Universiteit Antwerpen

Studying the pathogenesis of Onchocerciasis-associated epilepsy

Amber Hadermann

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Onchocerciasis-associated epilepsy (river epilepsy)

What is known?

What is not known?

Onchocerciasis-associated epilepsy (river epilepsy)

What is known?

Studies from the past

What is not known?

OAE-pathogenesis studies done in the past

1. Link between mf* load and epilepsy

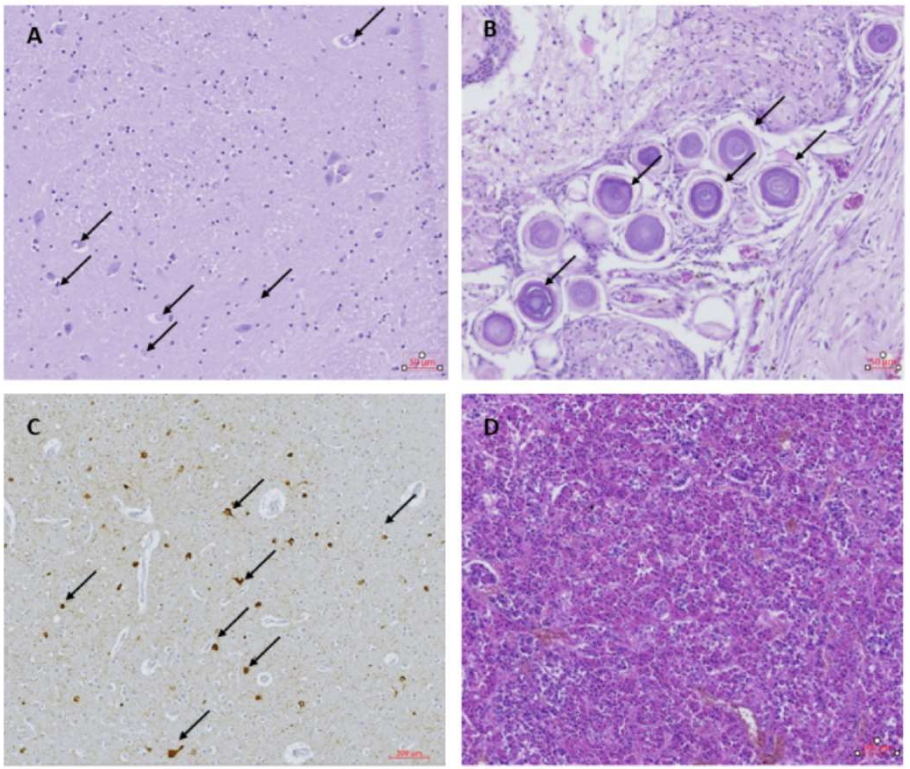
*mf = microfilaria

OAE-pathogenesis studies done in the past

1. Link between mf load and epilepsy

2. Ventriculitis scarring involving choroid plexus, gliosis and psammoma bodies found during **post-mortem** case study

Figure 1. (**A**) Thalamus with inclusions and pseudo-inclusions (black arrows); Hematoxylin & Eosin (H&E) staining. (**B**) Choroid plexus containing psammoma bodies (black arrows); H&E. (**C**) Frontal cortex with tau-reactive neurofibrillary tangles and threads (black arrows); AT8. (**D**) Pituitary gland; H&E.



OAE-pathogenesis studies done in the past

1. Link between mf load and epilepsy
2. Ventriculitis scarring involving choroid plexus, gliosis and psammoma bodies found during post-mortem case study
3. No mf nor OV-DNA found in OAE CSF*

Onchocerca volvulus is not detected in the cerebrospinal fluid of persons with onchocerciasis-associated epilepsy

An Hotterbeekx,[a] Stephen Raimon,[b] Gasim Abd-Elfarag,[c,d] Jane Y. Carter,[e] Wilson Sebit,[f] Abozer Suliman,[g] Joseph Nelson Siewe Fodjo,[a] Peter De Witte,[h] Makoy Yibi Logora,[i] Robert Colebunders,[a,*] and Samir Kumar-Singh[j]

Microfilariae in the cerebrospinal fluid, and neurological complications, during treatment of onchocerciasis with diethylcarbamazine

Duke B.O.L.; Vincelette J.; Moore P.J.

*CSF = cerebrospinal fluid

OAE-pathogenesis studies done in the past

1. Link between mf load and epilepsy

2. Ventriculitis scarring involving choroid plexus, gliosis and psammoma bodies found during post-mortem case study

3. No mf nor OV-DNA found in OAE CSF

4. Link between occurrence of febrile seizures and OAE
   →Genetic predisposition?

OAE-pathogenesis studies done in the past

5. Tauopathy?

Nodding syndrome in Uganda is a tauopathy

Michael S. Pollanen[1,2] · Sylvester Onzivua[3] · Janice Robertson[4] · Paul M. McKeever[4] · Francis Olawa[5] · David L. Kitara[6] · Amanda Fong[2]

Neuroinflammation and Not Tauopathy Is a Predominant Pathological Signature of Nodding Syndrome

An Hotterbeekx, PhD, Martin Lammens, MD, PhD, Richard Idro, PhD, Pamela R. Akun, MD, Robert Lukande, MD, PhD, Geoffrey Akena, MD, Avindra Nath, MD, Joneé Taylor, MD, Francis Olwa, MD, PhD, Samir Kumar-Singh, MD, PhD, and Robert Colebunders, MD, PhD

OAE-pathogenesis studies done in the past

5. Tauopathy?

6. Leiomodin-1 autoimmune disease?

Nodding syndrome may be an autoimmune reaction to the parasitic worm *Onchocerca volvulus*

Tory P. Johnson,[1] Richa Tyagi,[1] Paul R. Lee,[1] Myoung-Hwa Lee,[1] Kory R. Johnson,[2] Jeffrey Kowalak,[3] Abdel Elkahloun,[4] Marie Medynets,[5] Alina Hategan,[1] Joseph Kubofcik,[6] James Sejvar,[7] Jeffrey Ratto,[8] Sudhir Bunga,[8] Issa Makumbi,[9] Jane R. Aceng,[9] Thomas B. Nutman,[6] Scott F. Dowell,[10] and Avindra Nath[1,*]

No Evidence for the Involvement of Leiomodin-1 Antibodies in the Pathogenesis of Onchocerciasis-Associated Epilepsy

by An Hotterbeekx [1,2,*], Melissa Krizia Vieri [1], Melanie Ramberger [3], Ashraf Jozefzoon-Aghai [3], Michel Mandro [4], Floribert Tepage [5], Alfred Dusabimana [1], Samir Kumar-Singh [2], Maarten J. Titulaer [3] and Robert Colebunders [1]

OAE-pathogenesis studies done in the past

Protection or susceptibility to devastating childhood epilepsy: Nodding Syndrome associates with immunogenetic fingerprints in the HLA binding groove

Gil Benedek[1]*, Mahmoud Abed El Latif[1], Keren Miller[1], Mila Rivkin[2], Ally Ahmed Ramadhan Lasu[3], Lul P. Riek[4], Richard Lako[5], Shimon Edvardson[6], Sagit-Arbel Alon[7], Eithan Galun[2], Mia Levite[2,8]

5. Tauopathy?
6. Leiomodin-1 autoimmune disease?
7. HLA potential co-factor

OAE-pathogenesis studies done in the past

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Problem?

→ Samples Post-Epileptic-Onset

More representable sample

The ideal sample: Longitudinal CSF samples of (non-)OAE children

→ **Problem?** Ethics!

→ **Solution?** CSF children with febrile seizures

→ Follow-up over time for OAE

Onchocerciasis-associated epilepsy (river epilepsy)

What is known?

What is not known?

