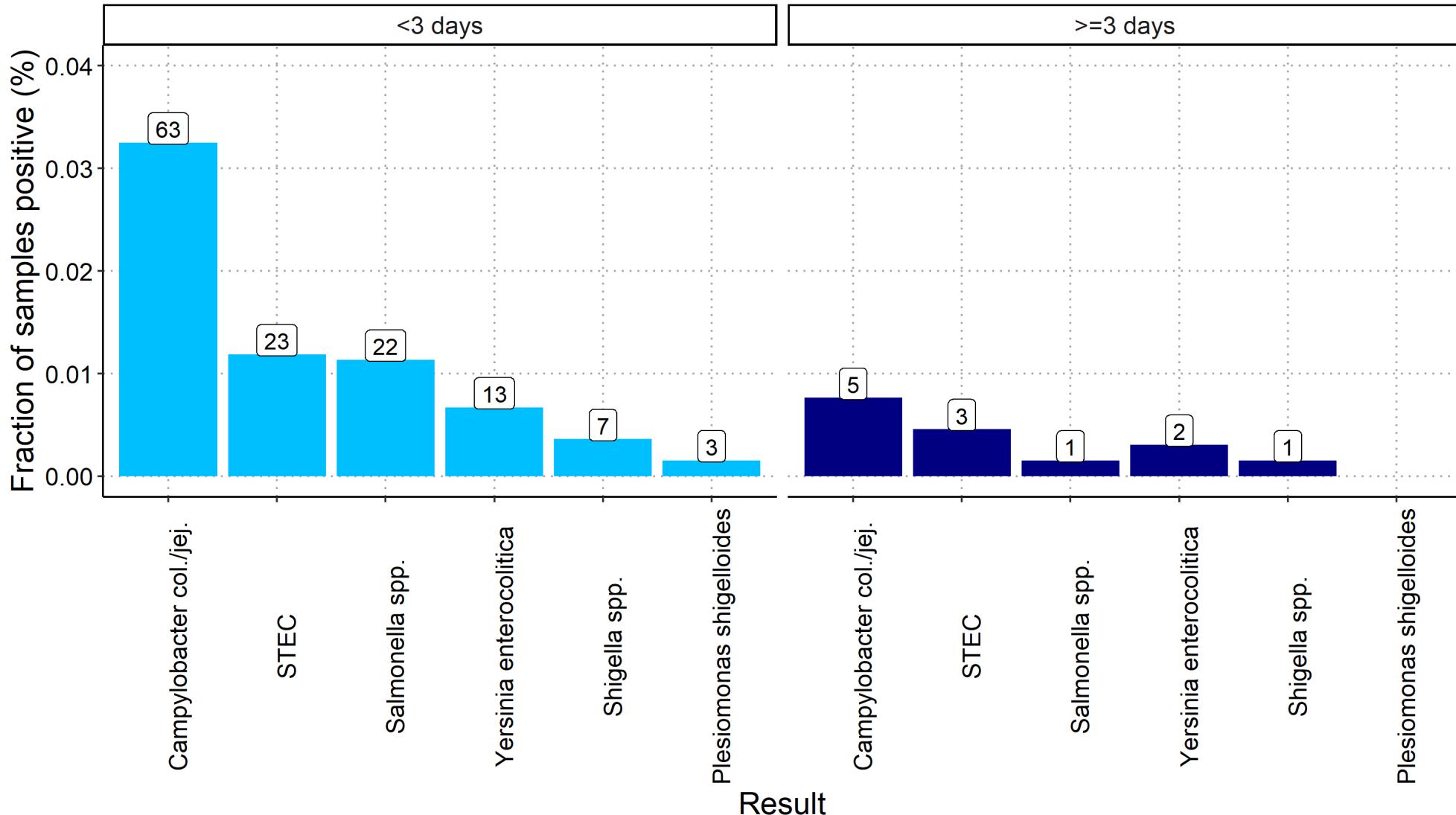
Welke kiemen >3d opgenomen?

Positive culture results in relation to duration of hospitalization



Welke kiemen >3d opgenomen?

