

Limitations and Future Considerations in Helminth Diagnostics

Daniel H. Paris

Department of Medicine, Swiss TPH
University of Basel, Switzerland



Swiss Tropical and Public Health Institute
Schweizerisches Tropen- und Public Health-Institut
Institut Tropical et de Santé Publique Suisse

Associated Institute of the University of Basel



Rickettsial diagnostics – moving forward

STIFA – From single titers to dynamic serology

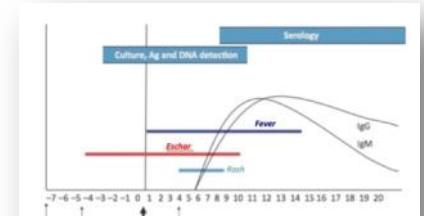
AG / NAAT combined w/serology – Improved ADM Dg

STIC – Robust criteria for diagnostic accuracy

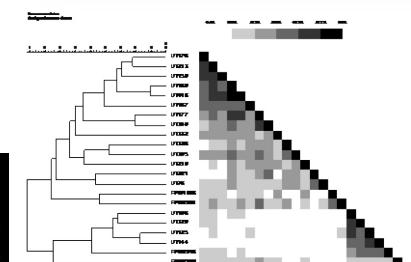
Bayesian Modelling - IFA results with false positivity?

PAST – Antigen detection using protein arrays

Ag cartography – Plotting antigenicity on “maps”



Dynamics



Aims:

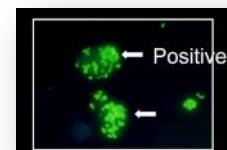
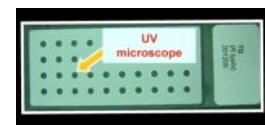
Simple, affordable point-of-care “RDT”

Standardized assay, non-subjective endpoint

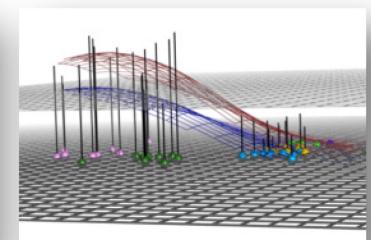
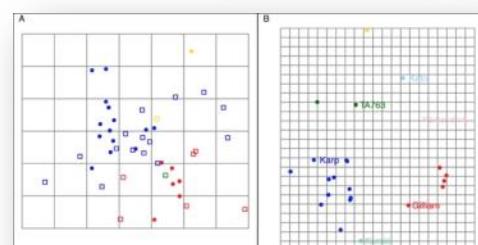
Covers antigenic heterogeneity / variation

High accuracy - Sn and Sp in endemic areas (!)

Coverage of complete disease course



Antigens



Strain Antigenicity

Blacksell et al., CID, 2007

Paris DH et al., CMI, 2009

Felgner P et al. PNAS 2009

McGready et al., PNTD, 2010

Paris DH et al., PNTD 2011

Paris DH et al., AJTMH 2013

Lim C. et al., PONE 2015

James S. et al., PNTD, 2016

Weitzel T et al., NEJM 2016

Paris DH et al., COID, 2017

Context of diagnostics ... schistosomiasis control

	Pre-control	Control	Elimination as public health problem	Interruption of transmission	Post-elimination
Programmatic steps	Situation analysis	Preventive chemotherapy (PCT)	PCT and other control measures	PCT, other control measures and surveillance-response	Continued surveillance-response
Target		100% geographical coverage; >75% national coverage; and heavy-intensity infections <5% in sentinel sites	Heavy-intensity infection <1% in all sentinel sites	Reduction of incidence of infection to zero	Incidence of infection remains zero (no autochthonous cases)
Diagnostic test accuracy	Sensitivity: +(+) Specificity: + Quantitative: + High throughput: +++	Sensitivity: ++ Specificity: ++ Quantitative: ++(+) High throughput: ++	Sensitivity: +++ Specificity: ++ Quantitative: ++(+) High throughput: ++	Sensitivity: +++ Specificity: +++ Quantitative: +(++) High throughput: +(++)	Sensitivity: +++ Specificity: +++ Quantitative: + High throughput: +(++)

Utzinger J et al., CMI 2015

2001 World Health Assembly
deworming of school-aged children

praziquantel (safe, effective, inexpensive)

Diagnosis unnecessary, not cost-effective
Little interest in diagnostics R&D

2020 WHO Roadmap to overcome NTDs
-- new era --

“Endgame” - Elimination of schistosomiasis
Importance of diagnostic tools - highly accurate diagnostic assays now required

Limitations of diagnostics ...

Questionnaires (blood in urine)

Microscopy

- Urine filtration, fecal smear
- FECT
- Kato-Katz
- FLOTAC
- Mini-FLOTAC

DNA detection

- PCR / LAMP

Antibody detection

- ELISA
- IHA / IFA

RDTs

- POC-CCA

Antigen detection

- UCP-LF CAA

Table 3. Diagnostic performance of selected signs and symptoms for the diagnosis of *S. mansoni* infection at the community level

Country	<i>S. mansoni</i> prevalence %	Questionnaire return rate ^a	No. of children interviewed	No. of children examined	Threshold or high-risk schools ^b	Questions (threshold ^c as %)	Diagnostic performance %			Reference	
							Sensitivity	Specificity	PPV ^d		
Côte d'Ivoire	54.4	121/134 (90)	12 227	5047 ^f	50	Blood in stool (22)	88	58	73	79	47
						Bloody diarrhoea (14)	88	58	73	79	47
						Schistosomiasis (4)	71	58	73	79	47
Democratic Republic of the Congo	31.2	136/160 (85)	19 362	5806 ^g	50	Blood in stool (19)	62	77	44	87	20
						Schistosomiasis (34)	62	89	62	87	20
Kenya	29.4	NA ^h	2913	2913 ⁱ	50	Blood in stool (25)	60	78	43	88	48
Ethiopia	20.9	142/161 (88)	13 756	8006 ^g	20	Blood in stool (15)	84	80	74	88	44
						Schistosomiasis (15)	77	98	96	87	44
						Bloody diarrhoea (25)	71	85	76	81	44

^a See footnote a, Table 1.

^b The threshold for high-risk schools is the prevalence level at which a school is said to be at high risk. These are the schools that the questionnaire aims to identify.

^c See footnote b, Table 1.

^d See footnote c, Table 1.

^e See footnote d, Table 1.

^f Kato-Katz thick smears (2 stool specimens; 1 slide each).

