Leiden University Medical Center

Outcome of the international pilot

Helminths External Molecular Quality Assessment Scheme (HEMQAS)

NAAT-based detection of Soil Transmitted Helminths (STH), *Stongyloides* and *Schistosoma* in clinical stool samples

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star worms
Stop Anthelminthic Resistant Worms

GHENT UNIVERSITY

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skml

Partnership for Treating and Preventing Intestinal Worms
ChildrenWithoutWorms

NEGLECTED TROPICAL DISEASES SUPPORT CENTER

Helminth External Molecular Quality Assessment Scheme (HEMQAS)

Following successes of protozoa EQAS

Distribution of real helminth-pathogens in stool

- Field samples (& non-endemic controls)
- No lyophilized material nor DNA in artificial matrix

- Ethanol preservative for long stability => global participation
- Participants: research laboratories; reference laboratories (control programs); clinical laboratories

Panel:

Hookworm (*N. americanus* & *Ancylostoma spp.*), *Ascaris spp.*, *Trichuris trichiura*, *Stronglyoides stercoralis*, *Schistosoma spp.*

HEMQAS pilot
Study design and sample validation

2017/2018: 12 stool samples

Microscopy pre-screened => diluted in ethanol

2x4 DNA (purified from isolated adult worms/L3 larvae; high and low concentration)

Sample validation: 6 expert laboratories, 3 continents (4-5 labs per target)

Stability and Homogeneity

- Targets with high parasite load were consistently detected in 5 aliquots
- Within accepted range of intra-laboratory variation in Cq

HEMQAS pilot
Principle of scoring system & expert outcome

Sample positive for specific target

- Homogenous & stable, detected by all expert laboratories (by PCR)

Sample negative for specific target

- Not detected by any of the expert laboratories (by PCR)

Sample educational for specific target

- Low concentration of a target – not detected by each expert laboratory
- NB: some of these were (low) microscopy positive!

HEMQAS pilot
Principle of scoring system & expert outcome

Outcome of 12 stool samples when tested by 6 experts laboratories:

- 1x completely negative
- 2x with positive target(s) only
- 5x with mixed positive target(s) & educational target(s)
- 4x with educational target(s) only

