Leiden University Medical Center

Stichting Kwaliteitsbewaking Medische Laboratoriumdiagno

Moleculaire diagnostiek van helminthen: een pilot rondzending voor detectie in feces

20 Maart 2018

Lisette van Lieshout
namens de sectie parasitologie van de SKML

Partnership for Treating and Preventing Intestinal Worms
ChildrenWithoutWorms

Stop Anthelminthic Resistant Worms

NEGLECTED TROPICAL DISEASES SUPPORT CENTER

Jaco Verweij

Piet Cools &
Bruno Levecke

GHENT UNIVERSITY

Bestaande EQAS voor feces protozoa PCR

Clin Chem Lab Med 2018; aop

Theo A. Schuurs [a], Rob Koelewijn, Eric A.T. Brienen [a], Titia Kortbeek [a], Theo G. Mank [a], Bert Mulder [a], Foekje F. Stelma [a], Lisette van Lieshout [a] and Jaap J. van Hellemond [a,*]

Harmonization of PCR-based detection of intestinal pathogens: experiences from the Dutch external quality assessment scheme on molecular diagnosis of protozoa in stool samples

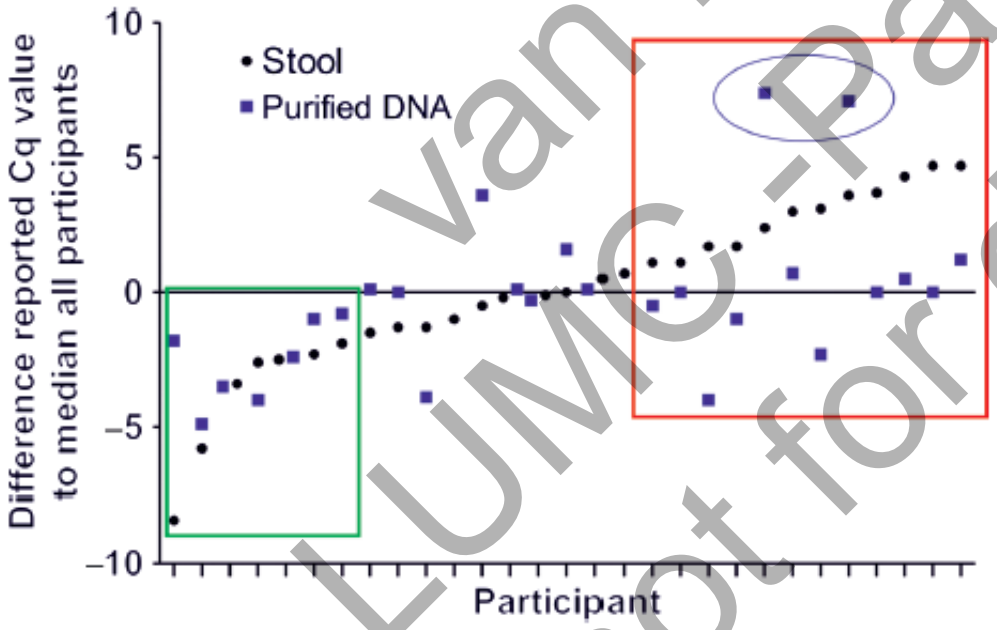


Figure 3: Reported Cq-values for Giardia lamblia in stool (black dots) and purified DNA (blue dots) specimens.
The reported Cq-values are plotted on the y-axis expressed as the reported Cq-value of the individual participant subtracted by the median of reported Cq-value of all participants. Each lane repre - sents a single participant and the order in which the participants are plotted on the x-axis differs from that in Figures 1 and 2.

Waarom een EQAS voor wormen PCR?

Voordelen microscopisch feces onderzoek wormen:

- Brede diagnostiek, zoveel verschillende species

Nadelen microscopisch onderzoek wormen:

- Komt relatief erg weinig voor in NL
- Hoe behouden van competentie
- €€€€

Waarom een EQAS voor wormen PCR?

Nadelen PCR

- Teveel potentiele targets (nooit compleet)
- €€€€; zeker indien nooit positief
- (Nog) Niet 24/7 of zelfs 8/5 beschikbaar
- Beperkt aantal centra heeft momenteel de capaciteit/kundigheid

Welke targets hoogste prioriteit?

- Welke in gebruik?
- Welke behoefte aan EQAS?

Welke helminth targets in een feces PCR?

Klinische setting?

- Welke het makkelijkst gemist?
- Welke klinisch het meest relevant?

Survey setting?

- “Identification of hot-spots” & “post-MDA monitoring”
- Welke het makkelijkst gemist?
- Welke het meest relevant?

Internationale markt voor een EQAS?

- Specifieke logistieke uitdagingen?
- Klinische materialen?

Is er behoefte aan helminthen PCR
Klinische setting in NL? Welk target eerst?

Transactions of the Royal Society of Tropical Medicine and Hygiene (2009) 103, 967–972

available at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/trstmh

REVIEW

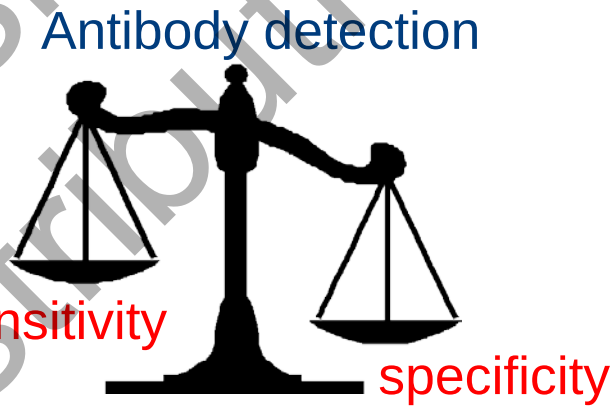
Strongyloidiasis—the most neglected of the neglected tropical diseases?