^g Kato-Katz thick smears (1 stool specimen; 1 slide).

^h NA = not applicable. Questionnaires were not distributed; the work was done by the research team in 46 schools.

ⁱ Kato-Katz thick smears (1 stool specimen; 2 slides).

Bulletin of the World Health Organization 2002, 80 (3)

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Antibody detection

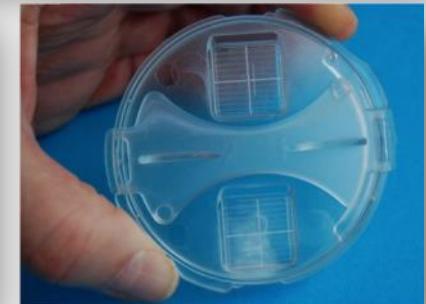
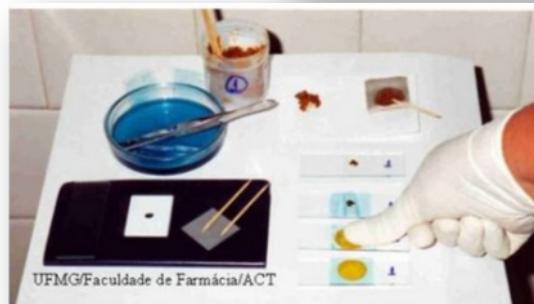
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KK “standard”:

*small amount of stool – good for high egg burden
high transmission setting
quantifiable egg counts - decrease upon therapy
BUT: problematic with low egg burden – detection 20-50 EPG*

Mini-FLOTAC:

Flotation of eggs - no centrifugation – detection 10 EPG

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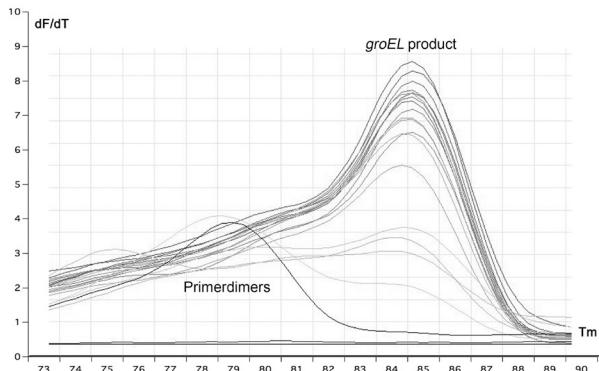
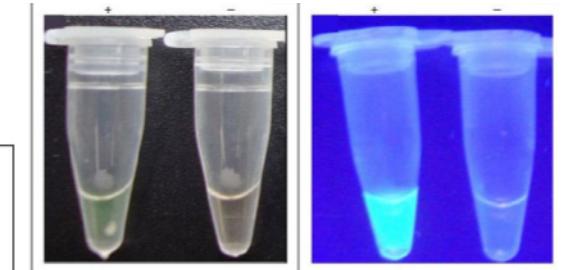
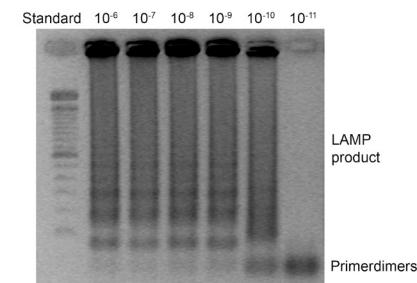
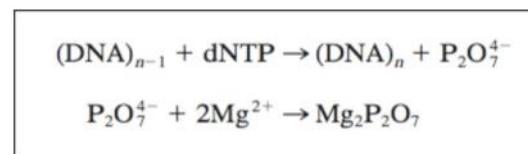
- ELISA
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Sn and Sp good, with low limit of detection

BUT: Poorly standardised for stool testing

"dead" eggs are PCR pos. (!)

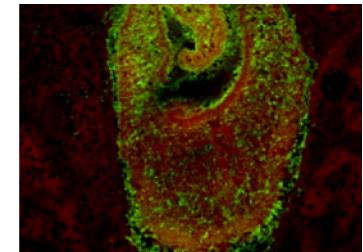
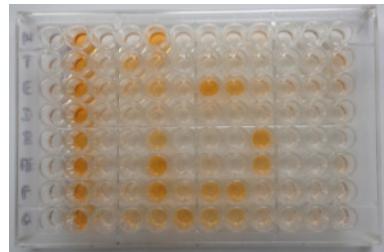
Risk of contamination (LAMP)

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Antigen detection

- #### • UCP-LF CAA

antigen on ELISA plate									
antibodies in serum	Antigen	Schisto (Sm Adult)	Schisto (Sm Egg)	Fasciol (F.hep. AG)	Echino (E.gran AG)	Strongy (S.ratti AG)	Filaria (A.vitelae AG)	Toxocar (T.canis AG)	Trichin (T.spiralis AG)
	Antibodies								
	Schistosomiasis							+	++
	Fasciolosis	+	+		+++	+	+++	+	
	Echinococcosis					+	+++		
	Strongyloidosis				+		+++	++	
	Filariasis				+++	+++		++	
	Toxocarosis								
	Trichinellosis		+++						

	B	C ₁	C ₂	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉
Trichinella spiralis	N		●	●								
Toxocara canis	T		●	●		●				●		
Echinococcus granulosus	E		●	●		●						●
Fasciola hepatica	D		●	●								
<u>Schistosoma</u> m. (Adult AG)	B		●	●				●	●		●	
<u>Schistosoma</u> m (Ei AG)	BE		●	●				●	●	●	●	
Filaria (A viteae)	F		●	●		●						●
Strongyloides stercoralis	O		●	●		●						●

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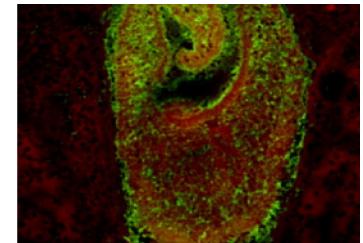
- ELISA
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ELISA:

screening tool – sensitive in unexposed

BUT: *cross-reactivity / cut off titers / species differentiation
active vs. past infection / endemic areas*

IFA:

confirmation, highly specific - laborious!