Positive PCR results in relation to duration of hospitalization



Discordanties - complementariteit



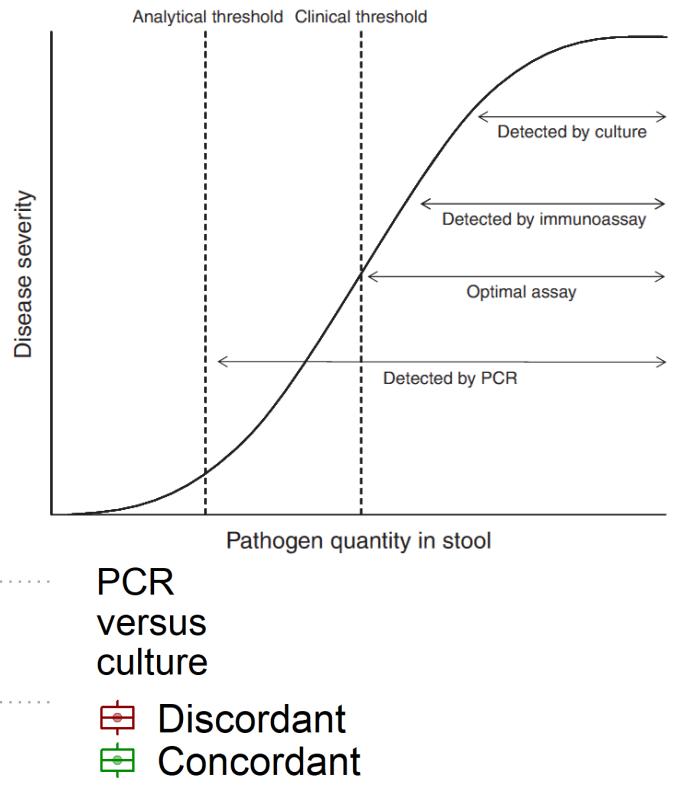
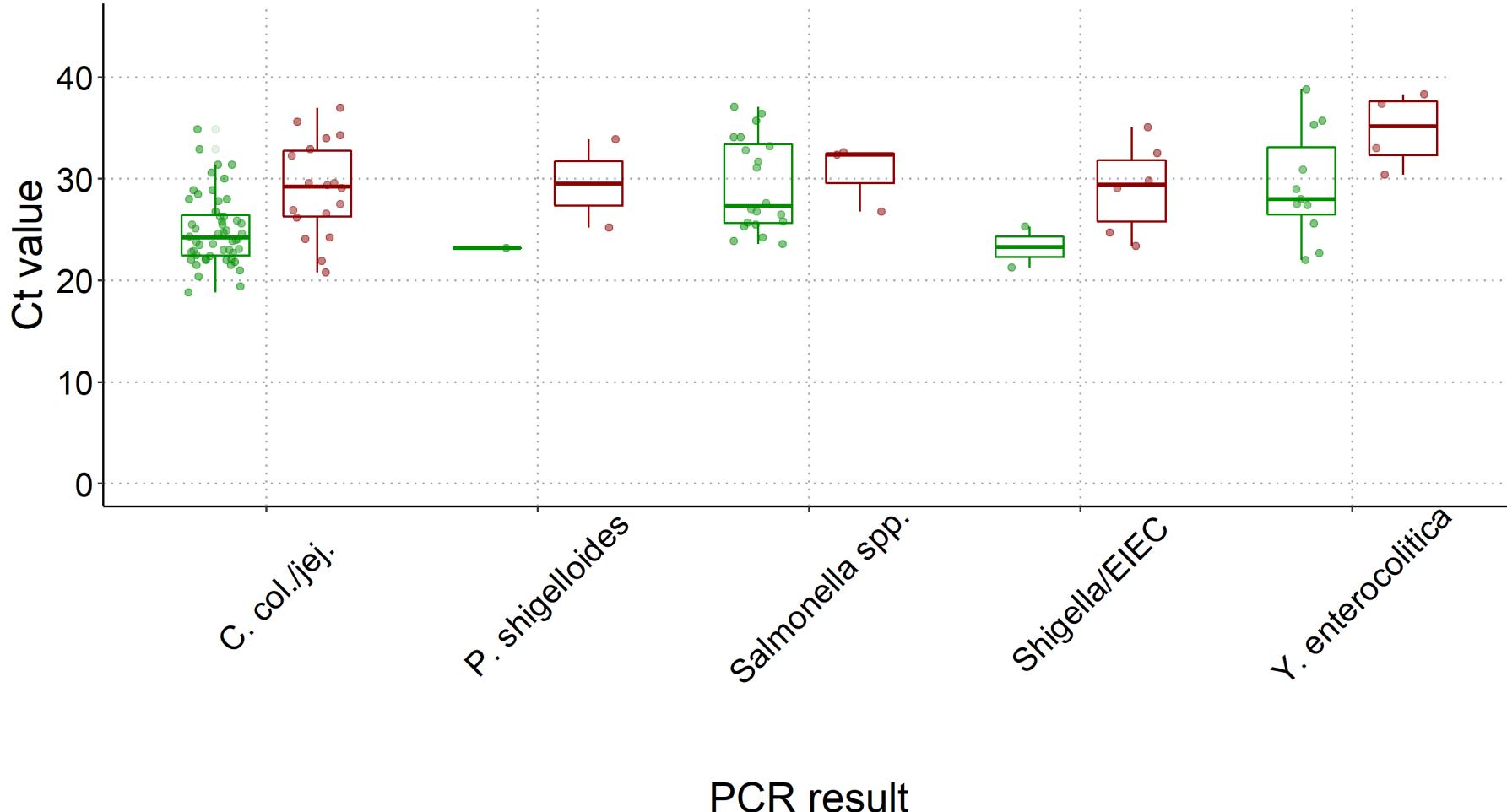
- Niet in PCR, wel in cultuur
 - Non coli/jej Campylobacter
 - Arcobacter
 - Aeromonas
- Niet in cultuur, wel in PCR
 - STEC
 - ETEC
 - EIEC