Annette Olsen[a,*], Lisette van Lieshout[b], Hanspeter Marti[c], Ton Polderman[b], Katja Polman[d], Peter Steinmann[e,f], Russell Stothard[g], Søren Thybo[h], Jaco J. Verweij[b], Pascal Magnussen[a]

Diagnosing *Strongyloides* in a Dutch setting

Serology

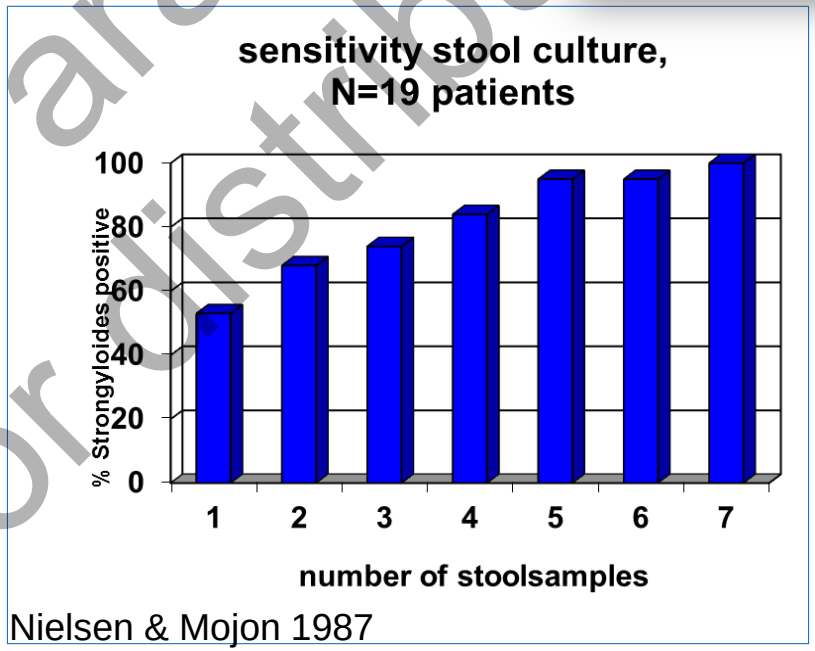
- Different formats (in-house, commercial)
- Screening of specific patients, migrants, chronic infections



Microscopy?

- Travelers (based on LUMC serology)
- Post-treatment monitoring (disseminated infections)

Diagnosis of *Strongyloides*
Microscopy based detection of larvae (no eggs!)



Nielsen & Mojon 1987

Differentiation between *Strongyloides* L1 and L3 larvae

L1 = rhabditoid larvae

lengths: 200-300 µm

short buccal cavity

large genital primordium

L3 = filariform larvae

lengths: 500-600 µm

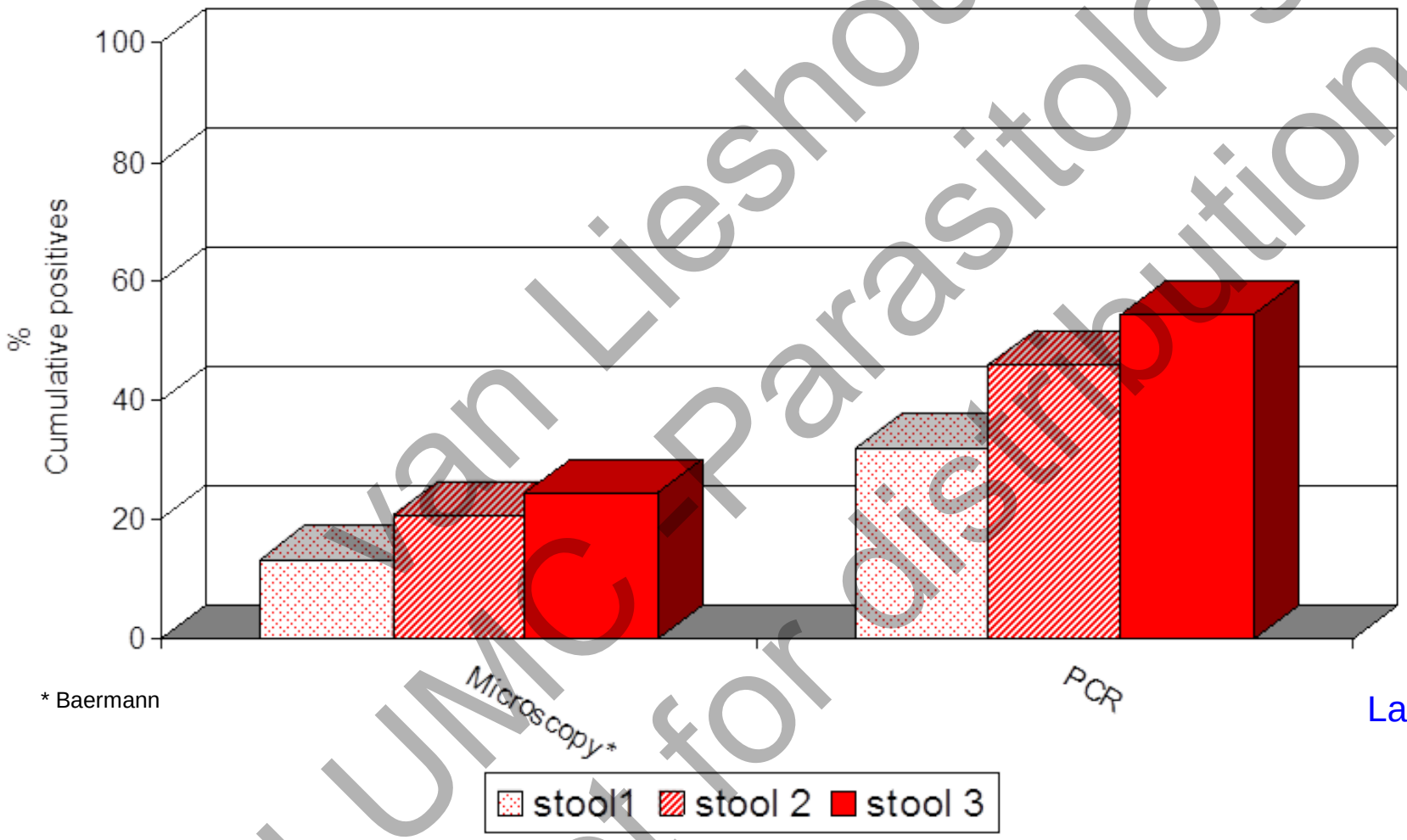
motile, slender

long oesophagus (>1/3)