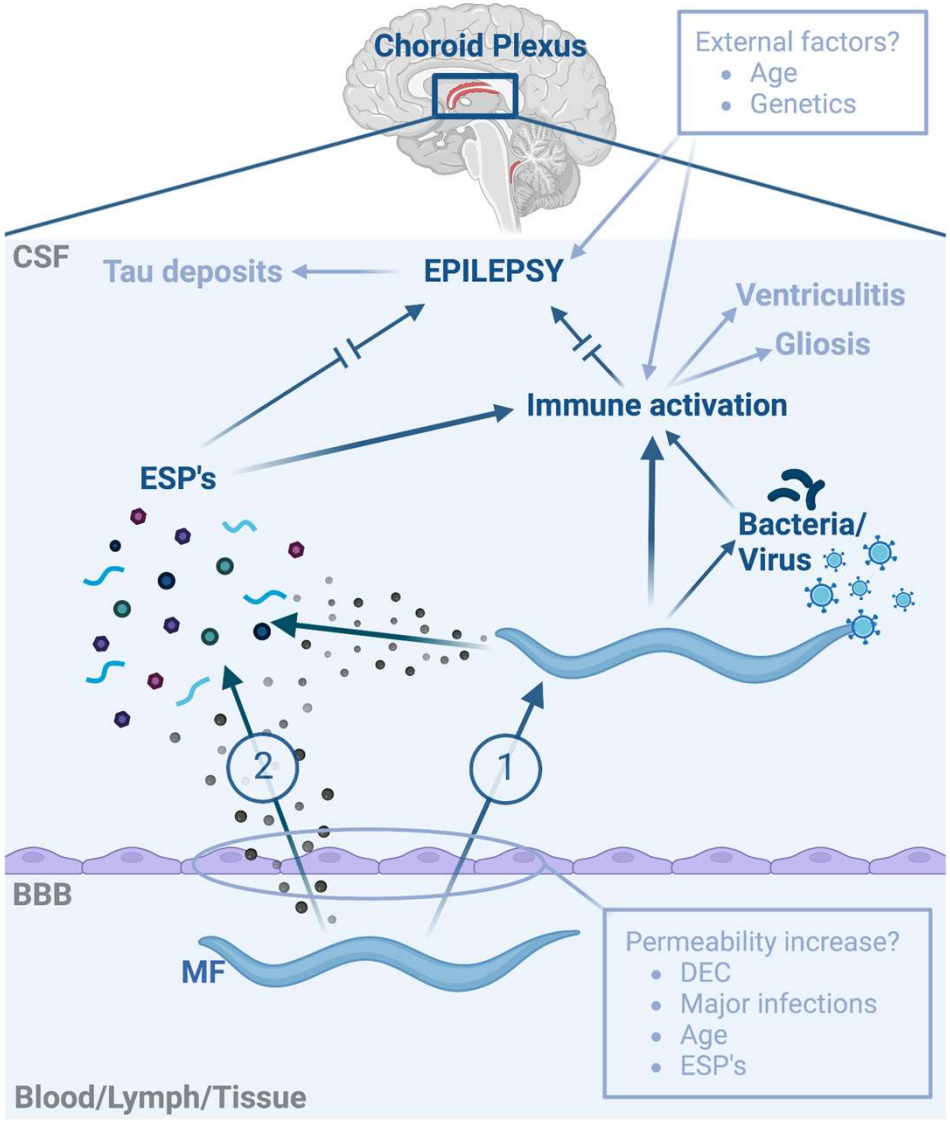
Hypotheses and ongoing studies

Main hypotheses

1. Microfilaria pass directly through the blood-brain barrier (**BBB**) to:
   A. Release Excretory/Secretory Products (**ESP**) that cause seizures either directly or indirectly
   B. Induce the immune system to cause seizures, directly or indirectly

2. Microfilaria release ESP's in the periphery that cross the BBB to cause seizures either directly or indirectly

+ Identify potential co-factors



Material and methods

- Metagenomic analysis → Fly (1) + Worm (2)
  A. Basic biology
  B. Patient samples (Serum/CSF)
  C. Link to epilepsy?

- Identify and characterize ESP's → Proteomics (3) + RNA (4)
  A. Basic biology all worm stages
  B. Patient samples (Serum/CSF)
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- Identify cofactors → HLA (5) + Parasitic (6) Tolarance?
  A. Link to epilepsy?

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  A. Link to epilepsy?

Metagenomics: Hypothesis

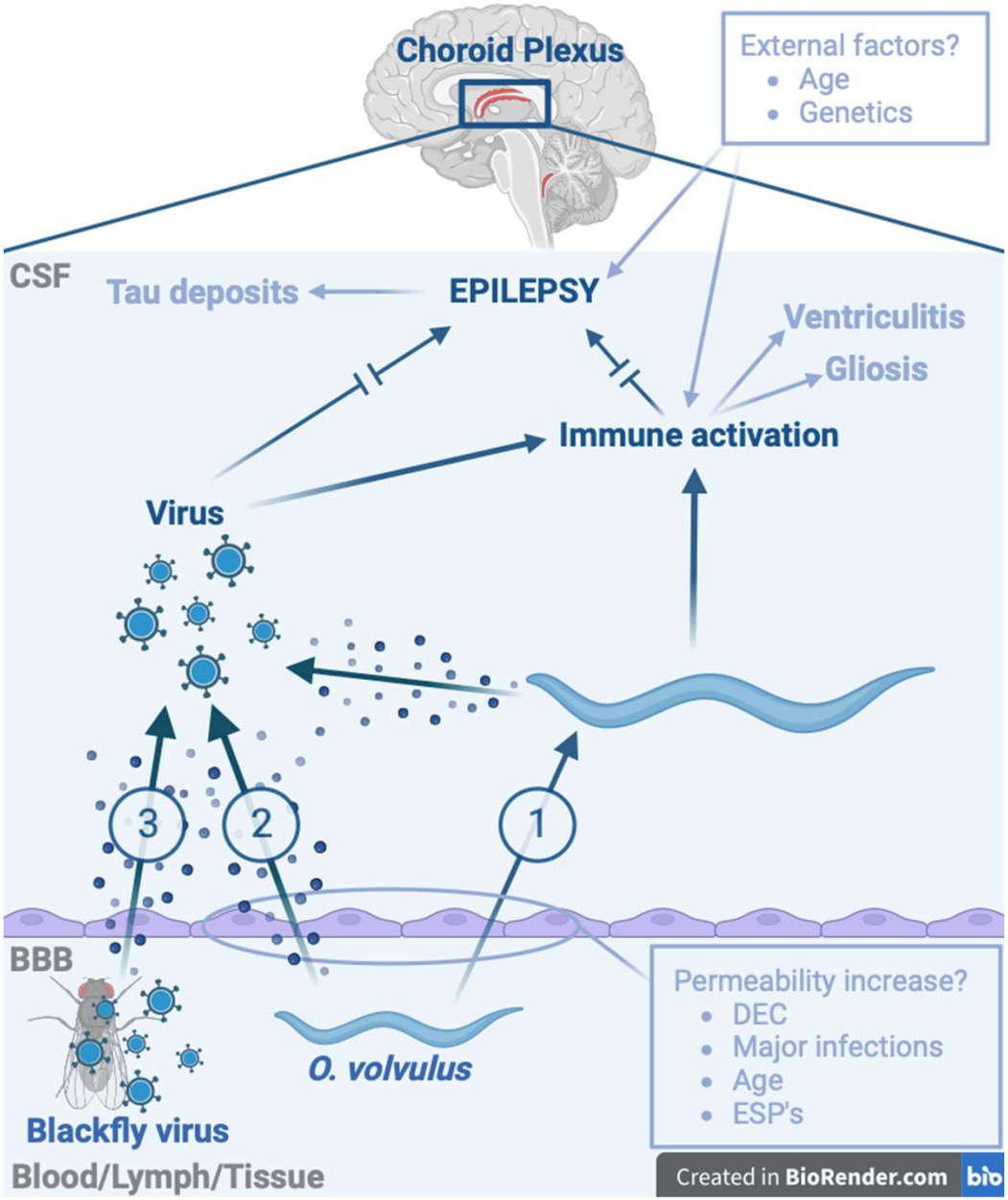
Emergence and re-emergence of mosquito-borne arboviruses