Post-PCR period (28/06/2021-18/04/2022, n= 2598)

Limited + reflex culture result

Discordanties – Ct waarde

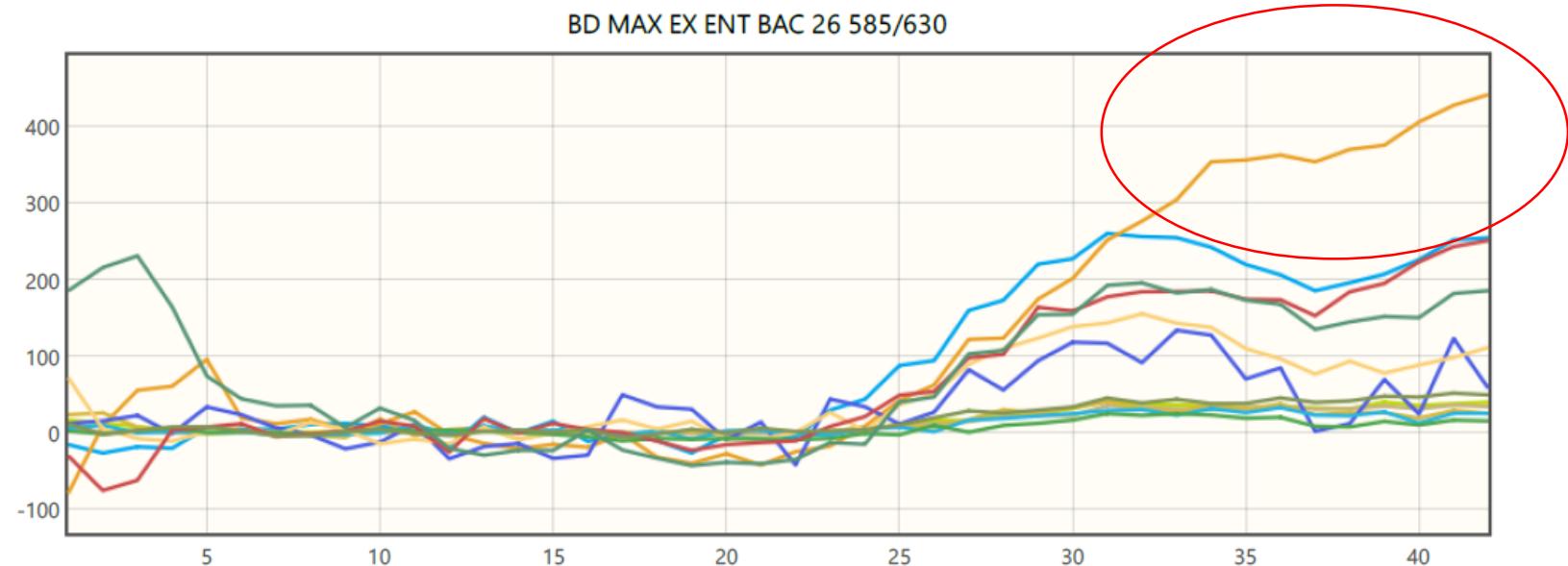
Relationship between Ct value and culturability (n=119)