no sheath, notched tail

Peru: *Strongyloides* survey
3 consecutive stool samples

PCR: Verweij *et al* (2009) – 18S ribosomal RNA gen



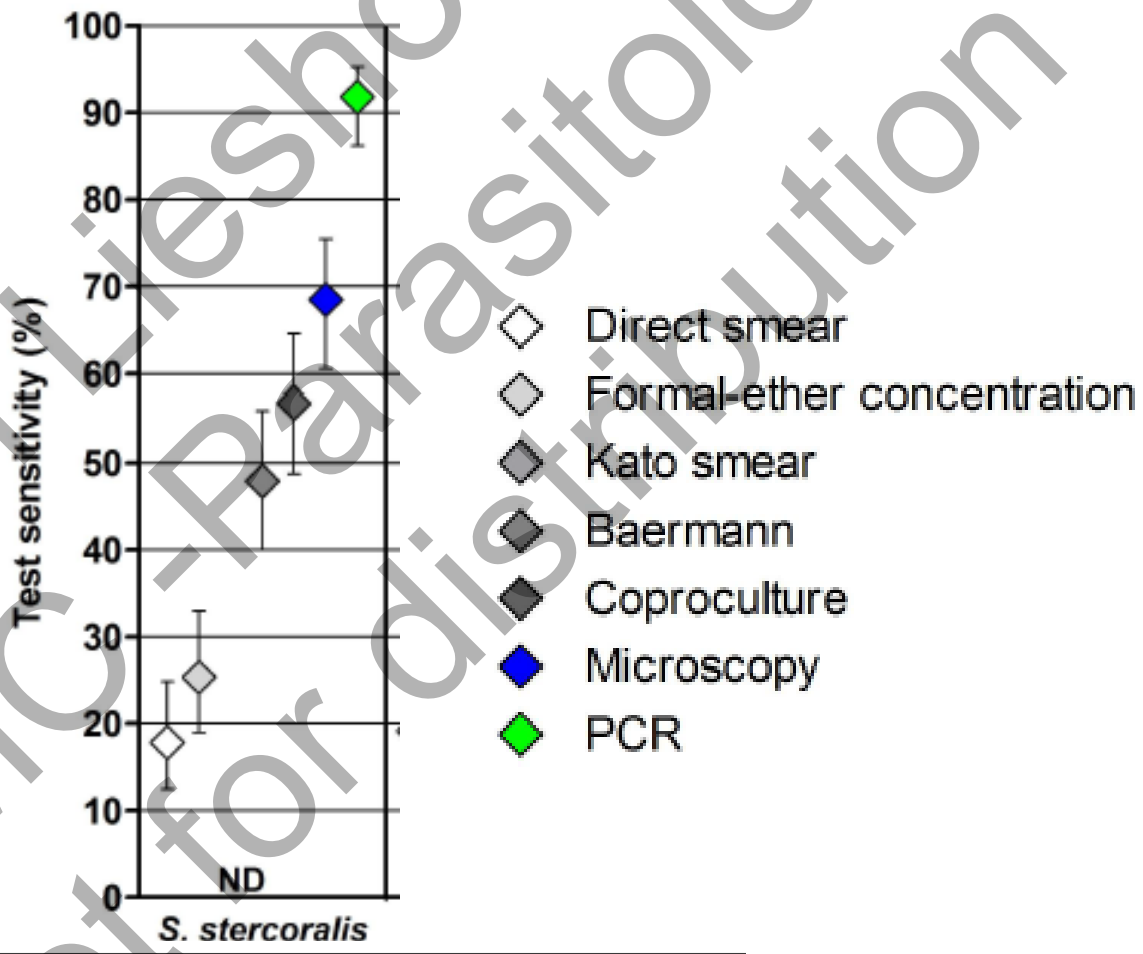
* Baermann

La Merced
(N=188)

Verweij *et al* (un published data)

Mozambique: *Strongyloides* survey
PCR > microscopy, but not 100% sensitivity

Meurs et al., 2017 Plos NTD
Strongyloides PCR: Verweij *et al* (2009) – 18S ribosomal RNA gen



PLOS Neglected Tropical Diseases | DOI:10.1371/journal.pntd.0005310 January 23, 2017

Results Antwerp Travel Clinic
N=2591 complete data

	Microscopy	PCR
E. histolytica/E.dispar	99	
E. histolytica		13
Giardia lamblia	95	149
Cryptosporidium	12	31
Strongyloides stercoralis	3 *	21**

*) Baermann performed in 121 clinically suspected cases only

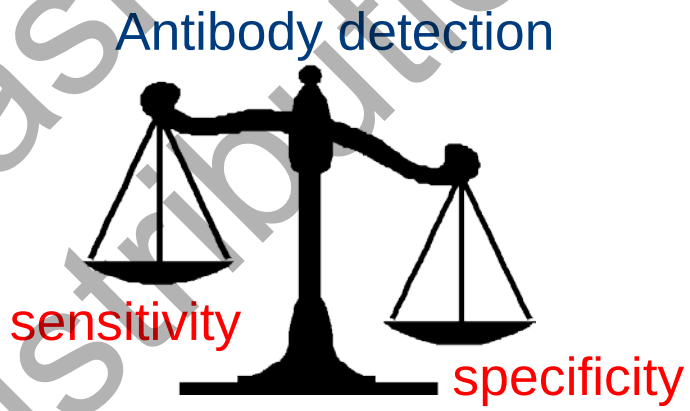
**) positive in microscopy N=3, serology N=7. Cases with eosinophilia N=5, clinical presentation N=4

(Ten Hove et al. 2009)

Diagnosing *Schistosoma* in a Dutch setting

Serology

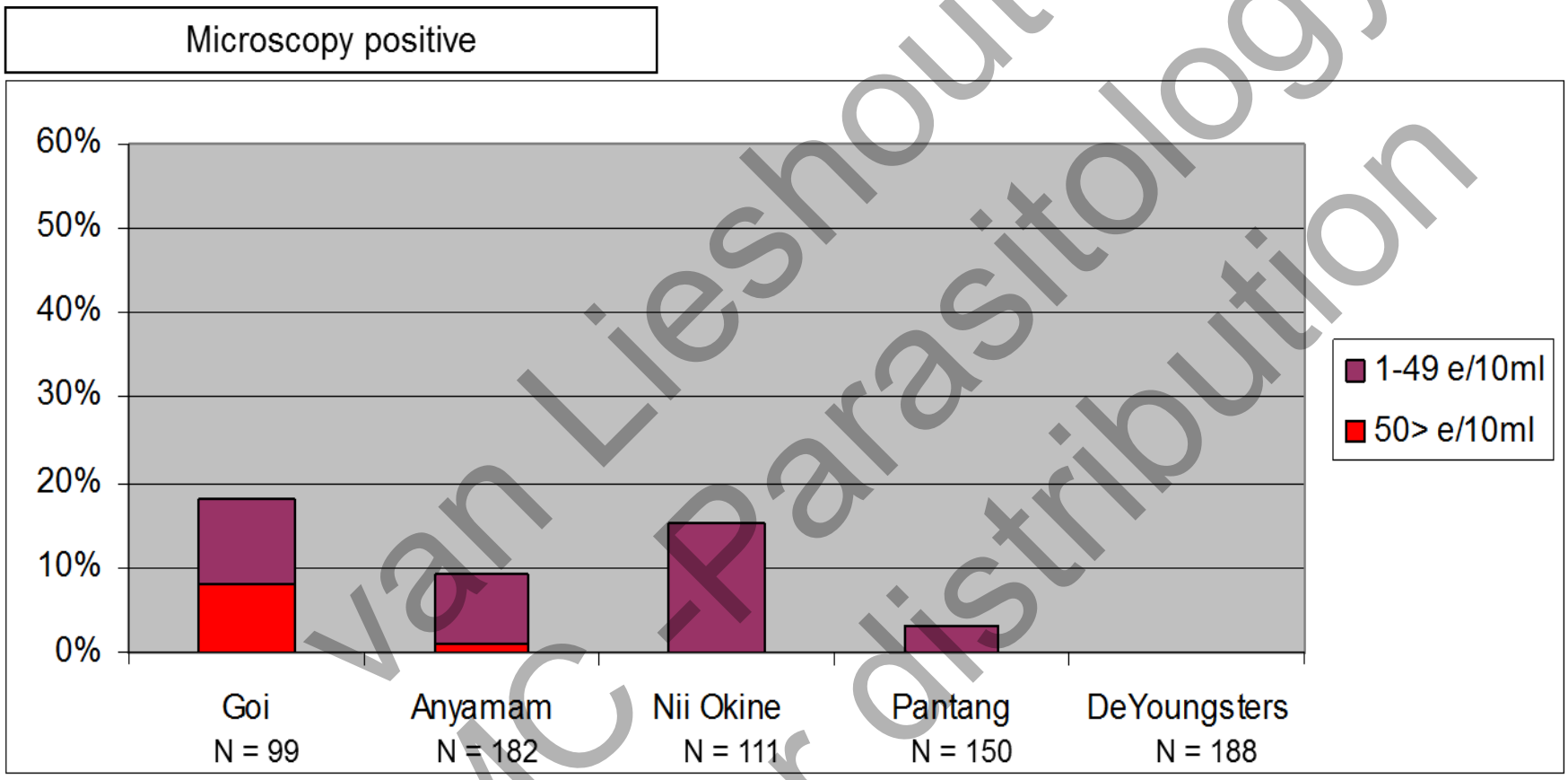
- Different formats (in-house, commercial)
- Screening of specific patients, travelers