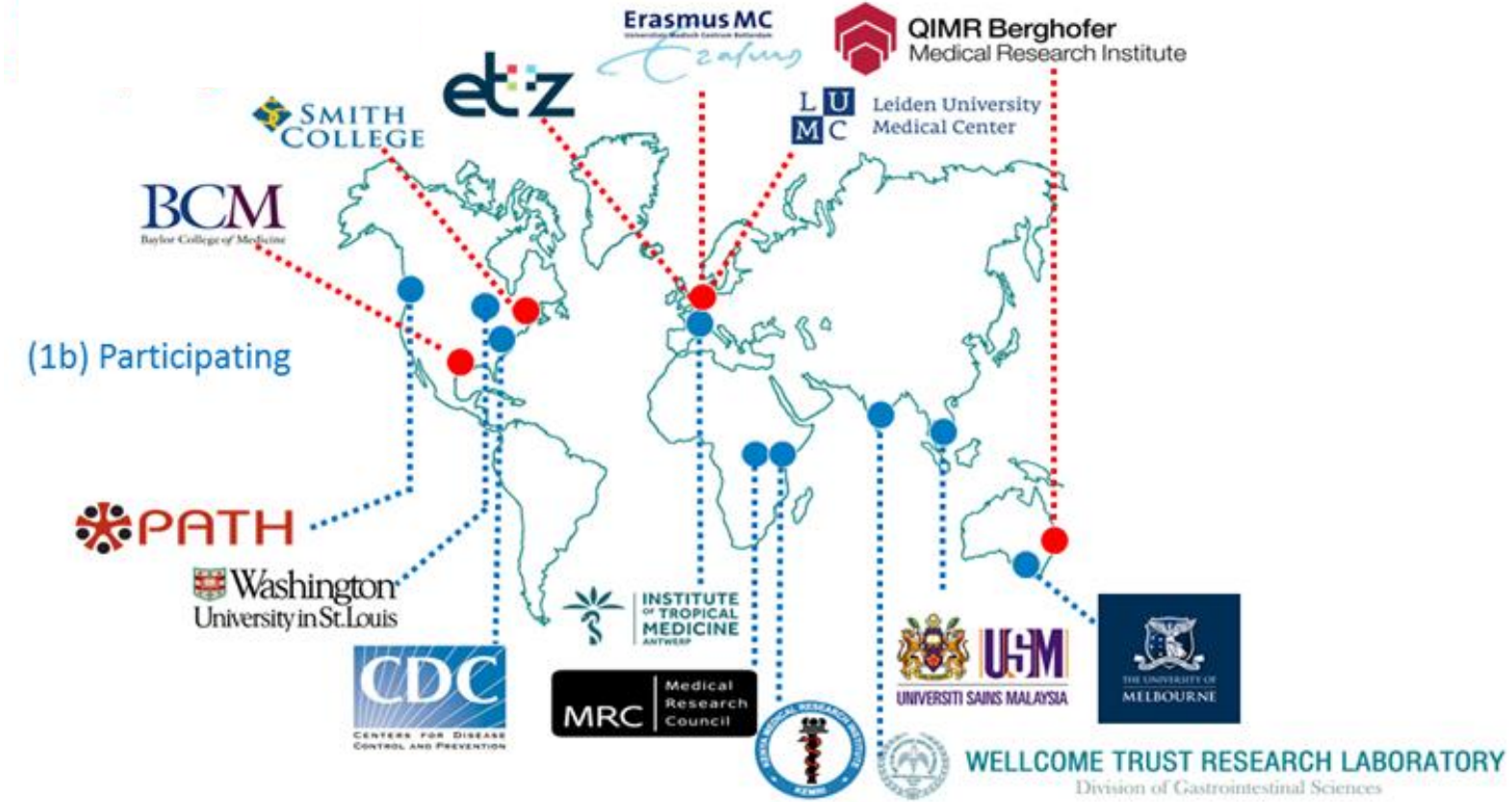
4 x DNA

Ascaris, Necator, Strongyloides, Schisto

Undiluted + 1:10

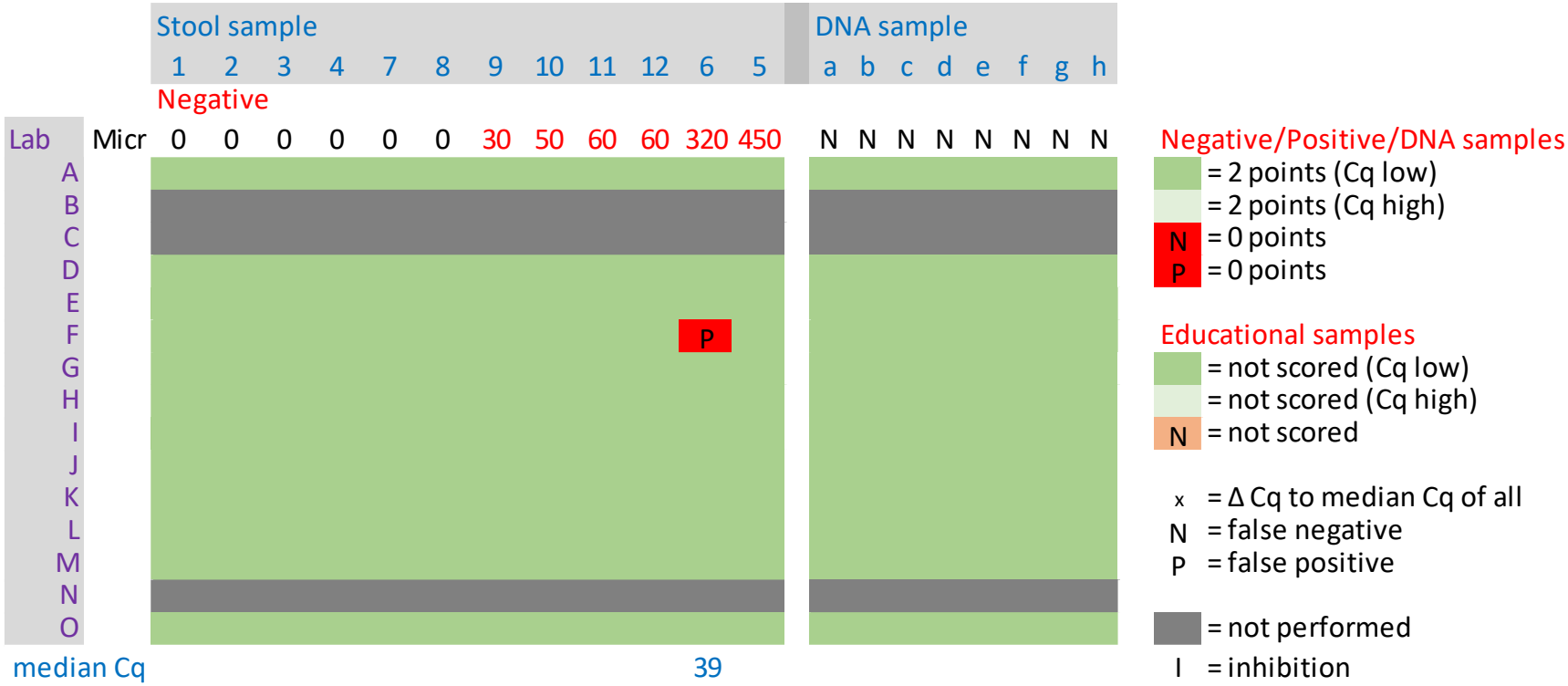
Sample	*Ascaris lumbricoides*	*Trichuris trichiura*	*Necator americanus*	*Ancylostoma duodenale*	*Strongyloides stercoralis*	*Schistosoma mansoni*
ST01	NEG	NEG	NEG	NEG	NEG	NEG
ST02	EDU	NEG	EDU	NEG	EDU*	NEG
ST03	EDU	EDU	NEG	NEG	NEG	NEG
ST04	NEG	POS	EDU	NEG	NEG	NEG
ST05	NEG	NEG	POS	NEG	NEG	NEG
ST06	POS	NEG	POS	NEG	EDU	NEG
ST07	POS	POS	NEG	NEG	NEG	NEG
ST08	EDU	EDU	NEG	NEG	NEG	NEG
ST09	EDU	EDU	POS	NEG	NEG	POS
ST10	NEG	EDU	NEG	NEG	NEG	NEG
ST11	POS	EDU	POS	NEG	NEG	POS
ST12	POS	EDU	POS	NEG	NEG	POS

Testing of samples: 15 laboratories, 5 continents
(including 6 expert laboratories)



HEMQAS pilot participants outcome

Ancylostoma



HEMQAS pilot participants outcome

Ascaris

	Stool sample												DNA sample							
	1	4	10	5	12	11	6	7	2	8	9	3	a	b	c	e	g	h	f	d
	Negative				Positive				Educational											
Lab	Micr	0	0	0	10	0	0	600	12700	0	0	10	3360	N	N	N	N	N	N	P	P
A						-3	-3	-7	-6	N	-4	0	-5							-3	-4
B																					
C																					
D						0	-2	-3	-4	N	-1	-1	-3							-1	-1
E						-2	0	-1	4	N	-1	-1	4							1	0,4
F						4	5	4	4	N	2	N	6							3	3
G						-13	-13	-12	-6	N	-15	-13	-7							-2	-3
H						N	3	2	N	N	3	1	N							-1	0,1
I						2	0	0	-1	N	2	0	0							0	-1
J						-1	0	1	1	N	0	0	3							0,3	0,1
K						0	-1	0	-2	N	-1		-1							0	0
L						0	2	0	4	N	3	1	N							0,5	-1
M						N	N	N	N	N	N	N	N							N	N
N																					
O						4	3	0	1	N	3	N	2							2	2
median Cq						33	31	33	27	-	32	36	29							33	30
Exp						1/5	4/5	4/5	4/5												