Yan-Jang S Huang [1], Stephen Higgs [2], Dana L Vanlandingham [1]

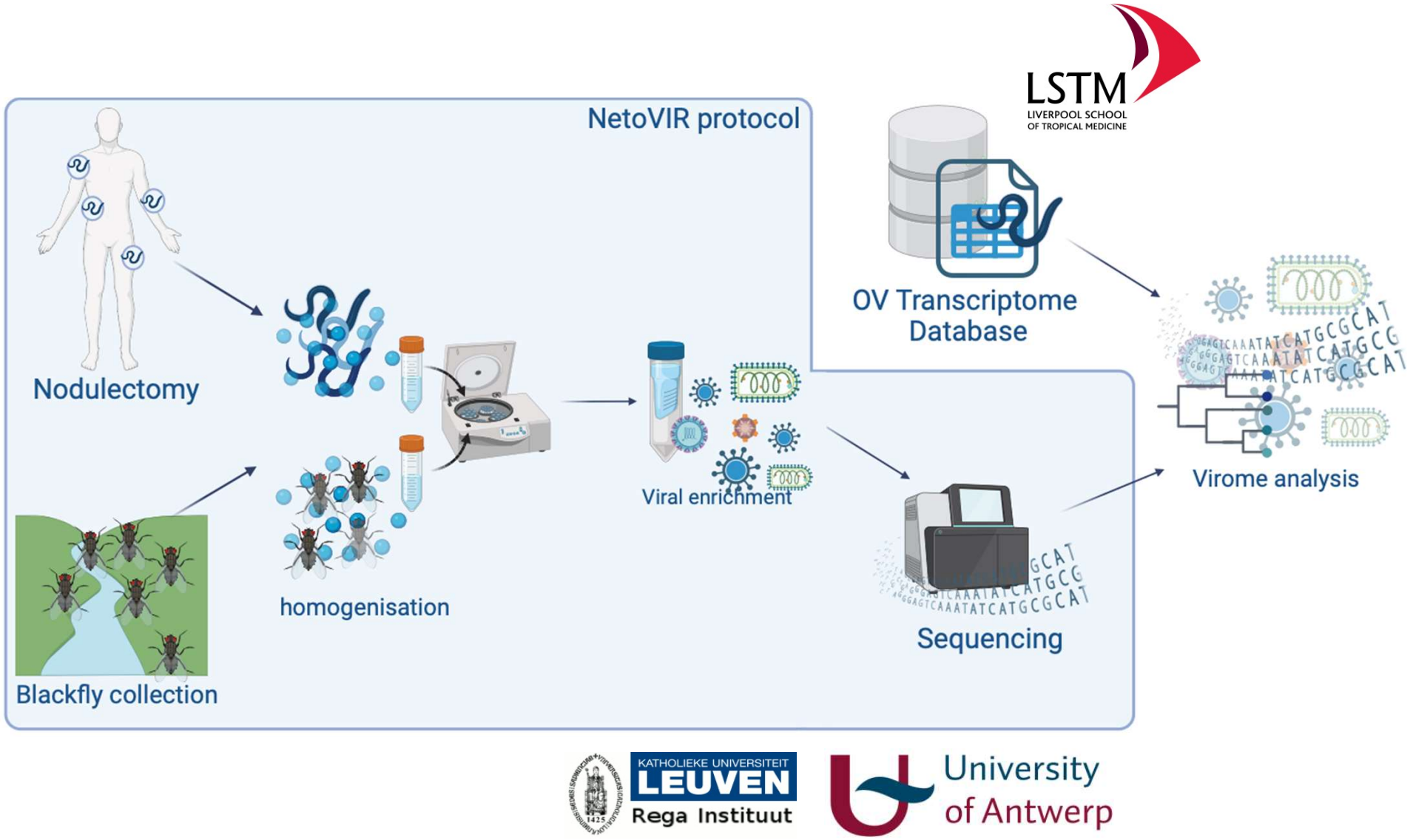
Leishmania RNA virus exacerbates Leishmaniasis by subverting innate immunity via TLR3-mediated NLRP3 inflammasome inhibition

Renan V. H. de Carvalho, Djalma S. Lima-Junior, Marcus Vinícius G. da Silva, Marisa Dilucca, Tamara S. Rodrigues, Catarina V. Horta, Alexandre L. N. Silva, Patrick F. da Silva, Fabiani G. Frantz, Lucas B. Lorenzon, Marcos Michel Souza, Fausto Almeida, Lilian M. Cantanhêde, Ricardo de Godoi M. Ferreira, Angela K. Cruz & Dario S. Zamboni