Microscopy?

- Migrants, chronic infection (based on LUMC serology)
- Post-treatment monitoring

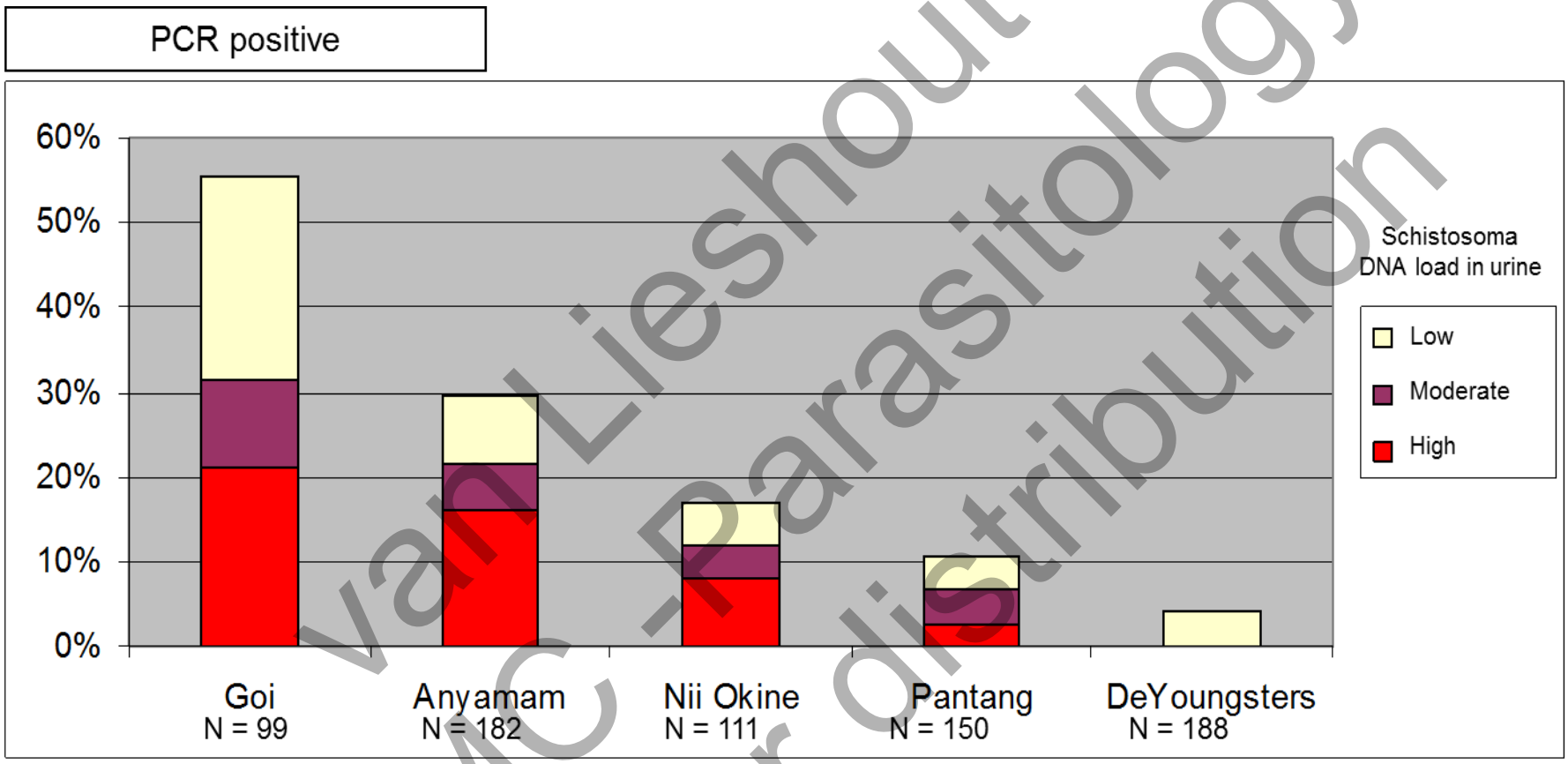
S. haematobium school children Ghana (N=730)



Microscopy: eggs detected in 10 mL of urine

Aryeetey *et al* (2013)

