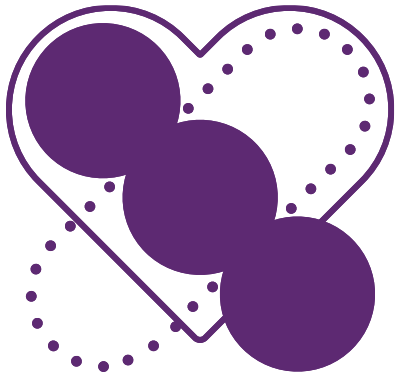
Imperial College London

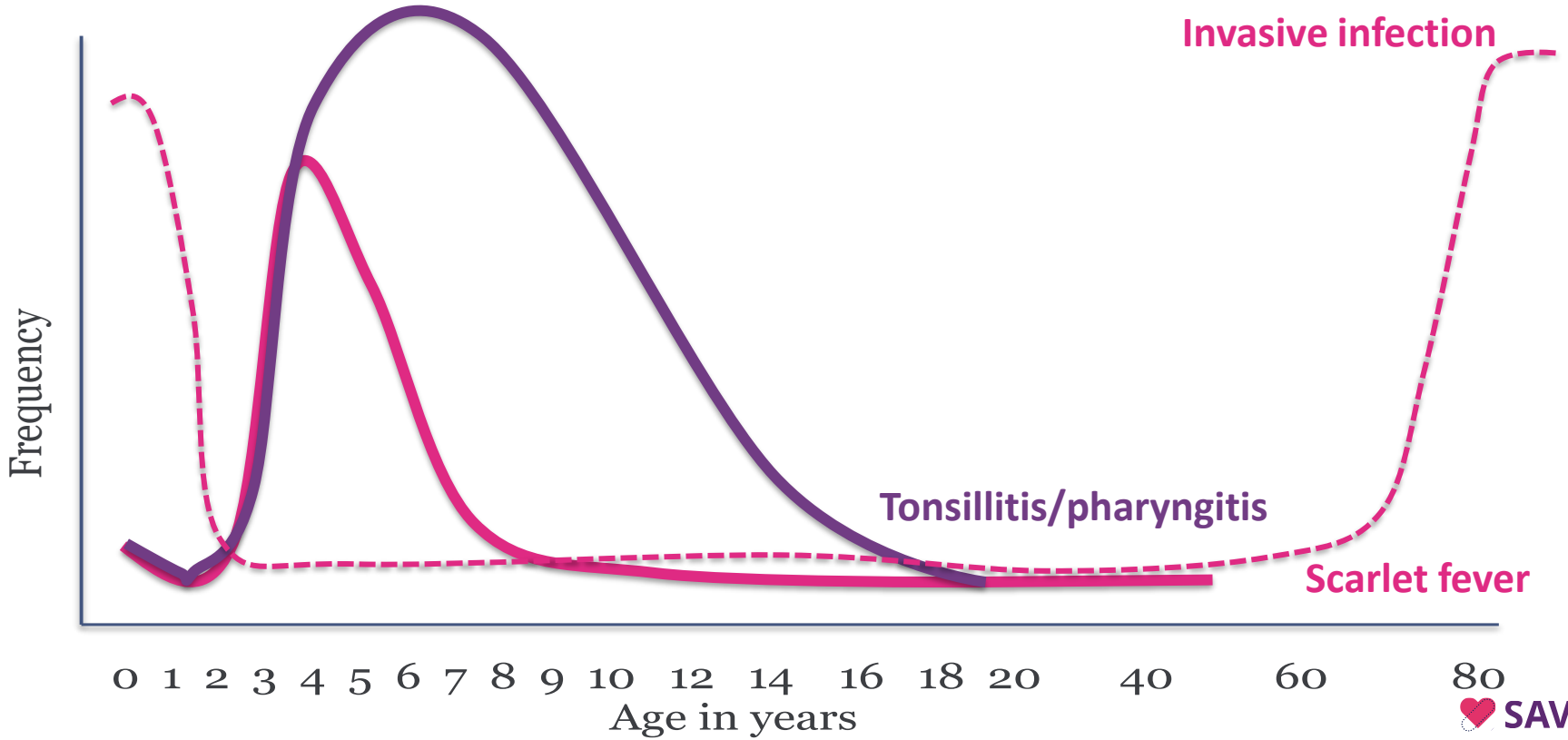


Strep A Vaccine Global Consortium

https://savac.ivi.int/

Immunity to Strep A is acquired with age

- **Underpins belief that we can vaccinate**
- **Understanding immunity might inform vaccination strategy**
  - Choice of what to vaccinate with
  - Route of vaccination
  - **Knowing what to measure- has it worked?**



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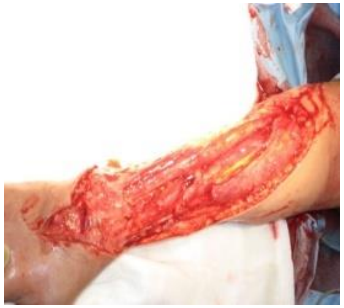
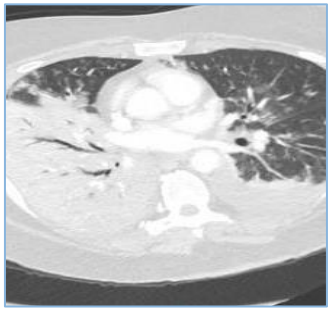
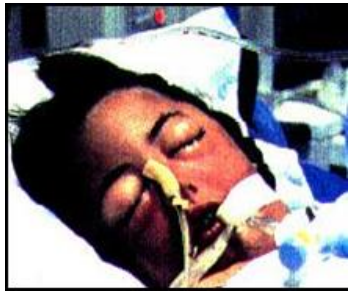
Immunity to invasive infection dominates knowledge