Metagenomics: Hypothesis

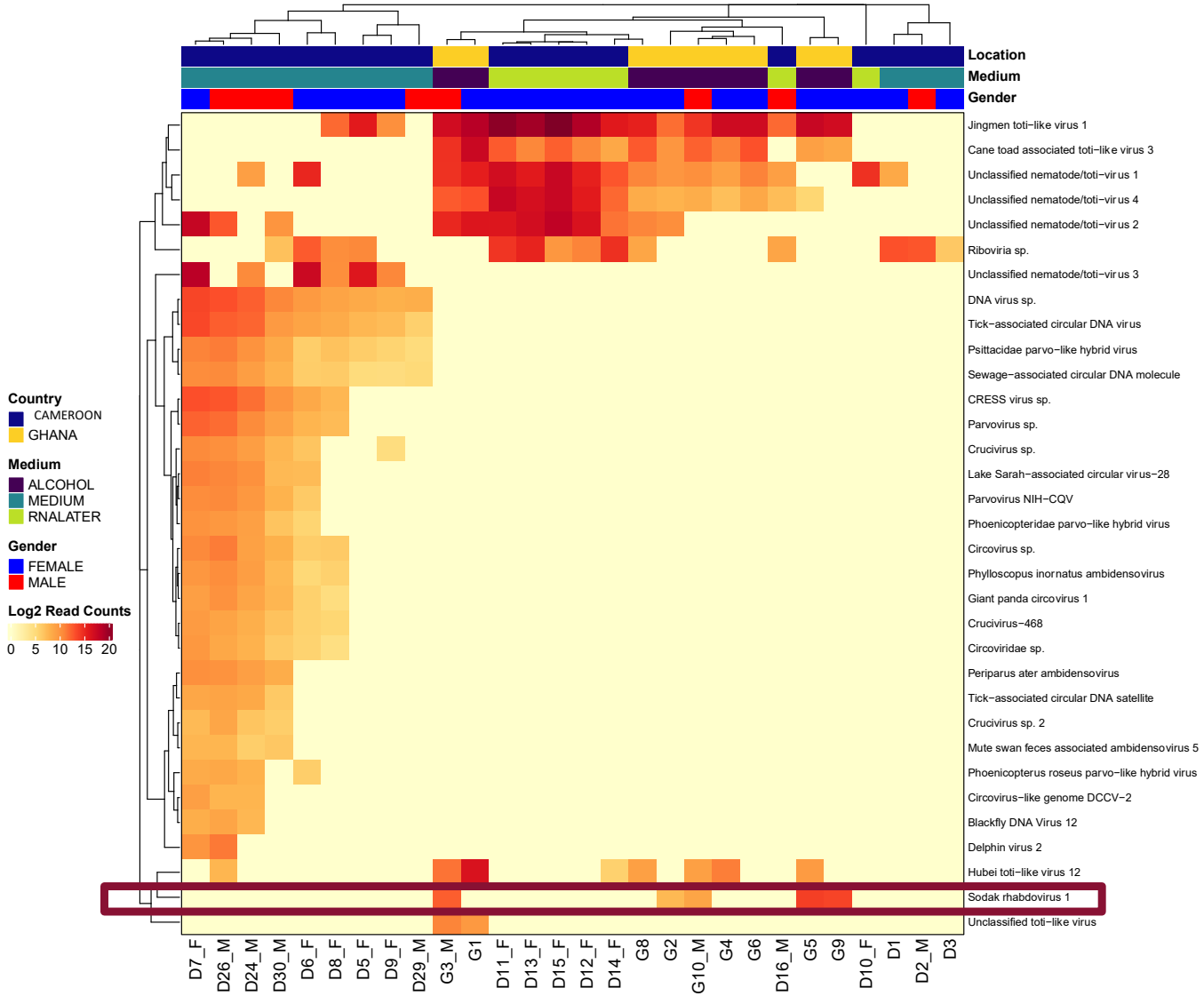


Metagenomics: Methods



*OV = *O. volvulus*

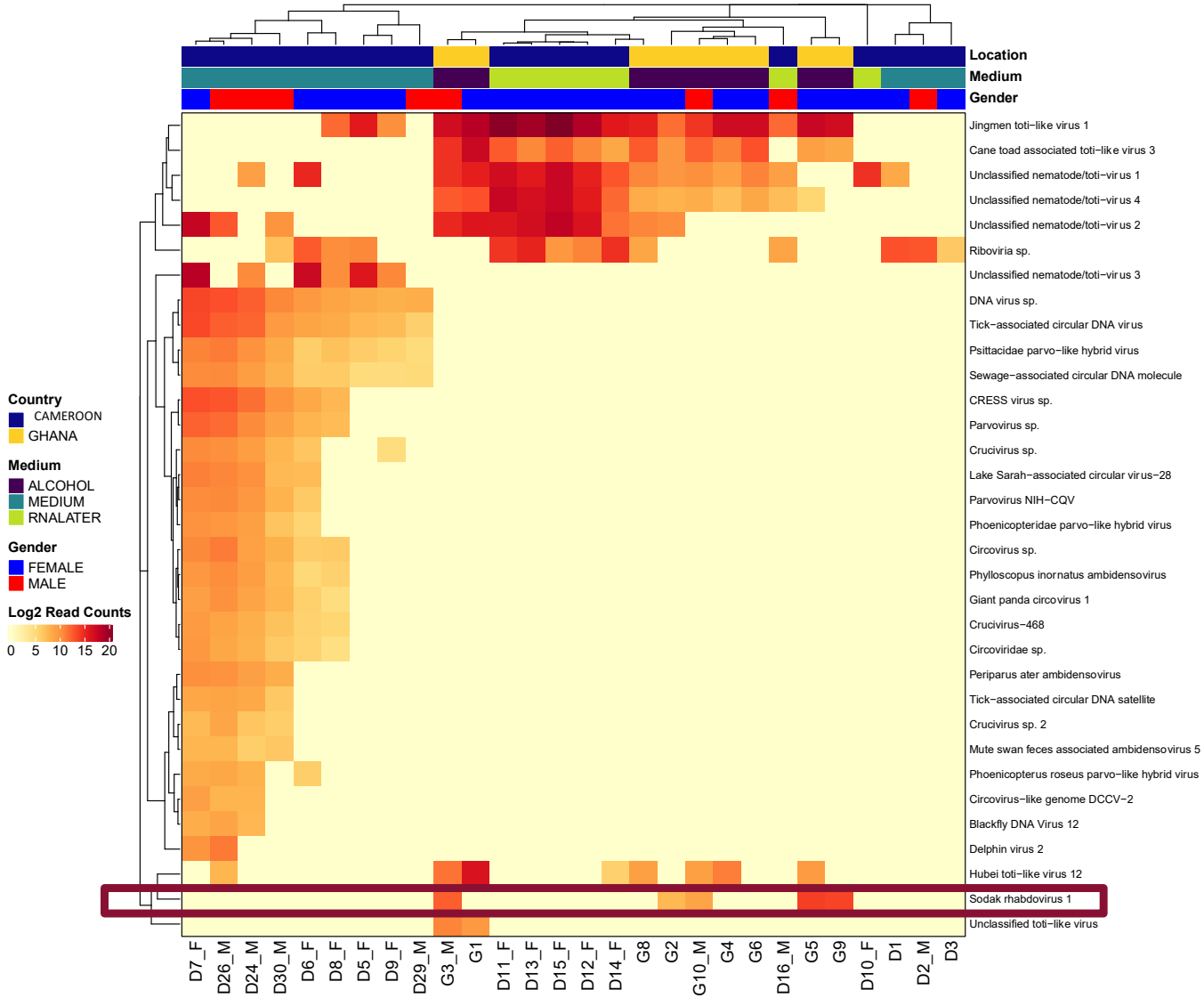
Metagenomics: Results *O. volvulus*



Novel Rhabdovirus

OVRV1

Metagenomics: Results *O. volvulus*



Novel Rhabdovirus

OVRV1

ALSO **found OVRV1 reads in *Simulium*** metagenomics

Metagenomics: Discussion

What is the impact of this virus?

↙ ↘

Able to infect human host?

↓

Directly impacting OAE?

dynamics?

Influencing infection

↓

Indirectly impacting BBB?

Metagenomics: Next Steps

- **Association:** Screening blood samples from OAE Case-Control studies for OVRV1 with ELISA and PCR.

- **Pathogenesis:** Screening CSF for presence of OVRV1 or immune response to OVRV1.

Research Foundation Flanders
Opening new horizons

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LIVERPOOL SCHOOL OF TROPICAL MEDICINE

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Rega Instituut

vliruos Scholarship in Belgium
SHARING MINDS, CHANGING LIVES

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European Research Council
Established by the European Commission

THANK YOU

amref health africa

r2hc Research for health in humanitarian crises | elrha

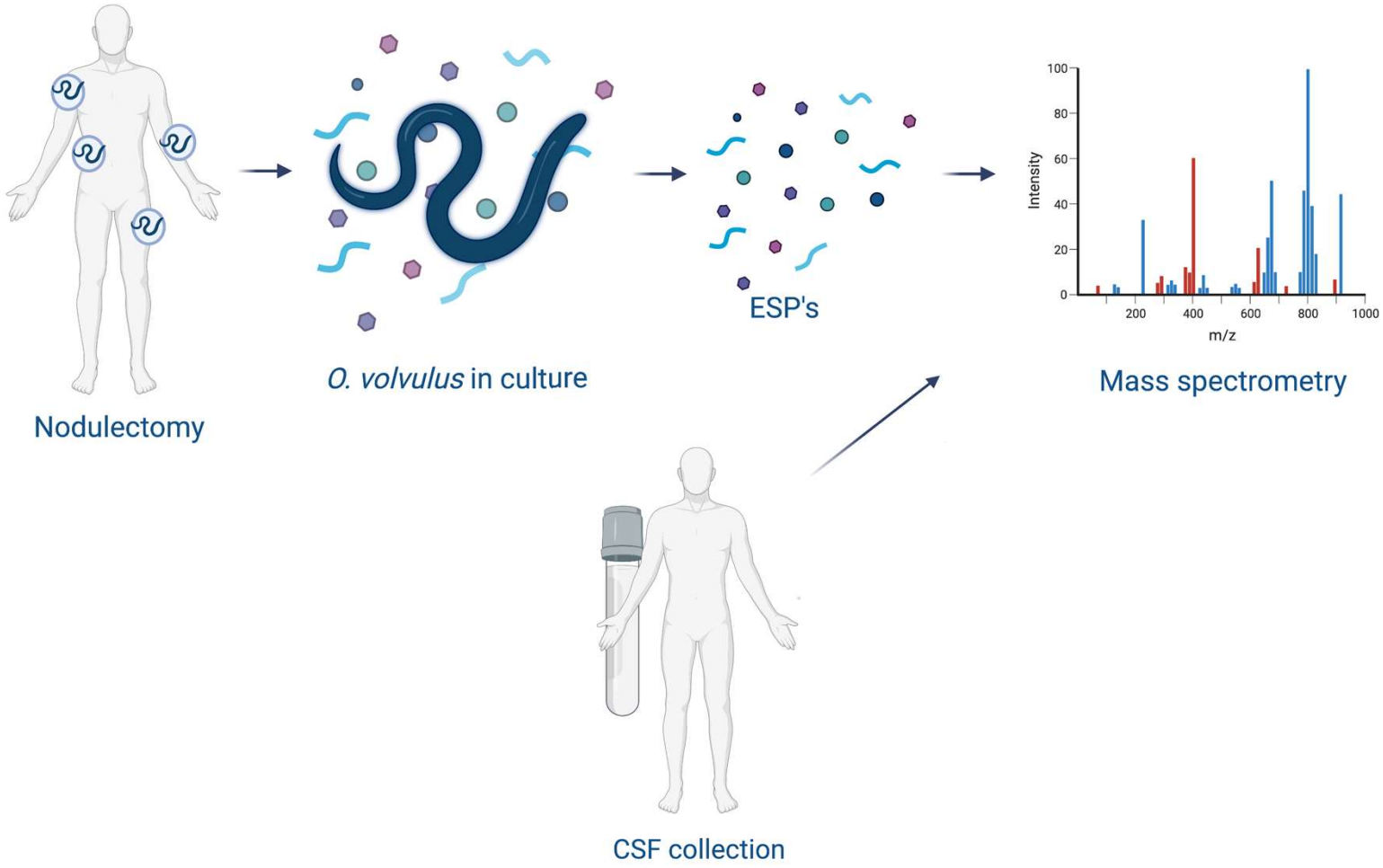
Ministry of Health
Promotes Health & Provides Care