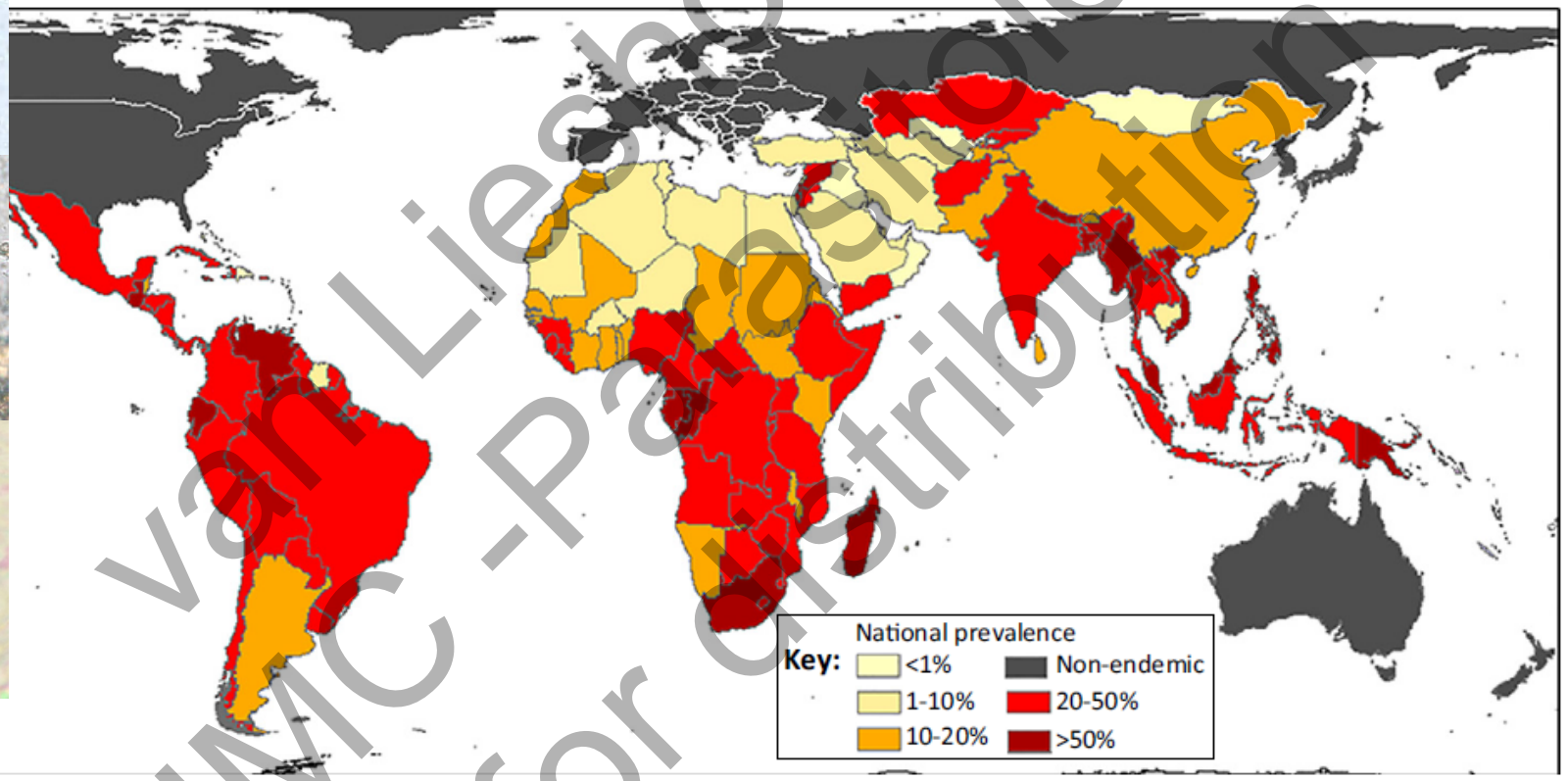
S. haematobium school children Ghana (N=730)



Schistosoma DNA isolated from 200 µl urine

Aryeetey *et al* (2013)

Distribution of Soil Transmitted Helminths
≈ 1.4 billion people infected



Trends in Parasitology

Figure 1. Global Distribution of Soil-Transmitted Helminths, 2010. Data from the *Global Atlas of Helminth Infection* were sourced to derive global estimates of soil-transmitted helminths (*Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus*, and *Ancylostoma duodenale*) [83]. Reproduced, with permission, from [83].

PCR vs microscopy – what is the truth prevalence?