Negative/Positive/DNA samples

- = 2 points (Cq low)
- = 2 points (Cq high)
- N = 0 points
- P = 0 points

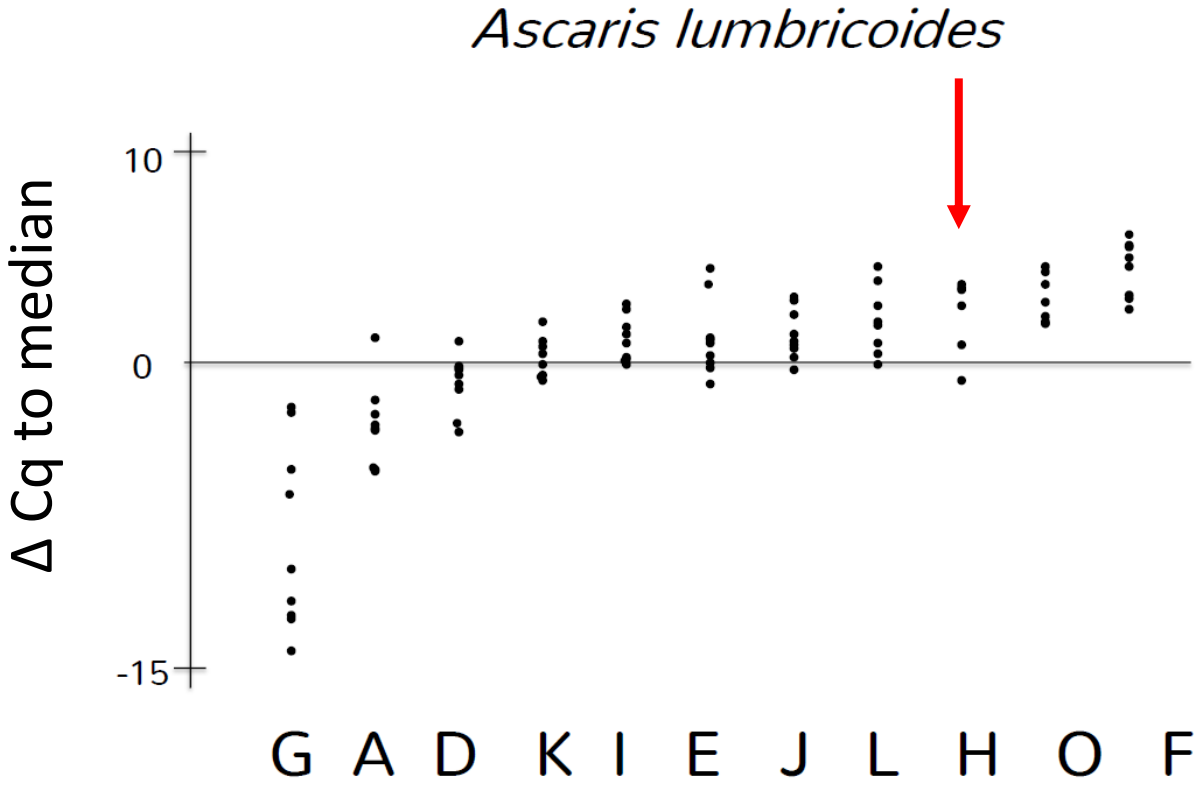
Educational samples

- = not scored (Cq low)
- = not scored (Cq high)
- N = not scored

x = Δ Cq to median Cq of all
N = false negative
P = false positive

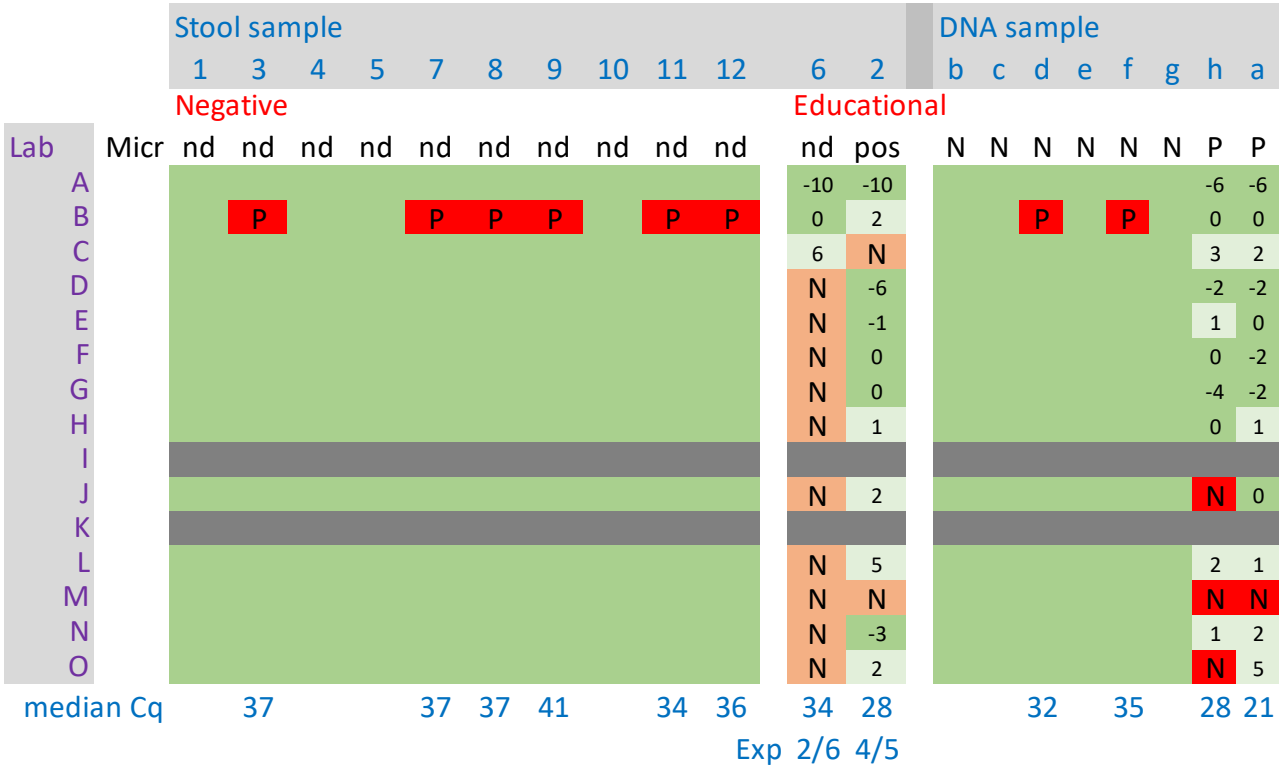
= not performed
I = inhibition

HEMQAS pilot participants outcome



HEMQAS pilot participants outcome

Strongyloides stercoralis



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HEMQAS pilot participants outcome

Trichuris trichiura

	Stool sample												DNA sample							
	1	2	5	6	4	7	3	12	9	10	11	8	a	b	c	d	e	f	g	h
	Negative				Positive		Educational													
Lab	Micr	0	0	0	0	40	280	0	0	10	40	40	60	N	N	N	N	N	N	N	N
A						-4	-2	-1	-1	-2	0	-1	-3								
B																					
C																					
D						-2	-1	1	4	2	3	4	0								
E						N	3	N	N	N	N	N	N								
F						N	0	N	N	N	0	0	1								
G						N	-2	N	N	N	N	N	N								
H						N	5	N	N	N	N	N	N								
I						4	6	N	N	N	N	N	N								
J						N	0	N	N	N	N	N	N								
K						N	0	N	0	N	0	0	0								
L						N	2	N	N	N	N	N	N								
M						N	N	N	N	N	N	N	N								
N																					
O						2	4	N	N	N	N	N	N								
median Cq						35	30	35	33	33	31	33	31								
Exp								1/5	2/5	3/5	3/5	3/5	3/5								