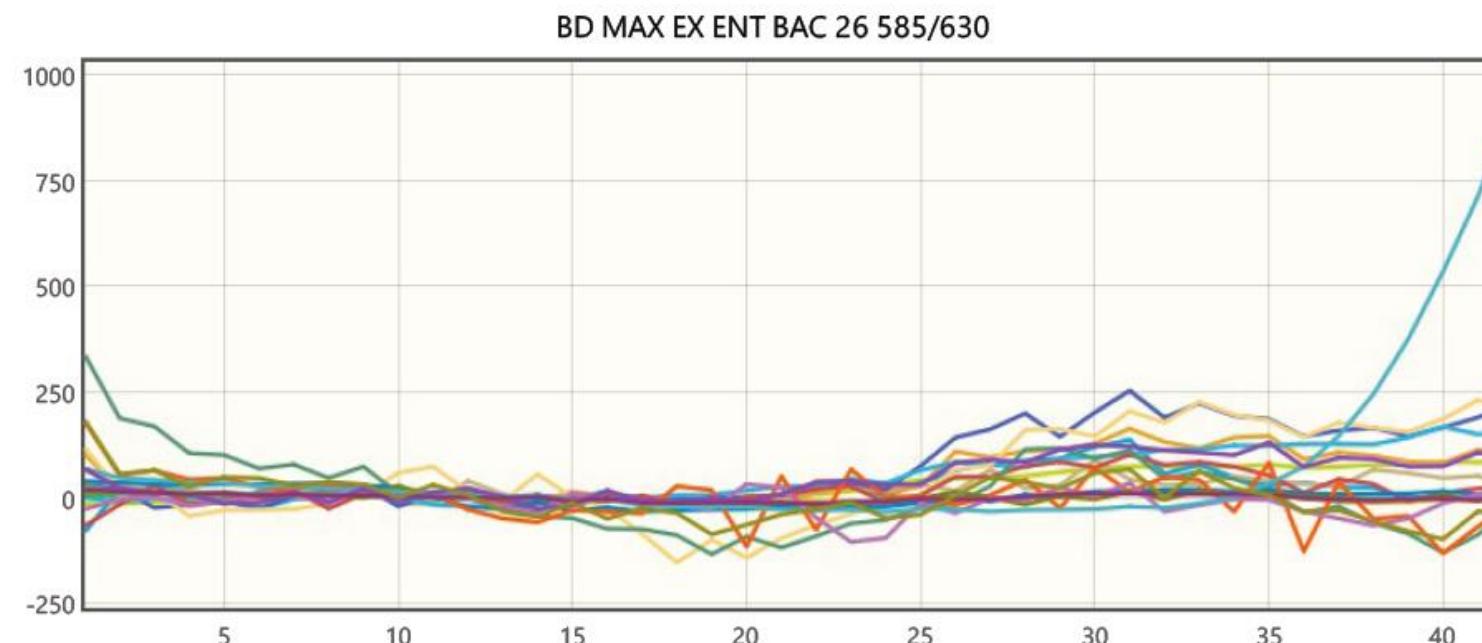
Vals-positieve resultaten

Position	Test Name	Sample Tube	Patient ID	Result	Ct 475/520	Ct 530/565	Ct 585/630	Ct 630/665	Ct 680/715
SP Status		PCR Status	Third Patient ID		Melt 475/520	Melt 530/565	Melt 585/630	Melt 630/665	Melt 680/715
Kit Lot	Master Mix Lot	Cartridge Barcode	Accession	- Shig NEG - STX NEG - Campy NEG - Salm NEG + Vibrio POS - Plesio NEG - ETEC NEG - Yersi NEG	-1.0	-1.0	-1.0	-1.0	27.3
A2	BD MAX EX ENT BAC 26	B33123838720230401KQ24	--		-1.0	-1.0	28.2	-1.0	27.7
Success		Success	--		--	--	--	--	--
K52125160920230121	M26115360420221217	C24125621120230930BJ28	1122836301		--	--	--	--	--