Antigens:

Egg or adult worm based

lack of ag standardisation / regions / amount

Larva for IFA

need to establish the cycle

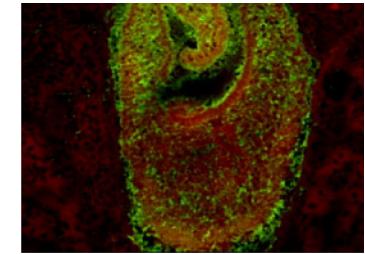
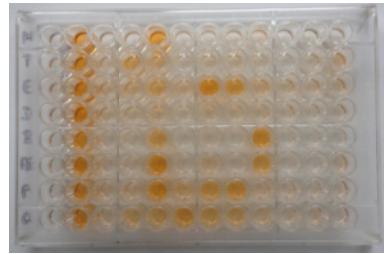
(dead larva antigens are different!)

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DNA detection

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Antibody detection

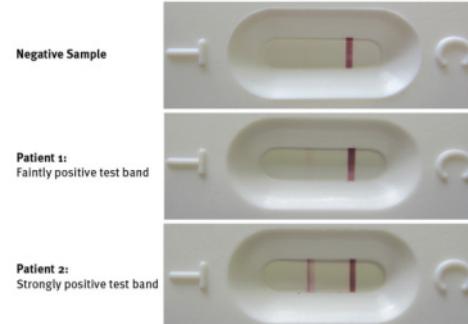
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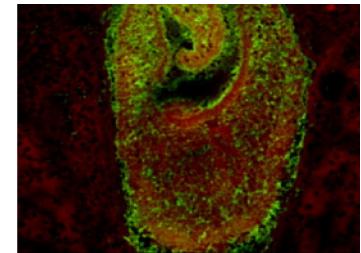
Rapid Medical Diagnostics, Pretoria, South Africa

Limitations of diagnostics ...

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DNA detection

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Antibody detection

- ELISA
- IHA / IFA

RDTs

- POC-CCA



“appropriate tool for monitoring schisto control programmes”

BUT:

false-positive results

- Urinary tract infection
- Hematuria
- Pregnancy
- Lewis-X trisaccharide
(common epitope on RBCs and anti-inflammatory cells)



Limitations of diagnostics ...

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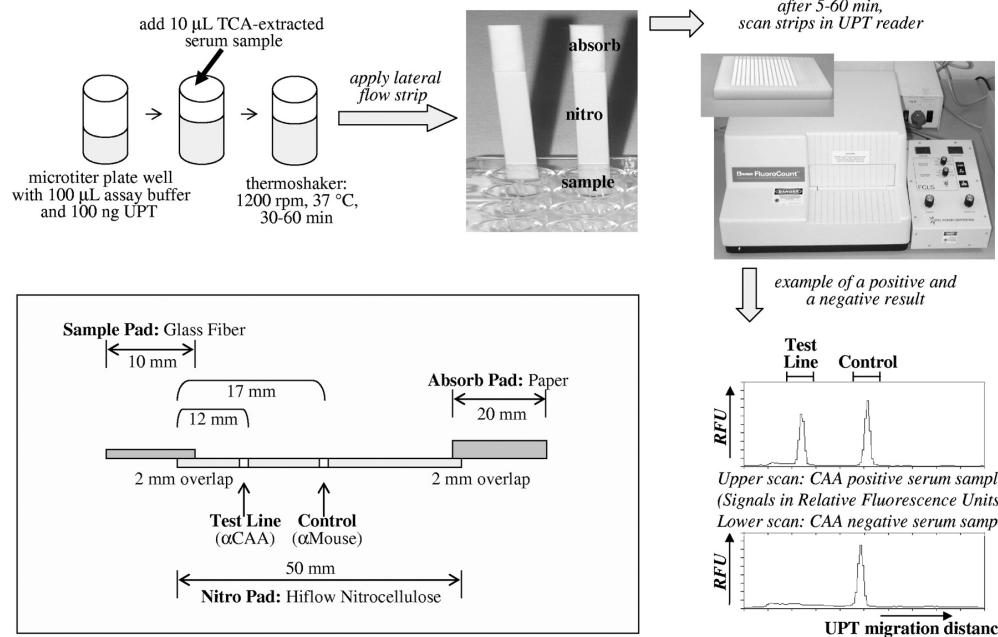
RDTs

- POC-CCA

Antigen detection

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up-converting phosphor-lateral flow (UCP-LF) reporter technology
to detect parasite-excreted circulating anodic antigen (CAA)



Promising ... might be up to 10-fold more sensitive than KK smears

Differential requirements of diagnostic tests

Diagnosis

- Acute infection / background titers
- Documentation successful therapy
- Chronic infection

Microscopy – egg detection

PCR – high sensitivity

LAMP – field deployment

Surveillance

- Reduction of positivity rates
- Re-emergence of positivity

RDTs (?) – CCA CAA ...
(ag-capture / semi-quant. / defined cut-off)