University of Antwerp

Metagenomics: Results

- **OVRV1**: identified in *O. volvulus* from **Ghana**, **Cameroon, Nigeria** and **Togo**.
- **Antibody response** against OVRV1 glycoprotein found in seropositive persons from Ghana, Cameroon, Nigeria and Togo.
- Reads **found in *Simulium*** metagenomics results
- **found across all life stage**s and most in the female macrofilairia reproductive organs.

Proteomics: Methods



Proteomics: Results

- Adult worm secretome currently being analysed and described
  - Secretome? Incl. whole protein and extravesicular (EV) level

→ **DISCUSSION**: Individual effect of the ESP's

- 9 CSF OAE patients → No *O. volvulus* proteins found

→ **DISCUSSION**: Do we expect to find something?

Universiteit Antwerpen

Novel *O. volvulus* rapid diagnostic tests

Amber Hadermann

Global Health Institute
University of Antwerp

Introduction: Current relevant *O. volvulus* diagnostics

Diagnostics	Strengths	Limitations
Skin Snips	- Specific - Current infection	- Invasive - Laboratory & Expertise needed - Ivermectin use - 24h incubation - Long/work intensive

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OV16 ELISA	- Past & current infection - Non-invasive	- Sensitivity - Laboratory & Expertise needed - Ivermectin use - Long/work intensive - No differentiation between Past & current infection

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***O. volvulus* polymerase chain reaction**	- Specific - Sensitive - Non-invasive - Current infection	- Laboratory & Expertise needed - Ivermectin use - Long/work intensive - Current infection

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***O. volvulus* polymerase chain reaction**	- Specific - Sensitive - Non-invasive - Current infection	- Laboratory & Expertise needed - Ivermectin use - Long/work intensive - Current infection
OV16 Rapid Diagnostic tests	- Fast - No laboratory needed - Past & current infection - Limited training - Non-invasive	- Sensitivity - No differentiation between Past & current infection

Introduction: WHO call for new diagnostics

- **Why?** Monitoring and evaluation

    1. Stopping MDA*
    2. Post-treatment surveillance
    3. Post-elimination surveillance
    4. Individual diagnosis and case management

- **Target?**
    - **Mapping:** > 60% sensitive and > 99.8% specific
    - **Stopping:** > 89% sensitive and > 99.8% specific

*MDA = mass drug administration

The Future: Rapid diagnostic tests (RDTs)

Diagnostics	Strengths	Limitations
OV16 Rapid Diagnostic tests	- Fast - No laboratory needed - Limited training - Non-invasive	- Sensitivity - No differentiation between Past & current infection

D|D|T|D
DRUGS & DIAGNOSTICS FOR TROPICAL DISEASES

GLOBAL ACCESS ga DIAGNOSTICS

Abbott

FIND Diagnosis for all

BILL & MELINDA GATES foundation

Feasibility assessment:

1. Individual RDT feasibility

2. RDT feasibility & Mass diagnostic surveys

3. RDT feasibility & Long term surveillance

Individual RDT feasibility

RDTs & Health care workers

RDTs are a part of most clinics

↓

Minimal training needed

&

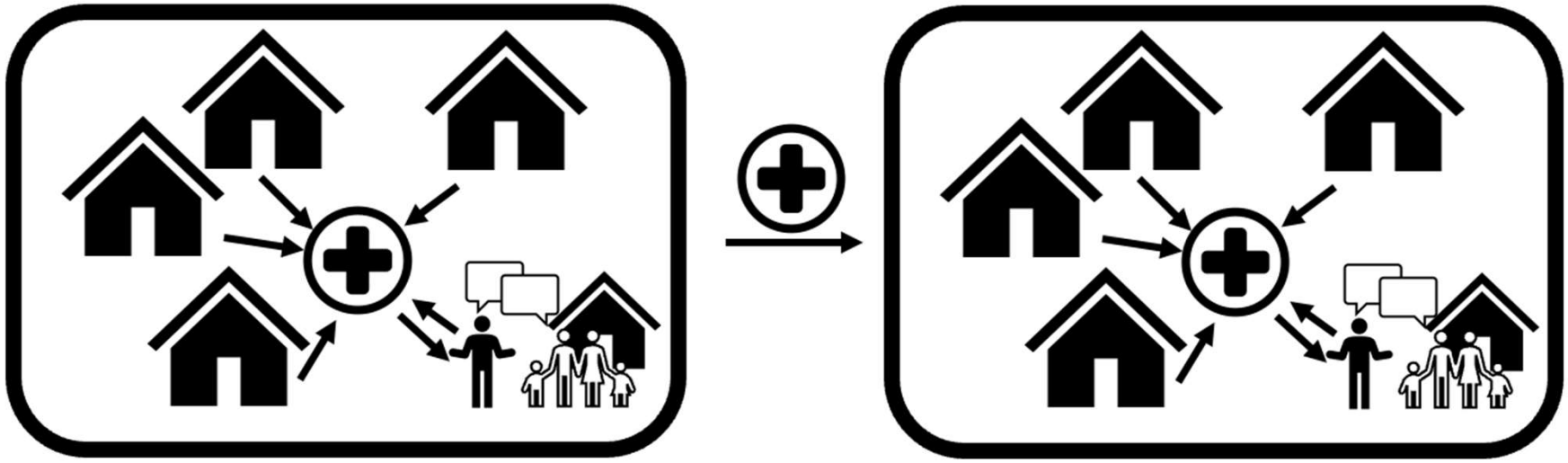
Feasible for all levels of HCWs*

Important design components

- Printing on cassette:
  - Amount of buffer/blood
  - Clearly defining of lines/holes
  - Read-out time
- Easy kit form incl.:
  - Extra buffer
  - Retractable lancets
  - Blood transfer devices