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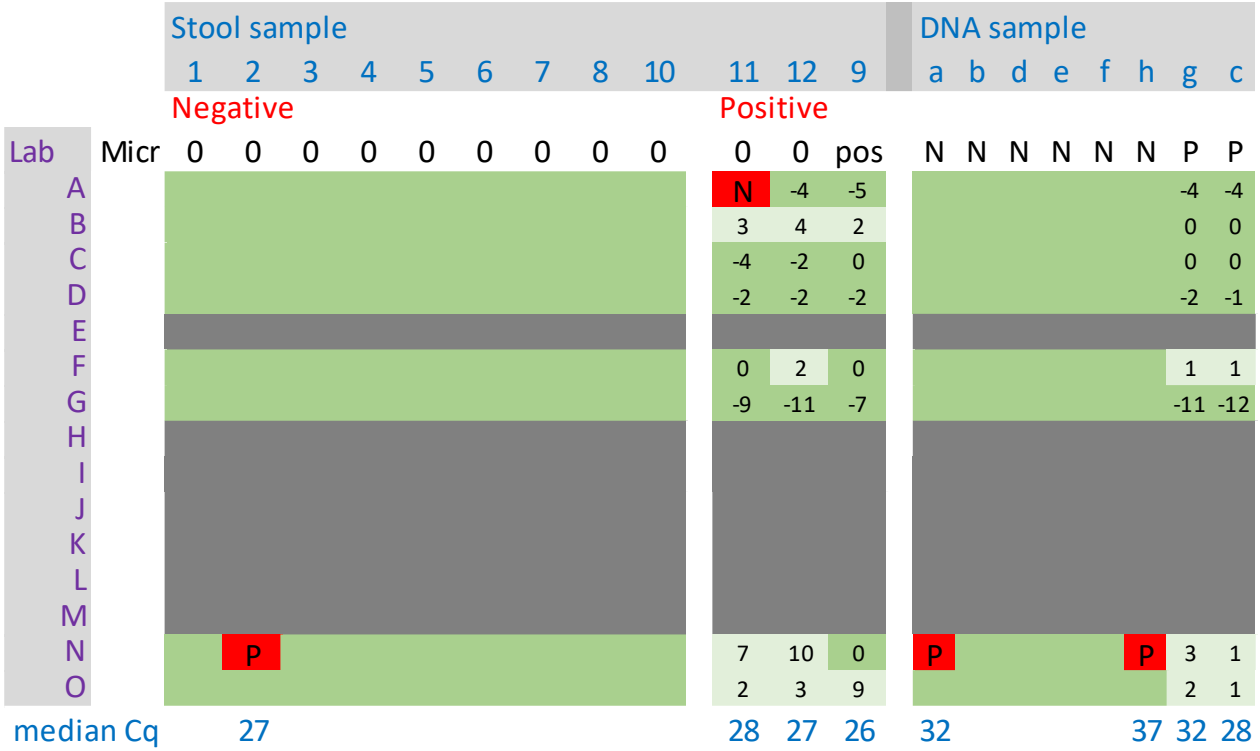
HEMQAS pilot participants outcome