Indonesia

Household Randomization

STOOL

Lab-Jakarta: FEC

Stool aliquot

Household Randomization

STOOL

Stool aliquot

Local: DS, FEC, KK, BM, CC

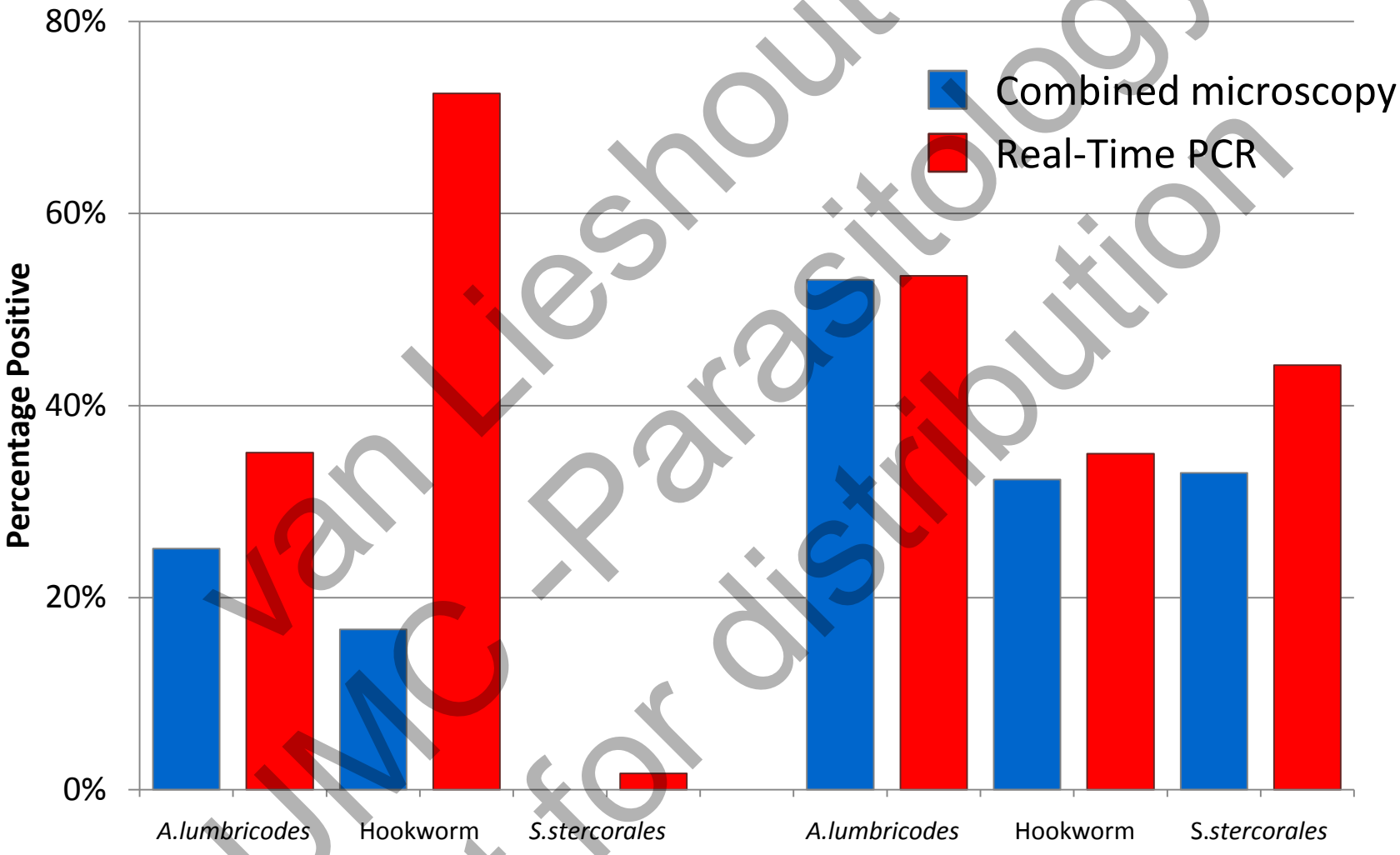
Mozambique

ANAS-PCR

LUMC Leids Universitair Medisch Centrum

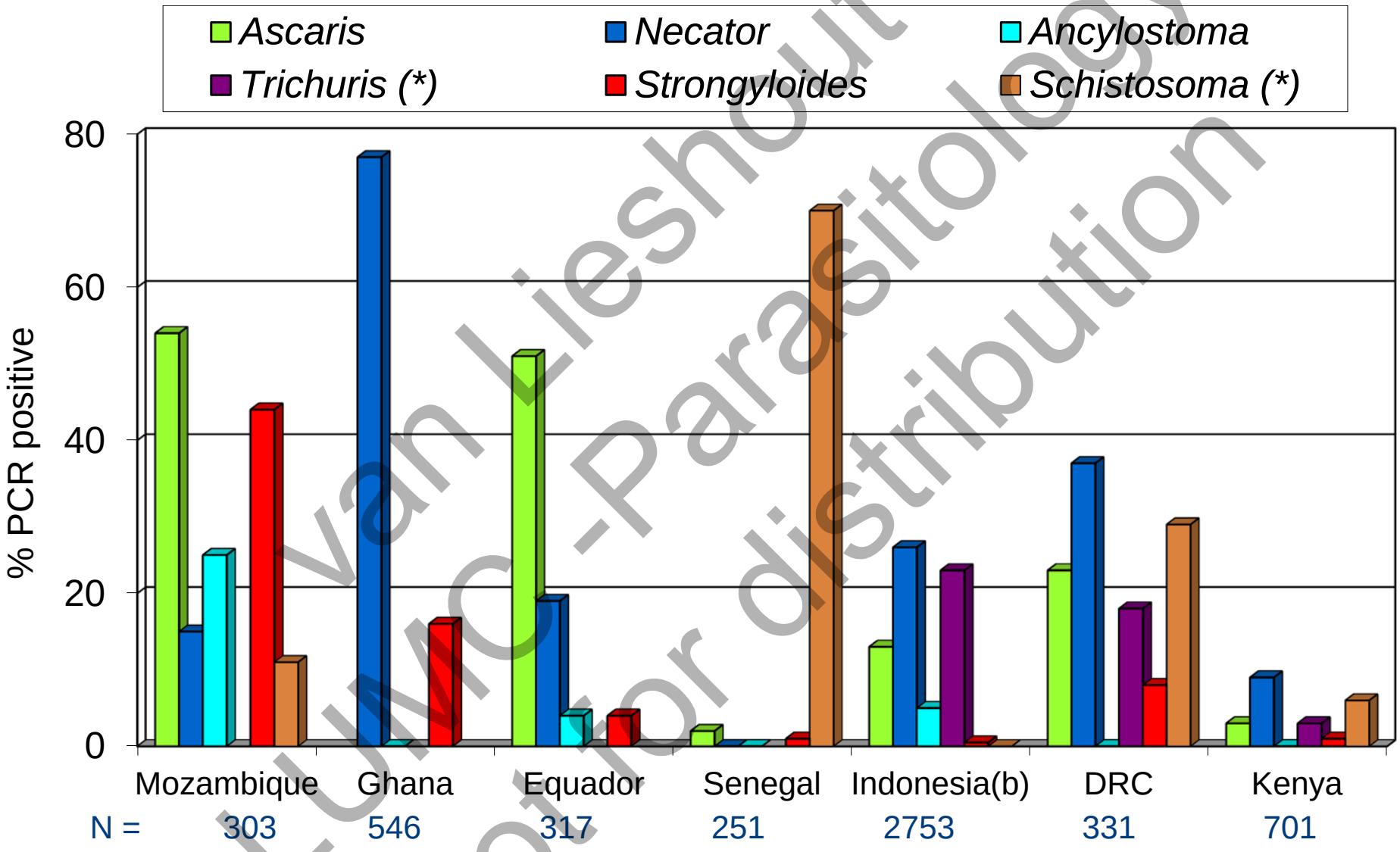
Kaisar *et al* (submitted)

PCR vs microscopy – what is the truth prevalence?



Kaisar *et al* (submitted)

Community-based surveys



(*= not tested at each survey)

LUMC multiplex real-time PCR 2007-2016 (unpublished data)

Welke helminth targets in een feces PCR?

Klinische setting?

- *Strongyloides stercoralis*
- *Schistosoma* (*S. mansoni*)

Survey setting?

- *Ancylostoma duodenale, Necator americanus*
- *Ascaris lumbricoides, Trichuris trichiura*

Internationale markt voor een EQAS?