Serology – ideal if “clean”
i.e. recomb. protein based assay

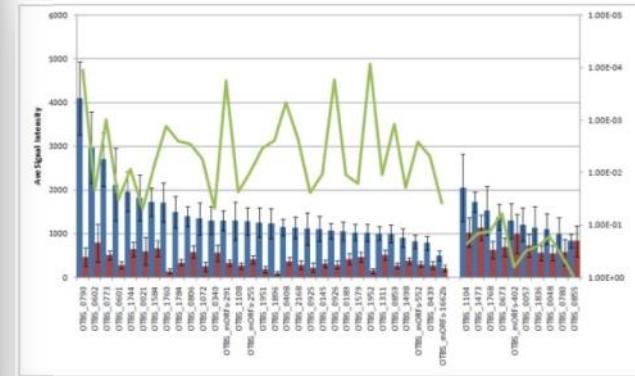
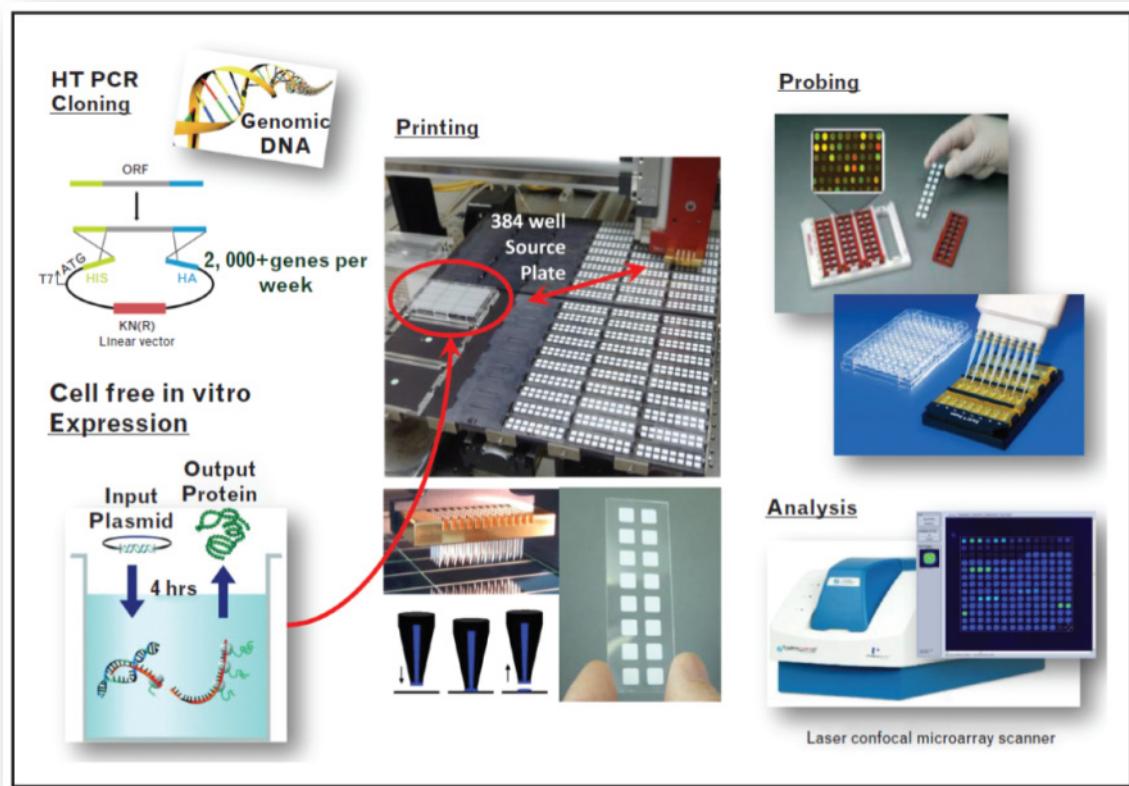
Eradication

- Documentation of true negativity
- New positives

High throughput vs. individual testing

Assay with good NPV, low LoD

Next Generation Protein Microarrays

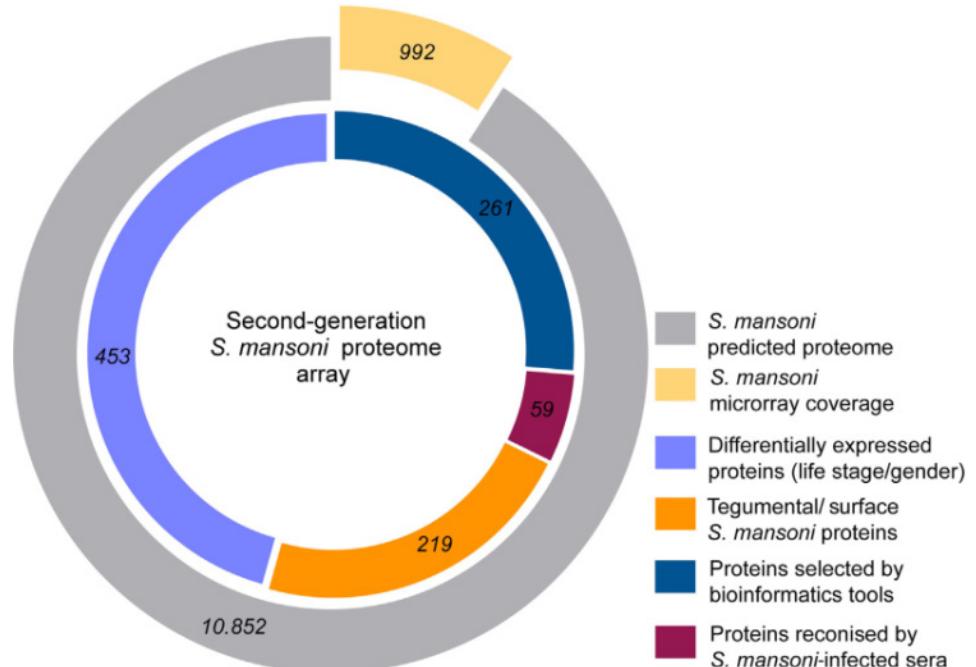


Schistosomiasis protein array

Proteome microarray consisting of 992 *Schistosoma mansoni* proteins

Selection on the basis of four characteristics:

1. Differentially expressed proteins from the tegument surface of *S. mansoni* (selection by seropositivity, qPCR, WB, ELISA)
2. Bioinformatic prediction by signal peptides and/or transmembrane motifs (cellular localisation)
3. Specific epitope prediction (software, i.e. IEDB)
4. Proteins recognized by *S. mansoni*-infected sera (from endemic areas)



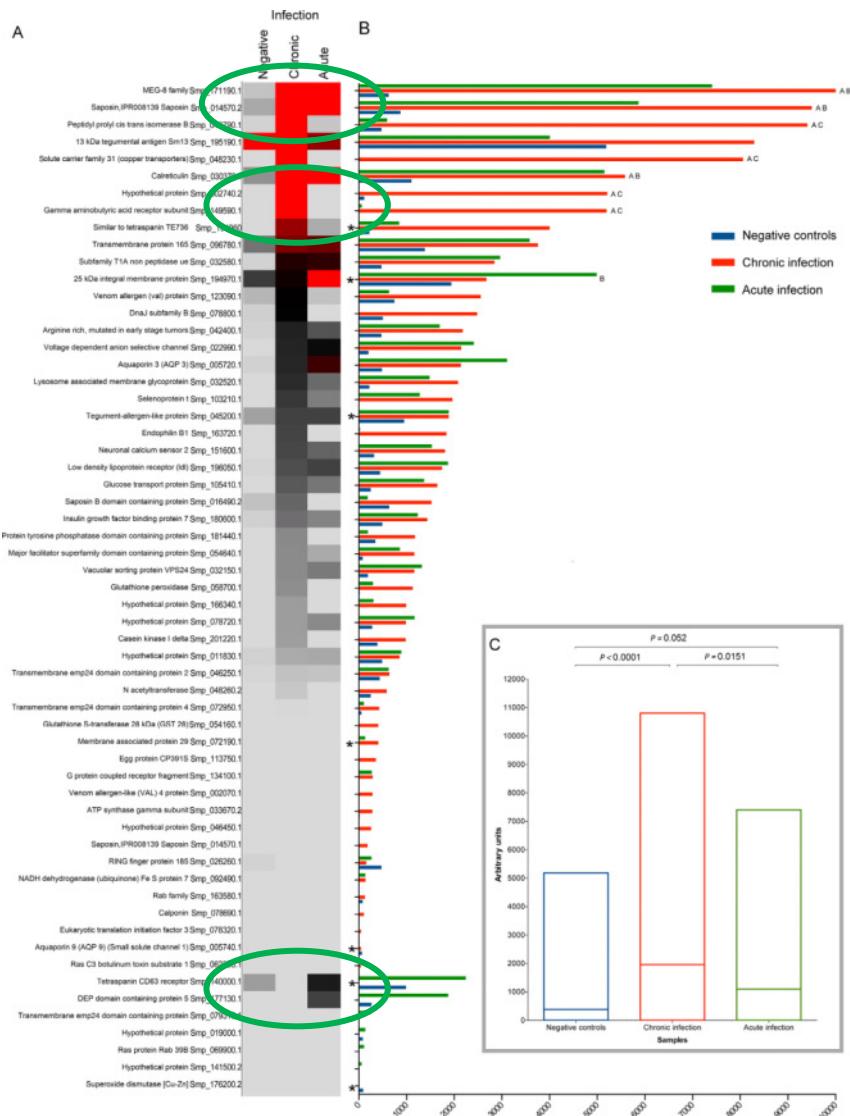
Next-generation proteome array for *Schistosoma mansoni*