*HCW = Health care worker

RDT feasibility & Mass diagnostic surveys



RDT feasibility & Mass diagnostic surveys

- The easy stuff: Performing test

- Difficulties:
  1. Performing X tests in parallel
  2. Time management

RDT feasibility & Mass diagnostic surveys

- The easy stuff: Performing test

- Difficulties:
  1. Performing X tests in parallel
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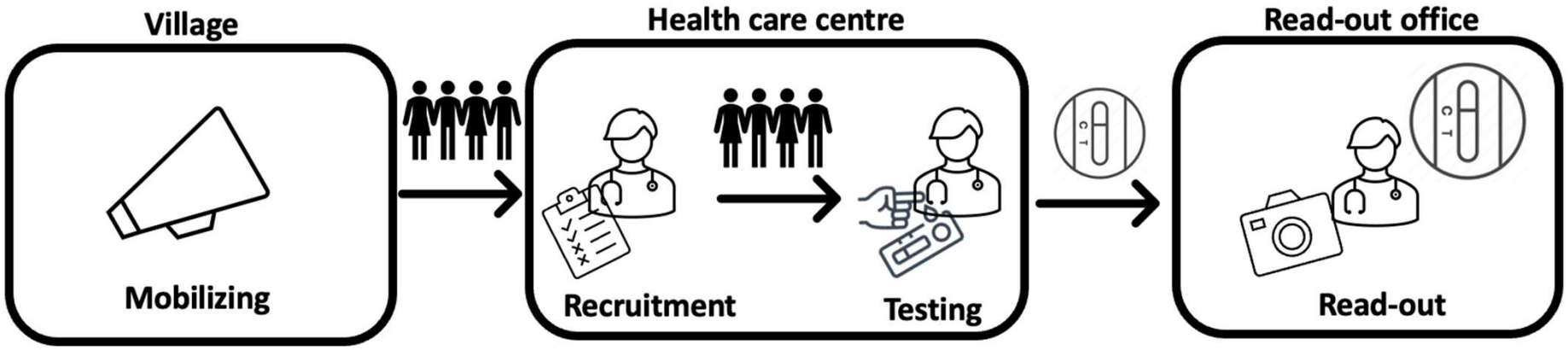
→ **Solution**: Minimum of 3
  1. Consent/Assent
  2. Performing test
  3. Time management

RDT feasibility & Long term surveillance



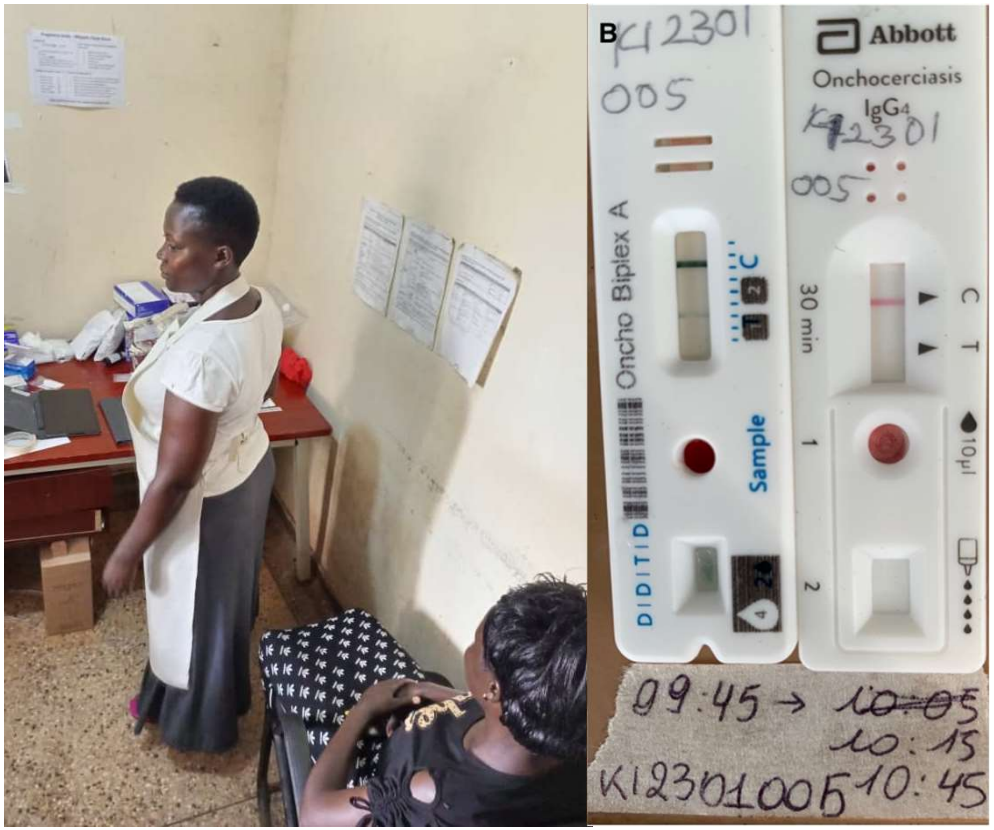
RDT feasibility & Long term surveillance

- The easy stuff: Performing test

- Difficulties:
  1. Less supervision
  2. Tests & routine work
  3. Time management

RDT feasibility & Long term surveillance

- The easy stuff: Performing test



- Difficulties:
  1. Less supervision
  2. Tests & routine work
  3. Time management

→ **Solutions**:

1. Cheat-Sheets
2. More extensive training – Theory/Patient/Individual problem solving
3. Writing down timings

Preliminary results: Comparing RDTs

1. Mass diagnostic survey in children – Maridi, South Sudan

2. Long term surveillance in pregnant women – Maridi, South Sudan

3. Mass diagnostic survey in Adults – Ntui, Cameroon

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1. Mass diagnostic survey in children – Maridi, South Sudan

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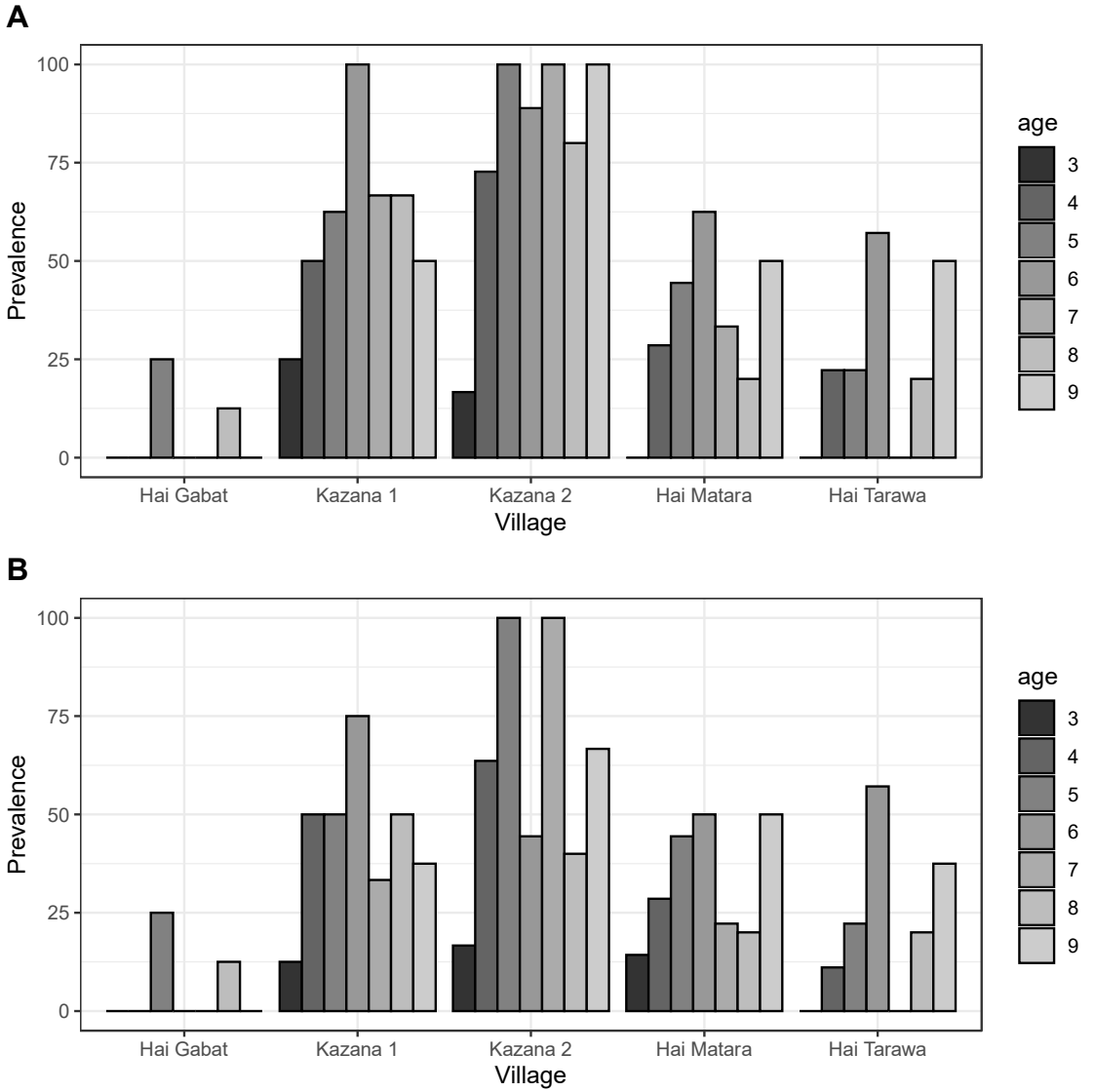
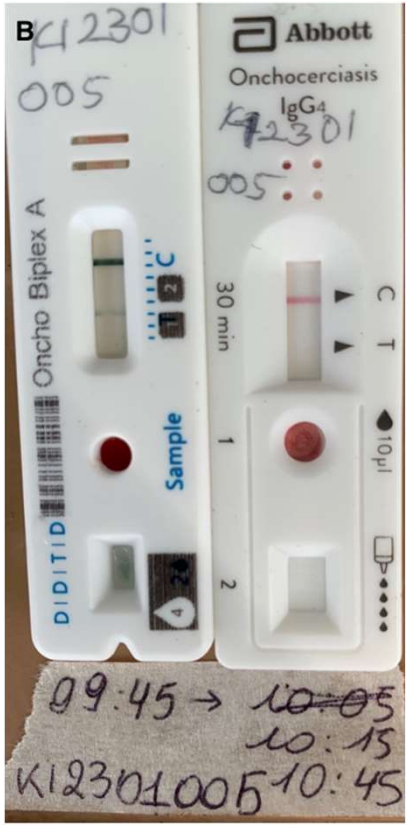
3. Mass diagnostic survey in Adults – Ntui, Cameroon