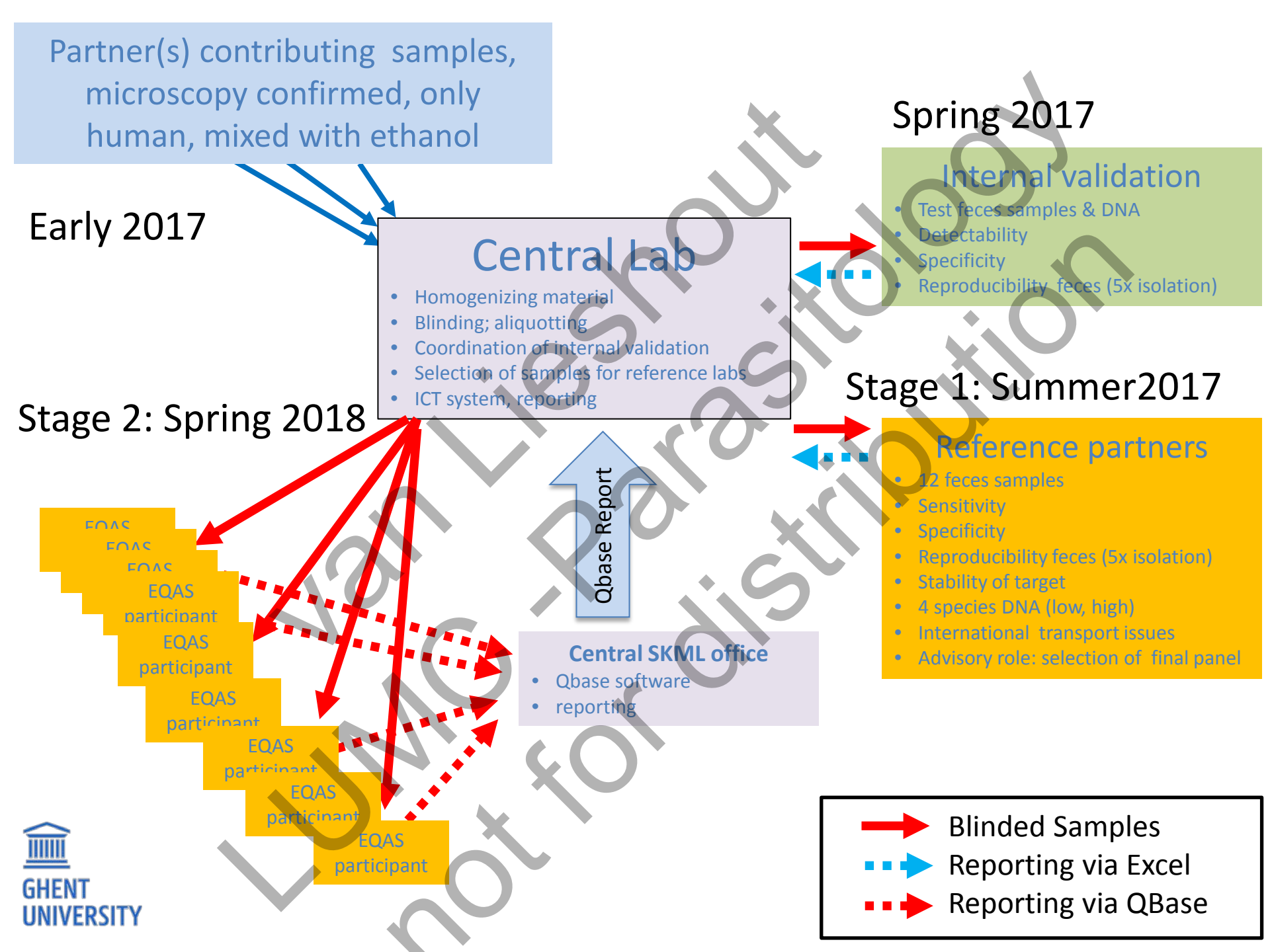
- Ja, ook in STH endemische gebieden
- Klinische materialen, maar ook DNA
- Feces in ethanol; stabiel voor opslag en transport

Pilot for helminth PCR EQAS – 2017/2018

HEMQAS

- Consortium of 18 academic, clinical, and public health laboratories and organizations from both endemic and non-endemic STH countries
- Clinical material from the field (mainly Gent)
  - Microscopy positive
  - Additional targets cannot be excluded
  - Negative material also included in the panel

- Central lab (NL) & 5 reference laboratories (NL 2x, USA 2x, Australia)
- Reproducibility, stability (12 feces in ethanol, 8 DNA)