Schistosoma



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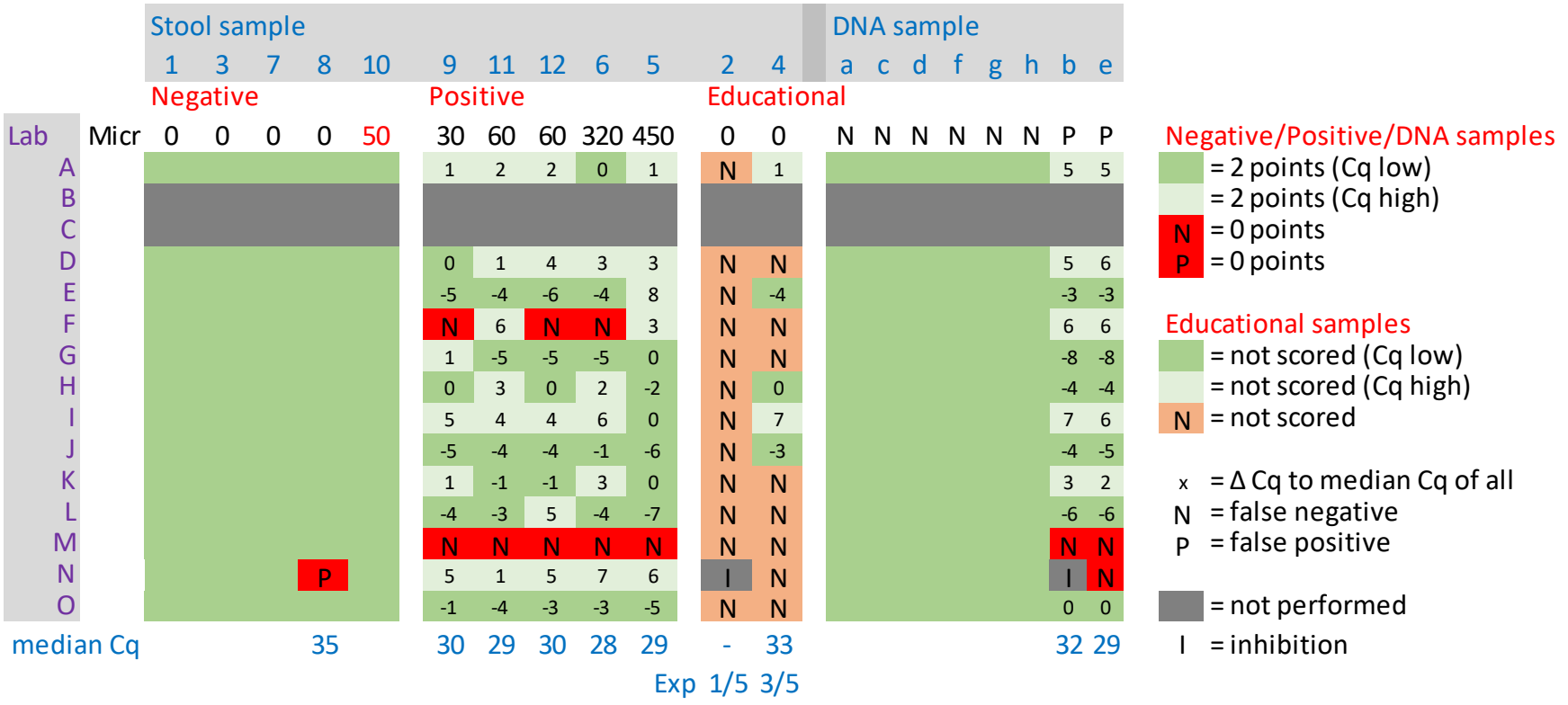
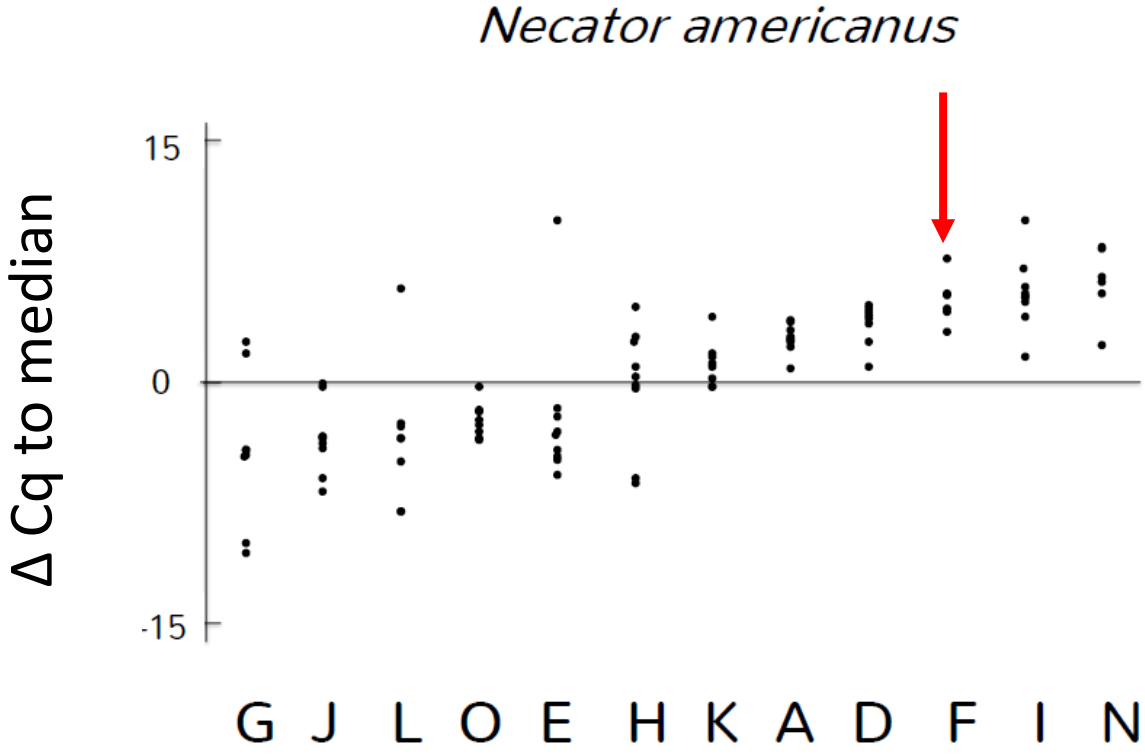
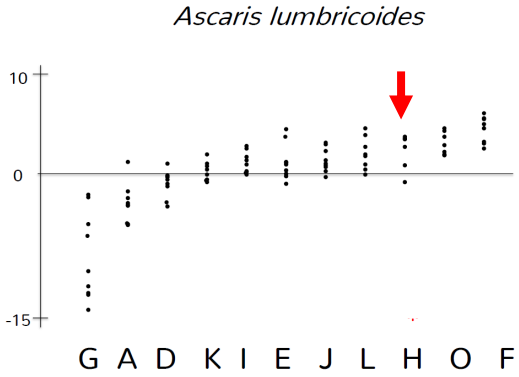
HEMQAS pilot participants outcome

Necator americanus



HEMQAS pilot participants outcome





HEMQAS pilot participants outcome

False negative results occurred

- mostly for *Trichuris* with max 92% positive score

Performance moderately related to intensity (microscopy) of infection

More failures seen in stool samples than in DNA samples

- isolation procedure crucial

False positive results occurred, in particular for *Strongyloides*

Clusters of poor-performers and good-performers.

- Cause of variations currently explored

HEMQAS pilot
Overall conclusions

- Proof of concept: validated, homogeneous samples
- Feasibility: world-wide EQAS distribution and reporting
- More variation in performance HEMQAS than GI protozoa
- Species-related performance differences, challenging samples
- Quantitative: reported Cq-values differ > 15 cycles

Need for quality control (and harmonisation) for NAAT methods

→ Yearly Helminth Molecular EQAS will start in 2019 by SKML

https://www.skml.nl/en/home/sections/parasitology

Acknowledgements – HEMQAS pilot

More info: www.skml.nl/en/home/sections/parasitology