Mass diagnostic survey in children – Maridi, SS

DDTD Biplex Type A

OV16 BIOLINE





(A) DDTD biplex A and **(B)** Ov16 SD Bioline RDT seroprevalence per village and per age

Mass diagnostic survey in children – Maridi, SS

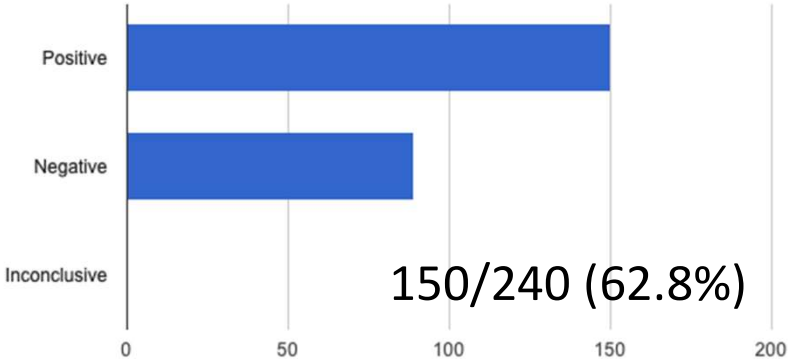
Table 2: Comparison characteristic related onchocerciasis prevalence between SD Bioline and DDTD Biplex RDT overall and per line.

	SD Bioline (OV16)		DDTD Biplex overall (OV16 + 2nd Antigen)		DDTD Biplex line 1 (OV16)		DDTD Biplex line 2 (2nd Antigen)	
Presence/Absence Characteristic / Characteristic	*Presence*	*Absence*	*Presence*	*Absence*	*Presence*	*Absence*	*Presence*	*Absence*
Dermatitis	37/87 (42.5; 32.1-53.6)	34/151 (22.5; 16.3-30.2)	45/87 (51.7; 40.8-62.4)	45/151 (29.8; 22.8-37.9)	40/87 (46.0; 35.4-57.0)	43/151 (28.5; 21.6-36.5)	23/87 (26.4; 17.8-37.2)	21/151 (13.9; 9.0-20.7)
	p-value= 0.002		*p-value*= <0.001		*p-value*= 0.01		*p-value*= 0.03	
	Test comparison: p-value$_{presence}$ = <0.001; *p-value*$_{absence}$ = <0.001				*Line comparison: p-value*$_{presence}$ = 0.01; *p-value*$_{absence}$ = 0.003			

→ Dermatitis (current infection marker) significantly linked to OV16 line not to added second line

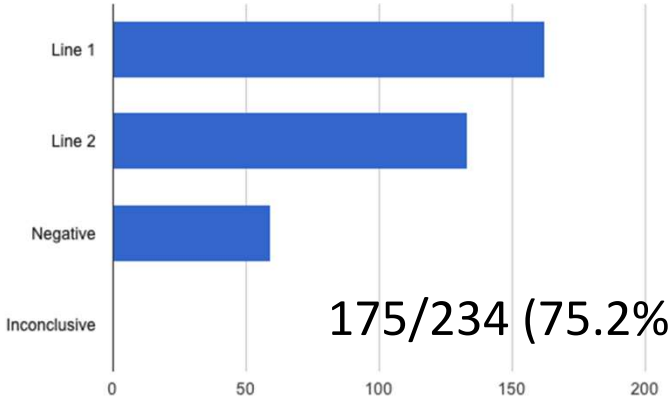
Long term surveillance pregnant women – Maridi, SS

OV16 SD BIOLINE



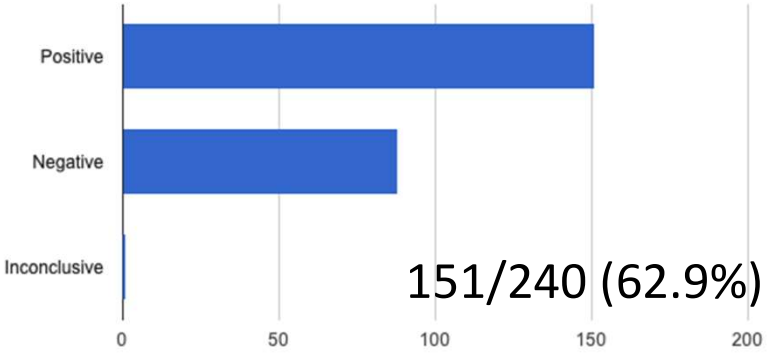
150/240 (62.8%)

DDTD Biplex Type A



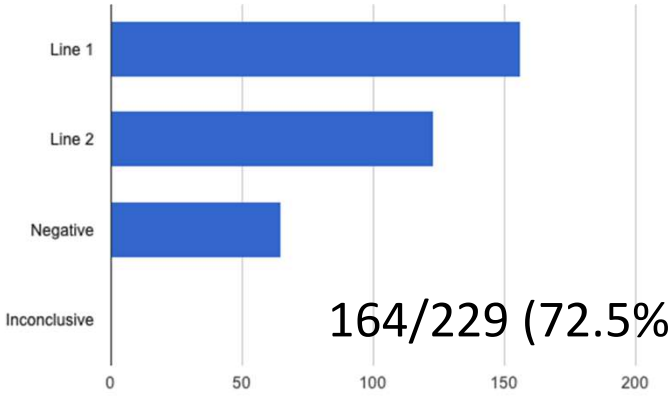
175/234 (75.2%)

OV16 GADx



151/240 (62.9%)