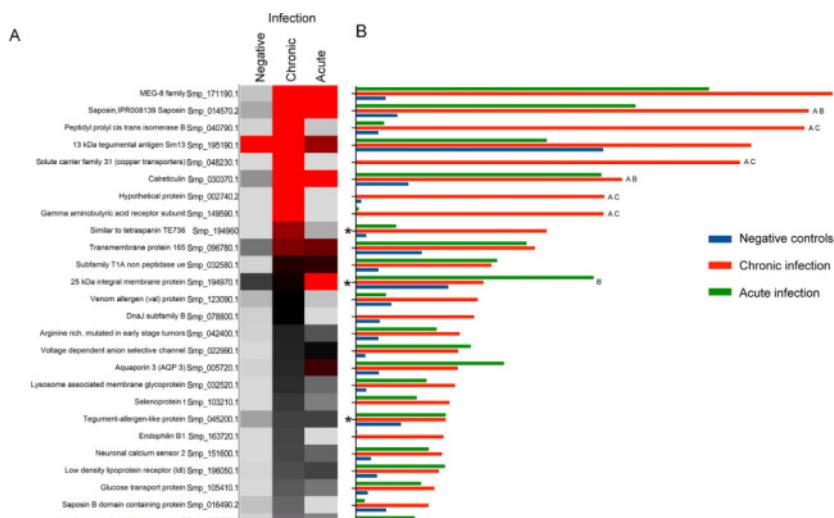
Belo Horizonte – Brasil

Previous study:
IgG1 vs. IgE and IgG3, IgG4 responses
-> Surface antigens

N=92 antigens – n=52 shown here



Next-generation proteome array for *Schistosoma mansoni*


Table 1

Comparison of antigens recognised by IgG in sera from individuals with chronic and acute *Schistosoma mansoni* between the expanded 992 antigen *S. mansoni* array and the previous 37 antigen *S. mansoni* array (Gaze et al., 2014).

Antigen	Description	Selection method	Immunoreactivity by infection status		
			Negative vs chronic	Negative vs acute	Chronic vs acute
SMP_171190.1	MEG-8 family	Bioinformatic	Yes	Yes	
SMP_014570.2	Saposin, IPR008139 Saposin	Bioinformatic	Yes	Yes	
SMP_040790.1	Peptidyl prolyl cis trans isomerase B	Bioinformatic	Yes		Yes
SMP_048230.1	Solute carrier family 31 (copper transporters)	Surface	Yes		Yes
SMP_002740.2	Hypothetical protein	Expression	Yes		Yes
SMP_030370.1	Calreticulin	Serologic	Yes	Yes	
SMP_149590.1 ^a	Gamma aminobutyric acid receptor subunit	Bioinformatic	Yes		Yes
SMP_194970.1 ^a	25 kDa integral membrane protein	Surface		Yes	
SMP_194960 ^a	Similar to tetraspanin	Expression			
SMP_045200.1 ^a	Tegument-allergen-like protein	Surface			
SMP_072190.1 ^a	Membrane associated protein 29	Surface			
SMP_005740.1 ^a	Aquaporin 9 (Small solute channel 1)	Bioinformatic			

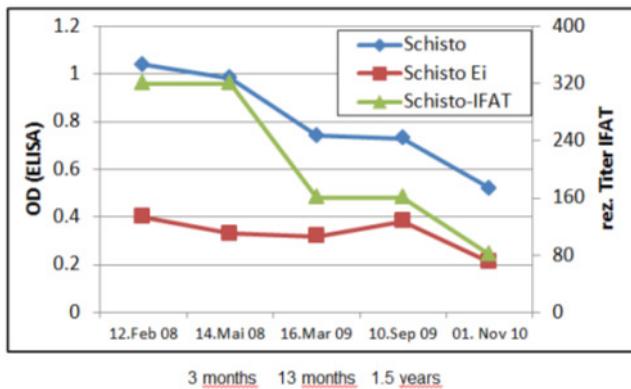
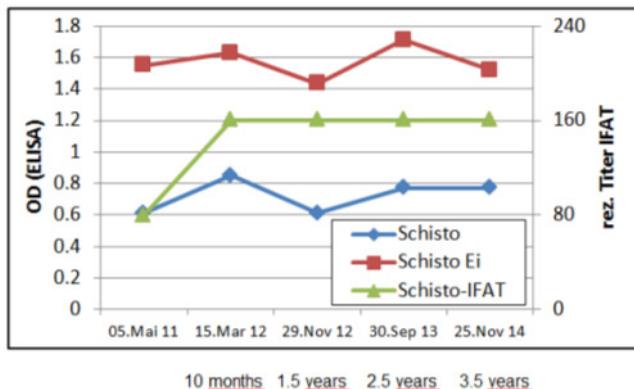
^a Proteins also used on the previous described array by Gaze et al. (2014).