Partner(s) contributing samples, microscopy confirmed, only human, mixed with ethanol
Early 2017
Central Lab
Homogenizing material
Blinding; aliquotting
Coordination of internal validation
Selection of samples for reference labs
ICT system, reporting
Spring 2017
Internal validation
Test feces samples & DNA
Detectability
Specificity
Reproducibility feces (5x isolation)
Stage 1: Summer2017
Reference partners
12 feces samples
Sensitivity
Specificity
Reproducibility feces (5x isolation)
Stability of target
4 species DNA (low, high)
International transport issues
Advisory role: selection of final panel
Stage 2: Spring 2018
EQAS participant
Qbase Report
Central SKML office
Qbase software
reporting
Blinded Samples
Reporting via Excel
Reporting via QBase
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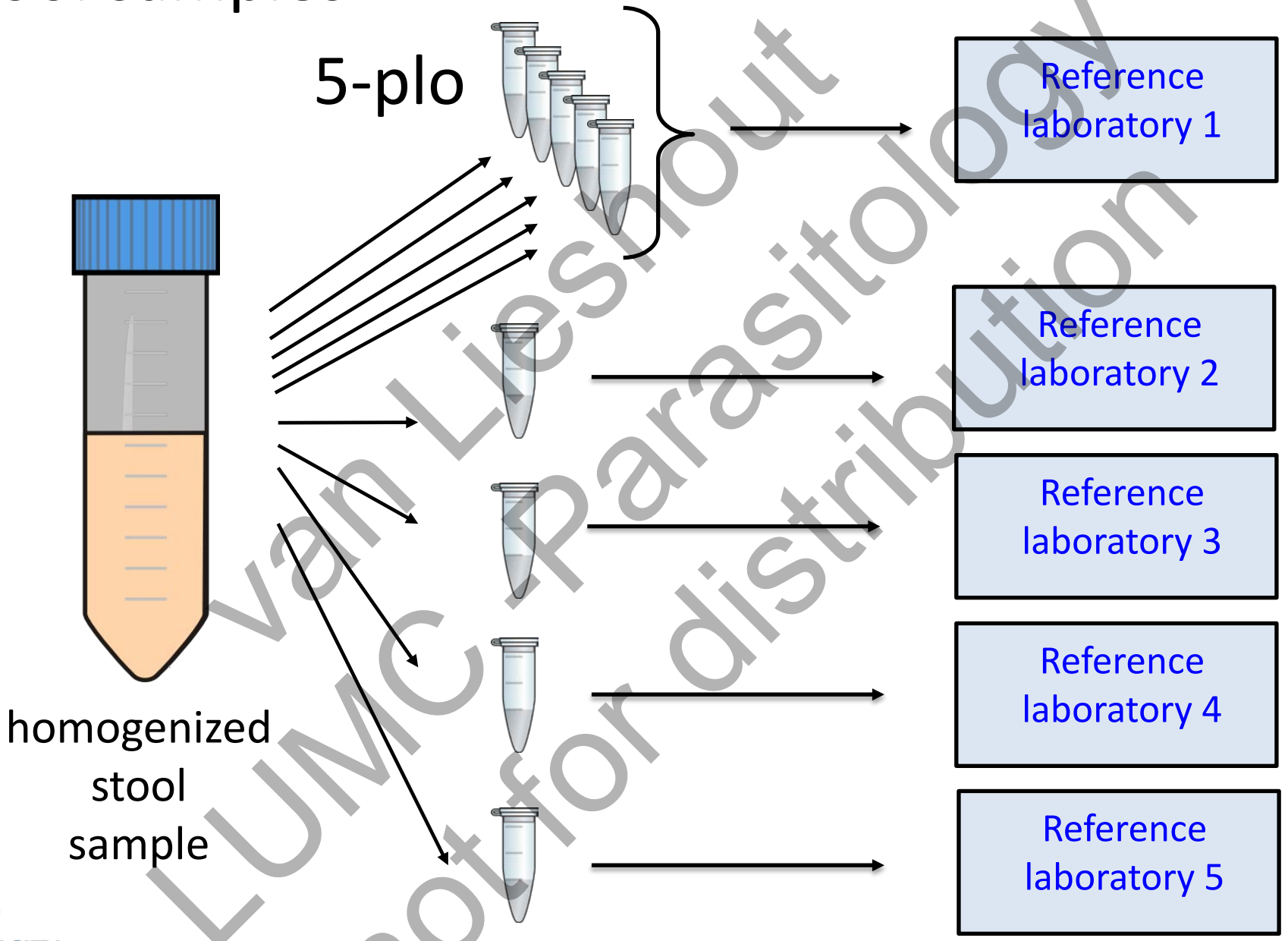
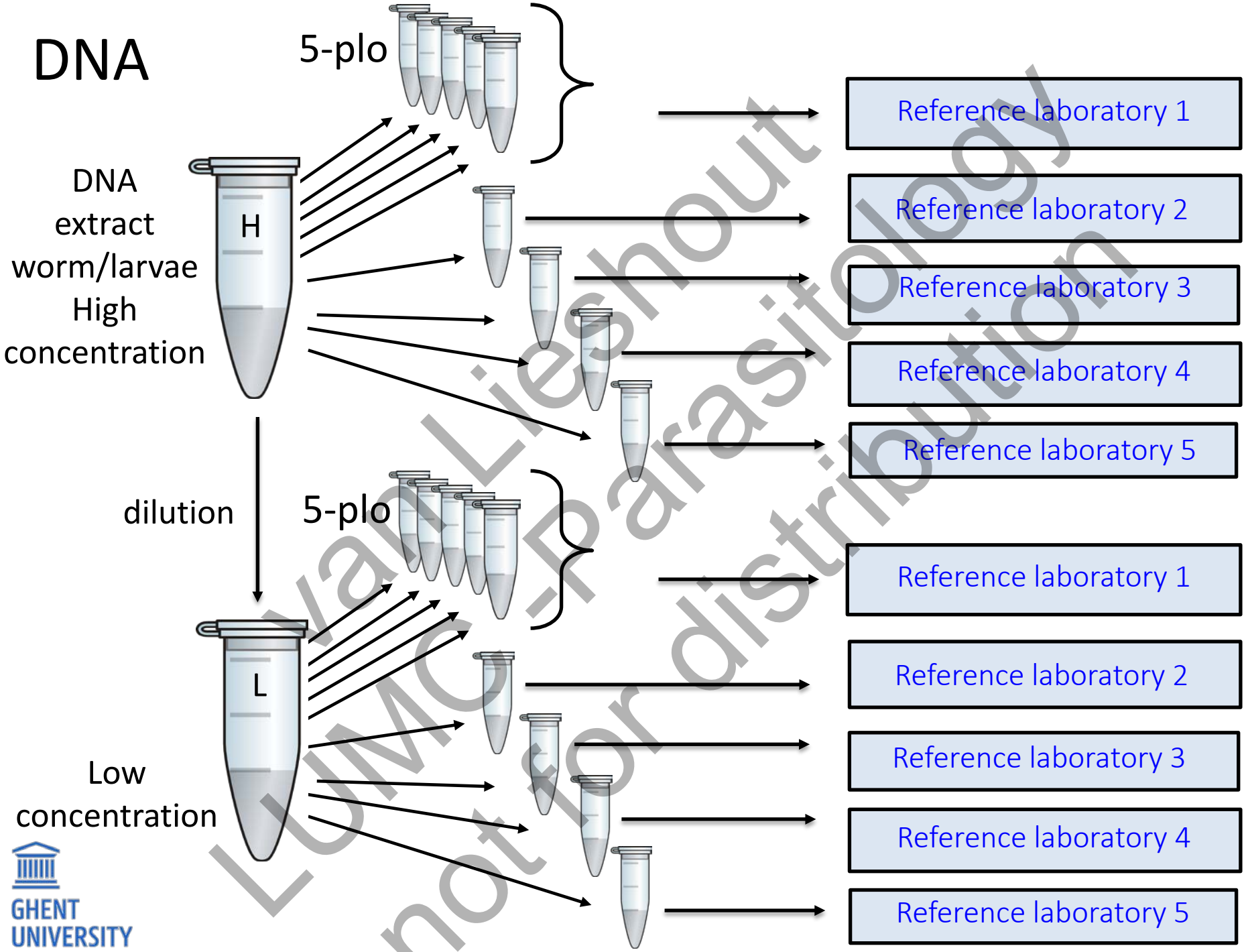
van Lieshout LUMC - Parasitology not for distribution

stool samples



DNA



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Pilot for helminth PCR EQAS – 2017/2018

Final judgement per sample

- Positive and reproducible for target X:
  - All 5-plo positive and within Cq range (X±2SD or 2Ct)
  - Positive by all 5 reference labs
- Negative for target Y:
  - All 5-plo negative
  - Negative by all 5 reference labs
- Educational for target Z:
  - Not uniformly positive (low loads)

Outcome of stage 1
Pilot for helminth PCR EQAS – 2017/2018

Reference labs

Each of 12 stool and 8 DNA samples suitable to be send out

Clear what is positive, negative, educational

Illustrative examples: sample A

- Microscopy: species X + species Y
- PCR: 100% species X + 60% species Y
- PCR: 100% species V + 100% species W; microscopy missed
- PCR: 0% species S + 0% species T
- All 5-plo accordingly

Outcome of stage 2
Pilot for helminth PCR EQAS – 2017/2018

Participating labs (including reference labs)

Received 12 stool (ethanol) and 8 DNA samples

Extensive Qbase questionnaire about used procedures

Summer 2018: analysis of data