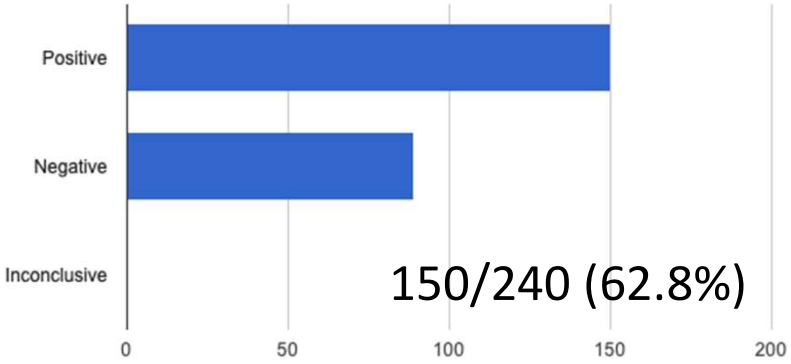
DDTD Biplex Type C



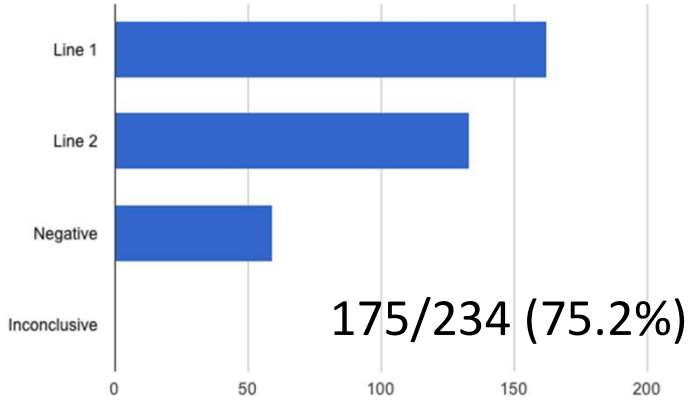
164/229 (72.5%)

Long term surveillance pregnant women – Maridi, SS

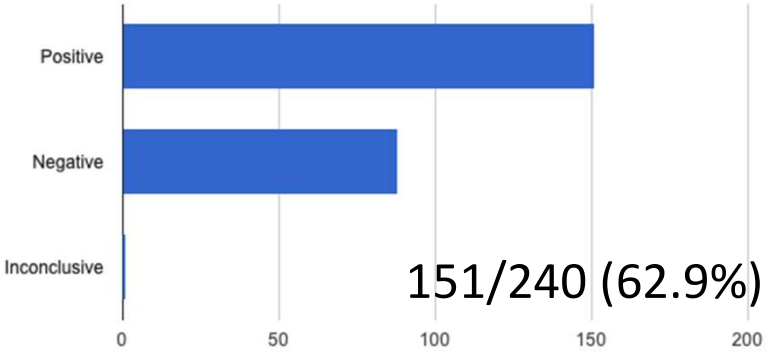
OV16 BIOLINE



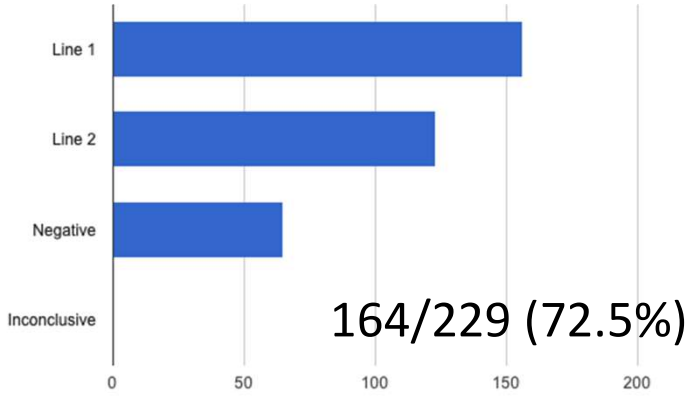
DDTD Biplex Type A



OV16 GADx



DDTD Biplex Type C



→ Significant difference between OV16 based test and Biplexes (p= 0.003)

→ Difference solely based on addition Second line

Conclusions & Discussions

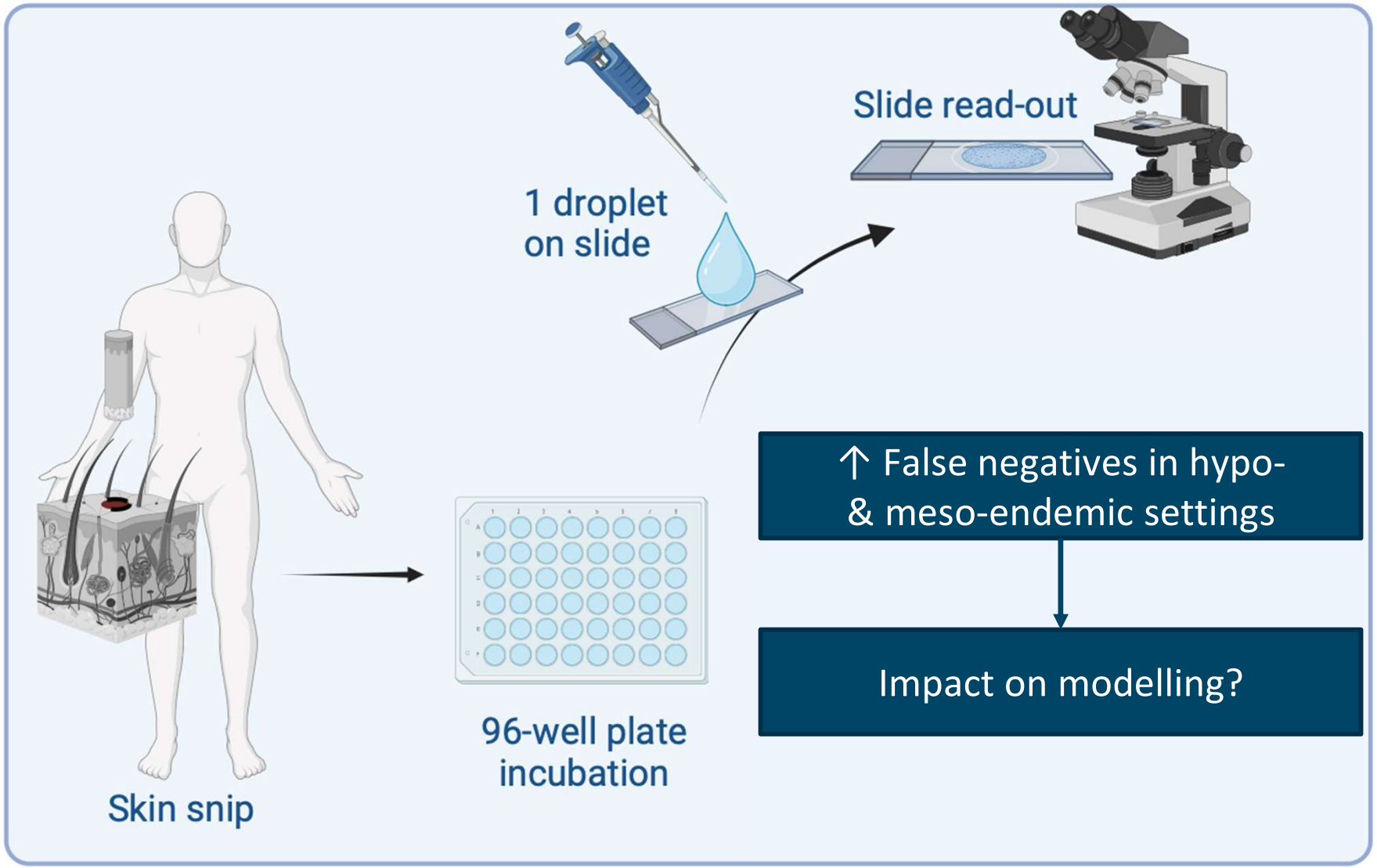
- Feasibility of RDTs itself is no problem

  → BUT the management of larger studies is

- DDTD Biplex RDTs **seems** to detect more OV-seropositive in comparison to Bioline and GADx OV16 RDTs

  → PCR, ELISA and Skin Snip data needs to be included

Skin snip methodology

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