n=7 **n=4** **n=4**

Questions

- (i) antibody signatures of acute vs. chronic infection (*S. mansoni*)
- (ii) antibody signatures of hepatosplenic schistosomiasis vs. chronic infection without hepatosplenomegaly
- (iii) antibody signatures of subjects with immunological resistance to *S. mansoni* after PZQ vs. those susceptible to reinfection

***Schistosomiasis:* High titres can persist over months to years after treatment**



! HOT ! S. mansoni protein array data

screened with sera to determine antibody signatures indicative of the clinical stages of schistosomiasis and to identify serodiagnostic antigens

Cohort of 120 Schistosomiasis cases treated with Praziquantel (PZQ)

- Heavy (>499 EPG, Eggs/gram feces)
- Medium (100-499 EPG)
- Low (up to 99 EPG) egg burden

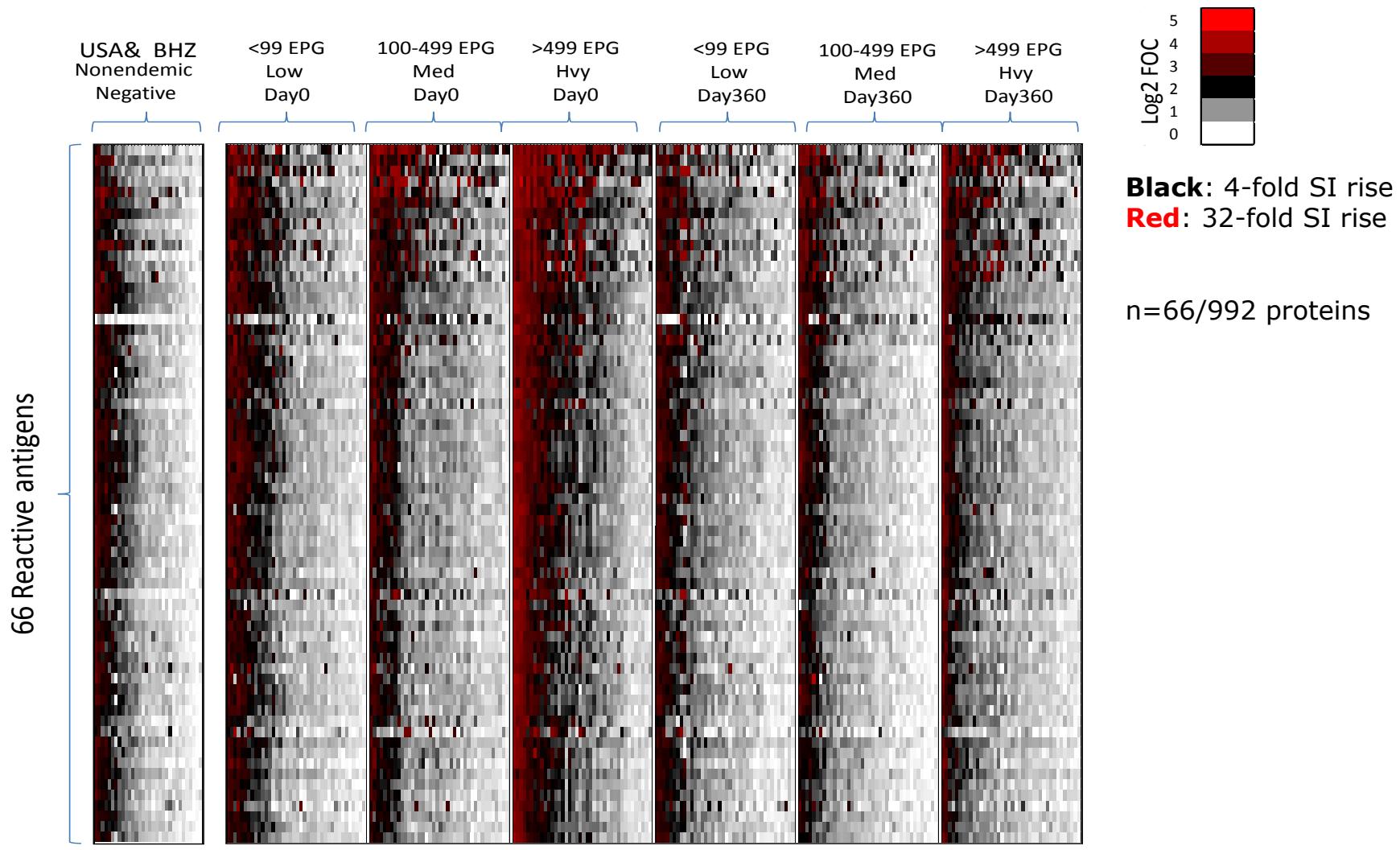
Serum specimens at D0 of PZQ treatment and D360 post-treatment

Sera were probed on the *S. mansoni* array

Breadth and intensity of Ab response directly proportional to egg burden

Ab responses declined after clearance of parasite with PZQ at D360

Antibody responses correlated with parasite burden



Selection process

Top 66 proteins

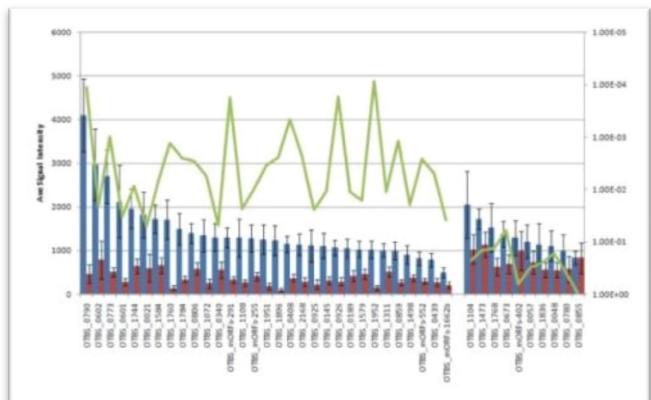
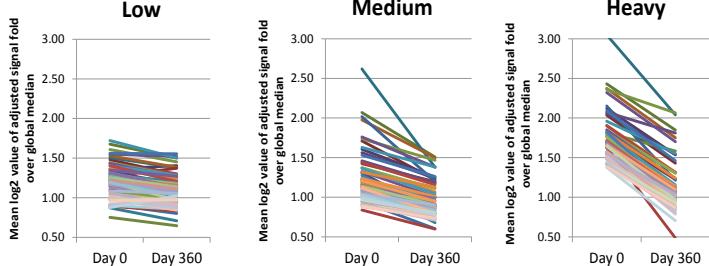
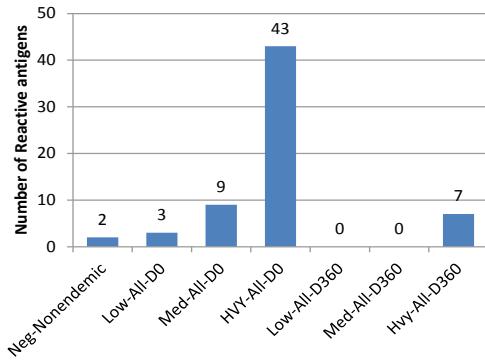
- Highest average signal intensity at D0
- Lowest signals at D360
- Ideal Ab kinetics 360 days after PZQ