Vals-positieve resultaten

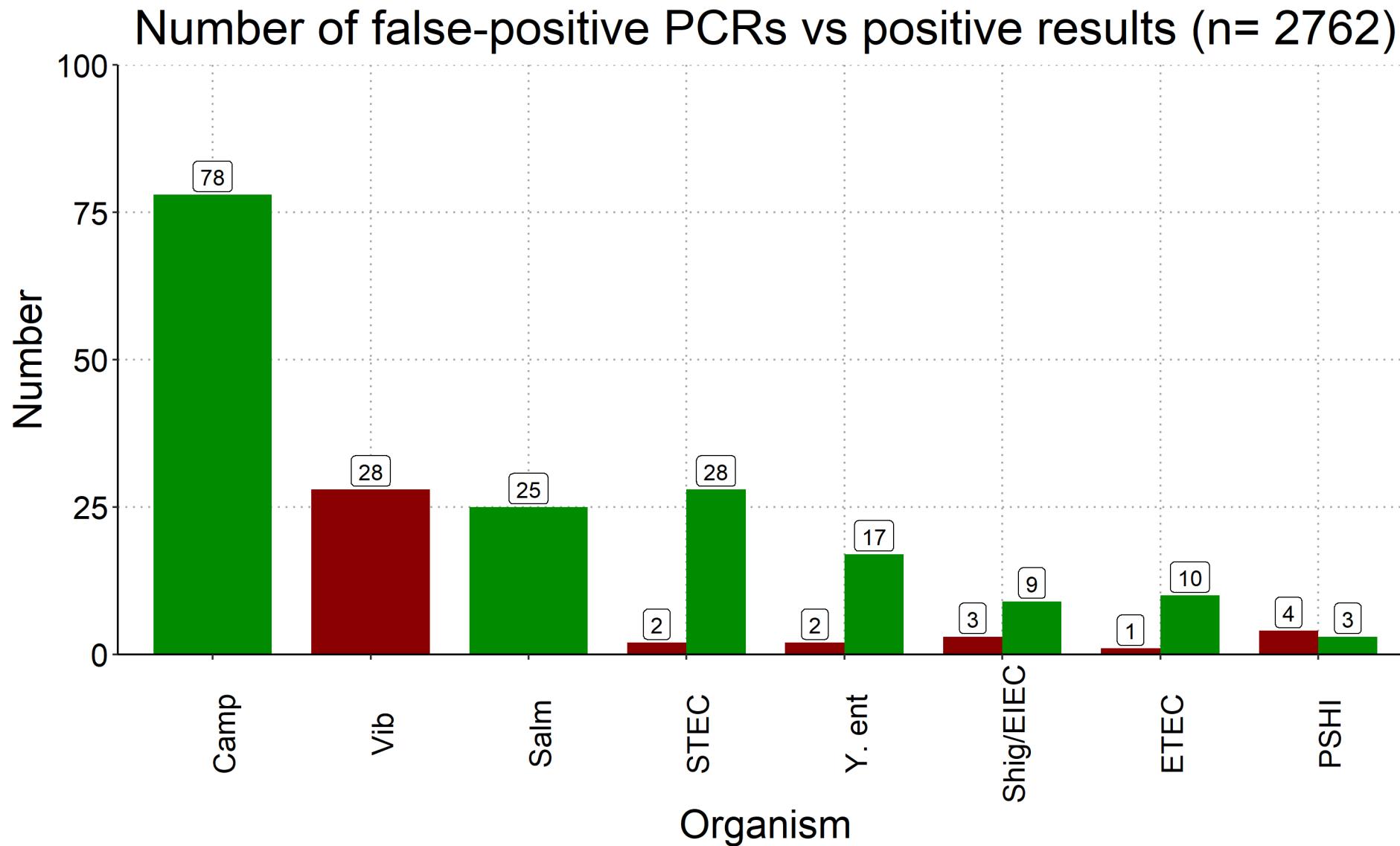


Vibrio spp. Ct 28.2



Shigella/EIEC Ct 36.3

Vals-positief gevlagde curves: frequentie?



Kost impact

$$\text{Inkomsten} - \text{Kosten} = \text{Winst}$$

	Traditionele cultuur	PCR*	Beperkte cultuur**
Reagentia	+/- €12	+/- €18	+/- €4
Personeelstijd	+/- €10	+/- €1	+/- €4
Som	+/- €22	+/- €19	+/- €8
			+/- €27



*Exclusief kost PCR toestel en onderhoudscontract

**Exclusief reflex cultuur (beperkt)

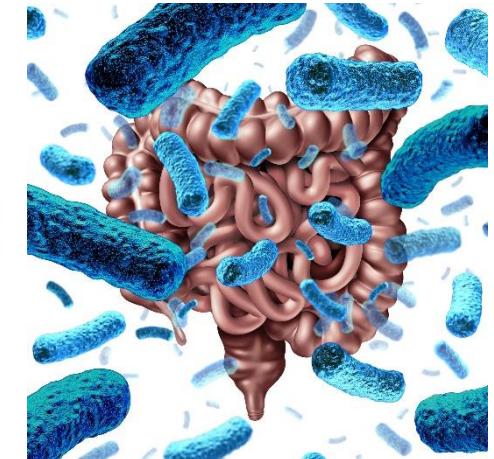
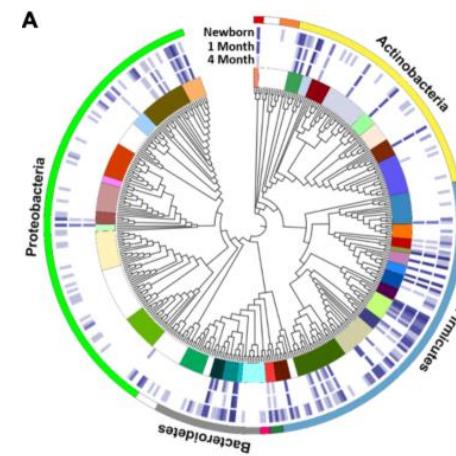
Inhoud

- Introductie
- Question 1) literatuur?
- Question 2) voor-nadelen PCR vs cultuur?
- Question 3) hoe beslissen welk panel?
- Question 4) evaluatie data voor-en na implementatie
- **Question 5) toekomst?**

Toekomst?

- Nomenclatuur hervorming?
- Ct waarde
 - Standaard semi-kwant interpretatie (~ SARS-CoV-2)?
- PCR panel gebruik
 - Centraliseren cultuur expertise?
 - Kwaliteitscontrole
- Emerging pathogens
- Metagenomics
 - ~microbioom onderzoek

RIZIV: "549835 549846 Kweek die tenminste het opzoeken van Salmonella, Shigella, Yersinia en Campylobacter omvat, met identificatie van de kiemen in faeces"



Samenvatting

- Acute gastro-enteritis
 - Testindicatie!
 - Diagnostiek
- Voor-nadelen PCR
 - Snelheid, standaardisatie
 - Kostprijs, interpretatie
- Implementatie AZ St-Lucas
 - Hogere gevoeligheid, kortere TAT
 - Complementariteit cultuur
- Toekomst