Top 43 differentially reactive antigens

- Significantly more reactive in the heavy egg burden grp compared to other grps / controls
- Heavy burden grp reacted to more antigens (breadth)

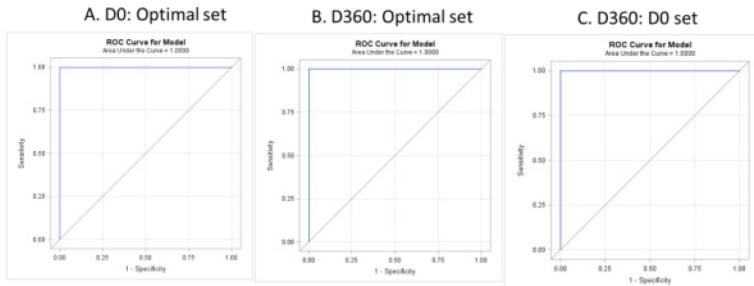
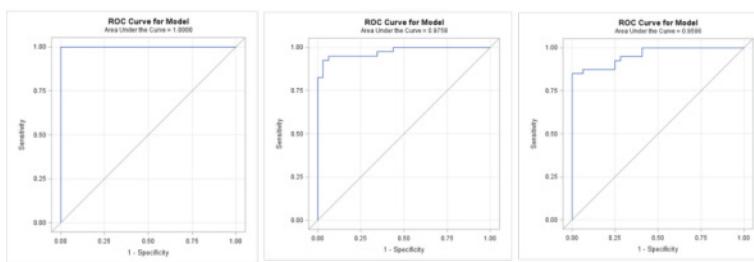
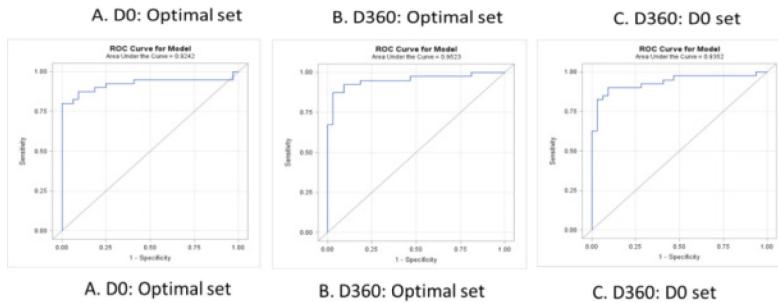
Top 18 sero-reactive antigens

- Best combination of high signals intensity and best p-values between grps
- Best correlation between signal intensities, parasite burden and a significant decline after treatment



ROC curves

optimised antigen "sets"



Non-Endemic vs. Low Intensity egg burden

Non-Endemic vs. Medium Intensity egg burden

Non-Endemic vs. High Intensity egg burden

From the top 18 featured proteins:

6 antigens were found to compose the optimal set for low burden

4 antigens for the medium burden

2 antigens for heavy burden

Multiplex Serosurveillance Arrays

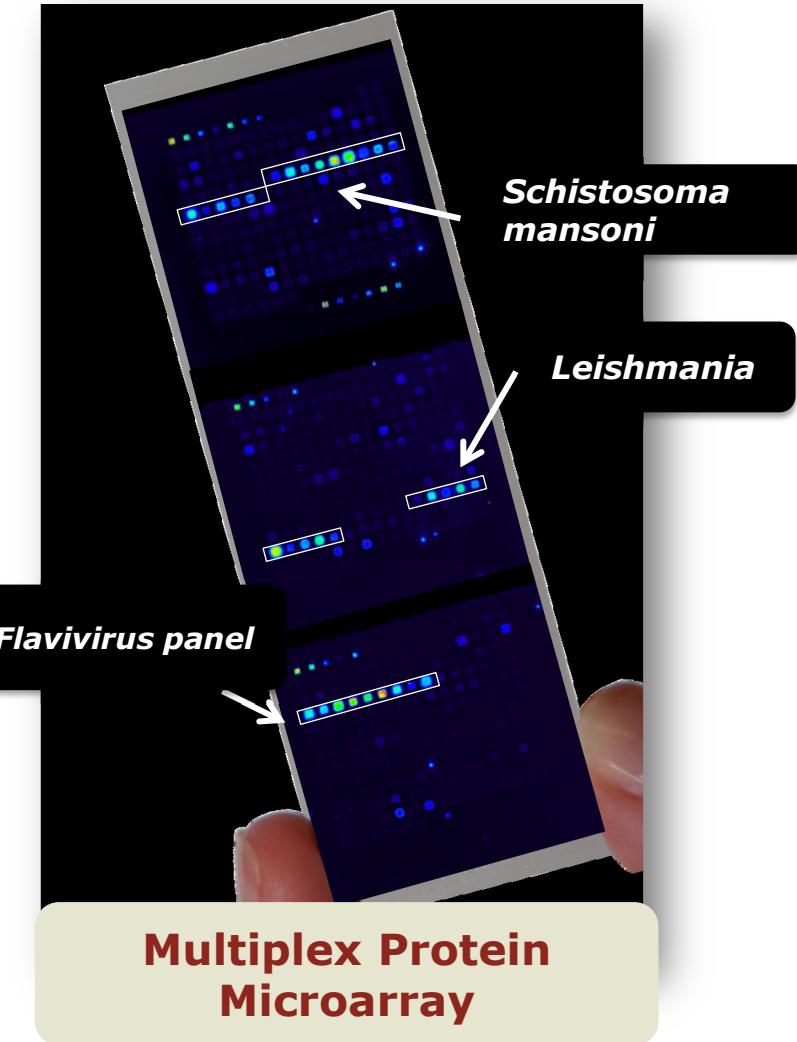
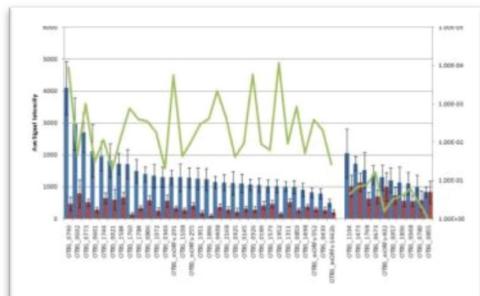
Endemic diseases / Fever panels
Migration medicine

Sero-Surveillance - Diagnostics
(combined with PCR panels)

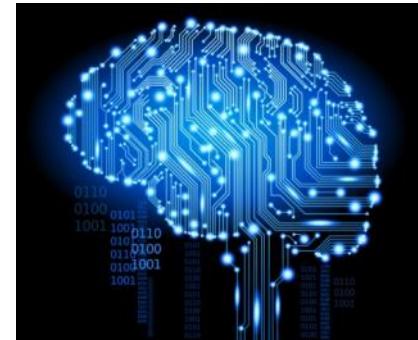
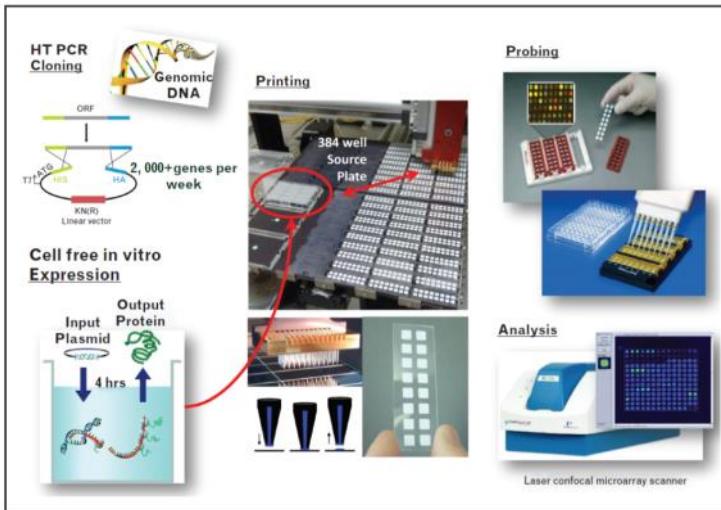
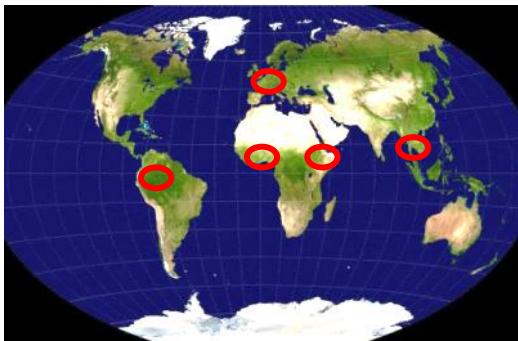
Support vaccine design

- Ab isotypes (IgE, IgG1, IgM etc.)
- Ab dynamics
- Cross-reactivities
- Diagnostic vs. vaccine ag
- Protective responses
- Predictive markers
- etc.

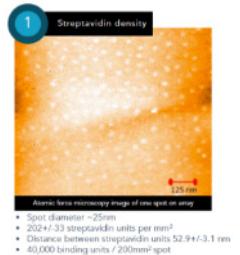
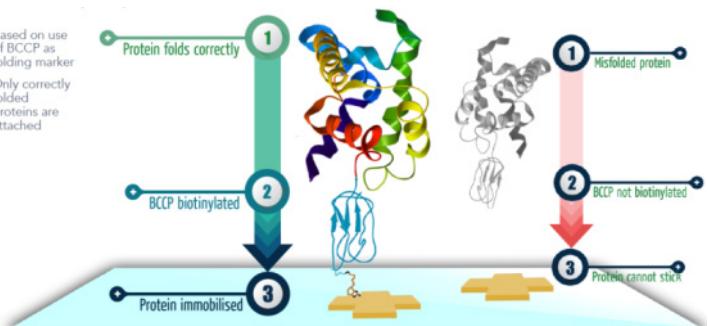
		antigen on ELISA plate								
		Schistosoma	Bilharzia	Bilharzia	Fasciolidae	Schisto	Strongylo-	Filaria	Toxocar-	Toxocar-
Antigen	Antibodies	2nd anti-	2nd anti-	2nd anti-	2nd anti-	2nd anti-	2nd anti-	2nd anti-	2nd anti-	2nd anti-
Schistosomiasis	Specific	+	+		+++	*	+++	+	+	++
Bilharzioses	Specific					+	+++			
Bilharzioses	Cross-reactive					+	+++			
Filariasis	Specific					+	+++			
Toxocarosis	Specific					+++	+++	+++	+++	+++
Toxocarosis	Cross-reactive					+++	+++	+++	+++	+++



Next steps ...

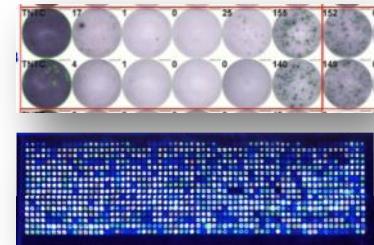
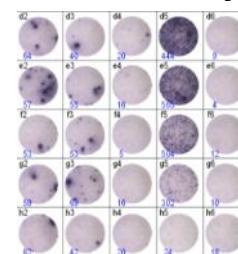


- Based on use of BCCP as folding marker
- Only correctly folded proteins are attached



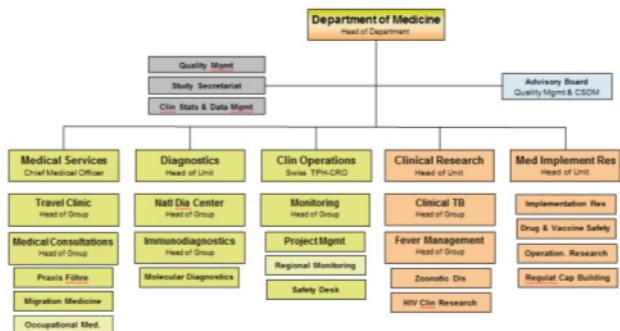
Soluble Proteome

T-Cell Assays



Thank you!

“New” Dept. of Medicine (MED)



Prof Phil Felgner, UCI
Dr Huw Davies, UCI