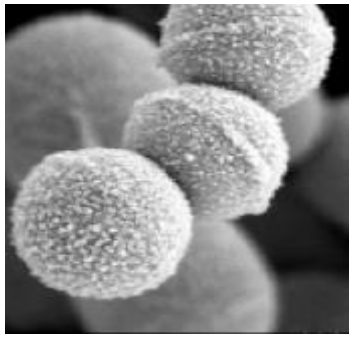
Soft tissue infection-
- Cellulitis
- Nec. Fasciitis
- Myositis



- Pneumonia
- Bacteremia
- Puerperal Sepsis



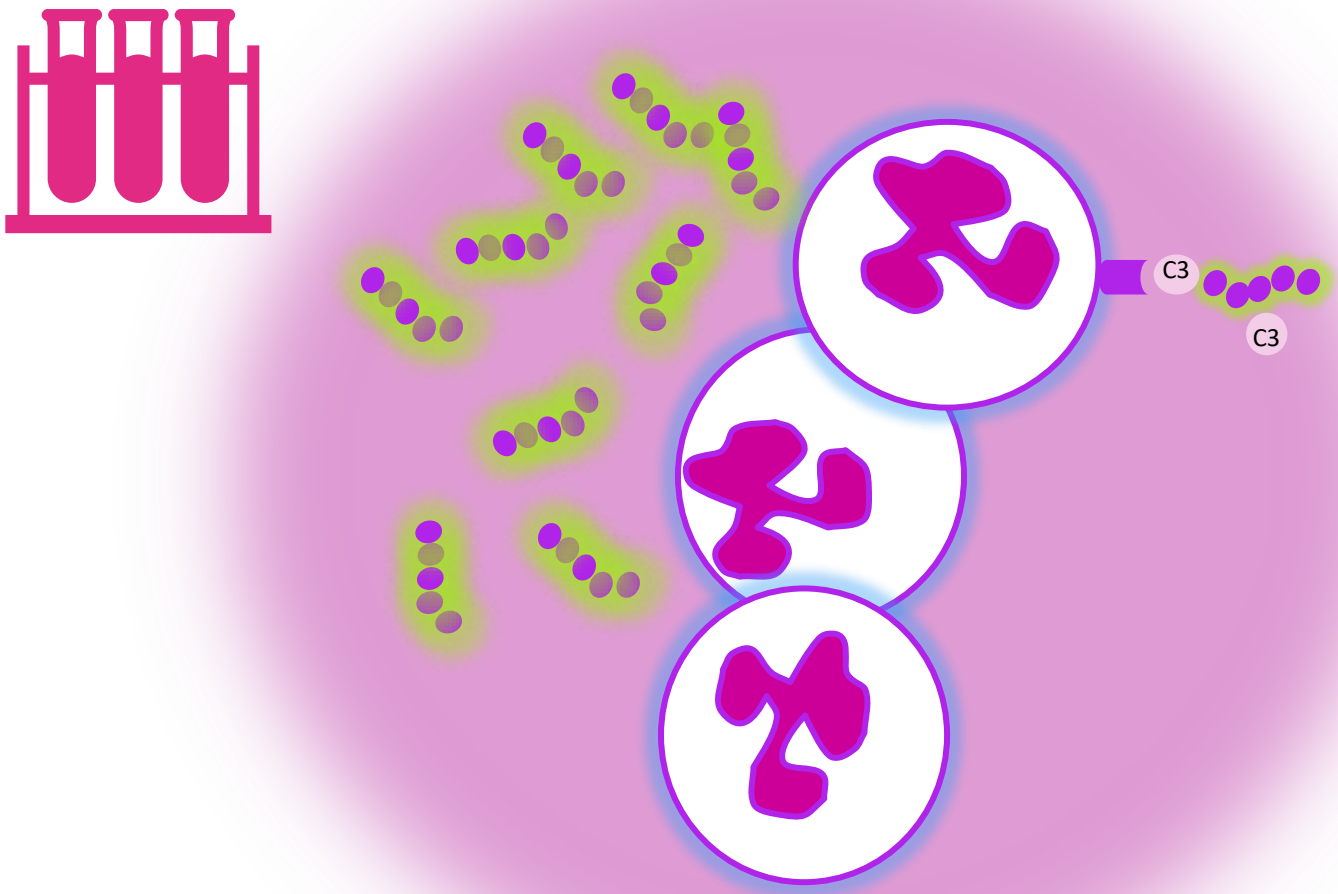
Toxic shock syndrome



Unique ability to grow in non-immune human blood

Strep A survival and growth in human blood

Whole blood without specific antibody



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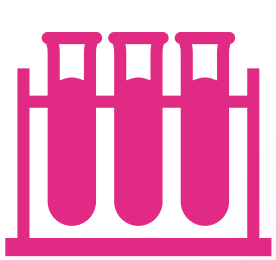
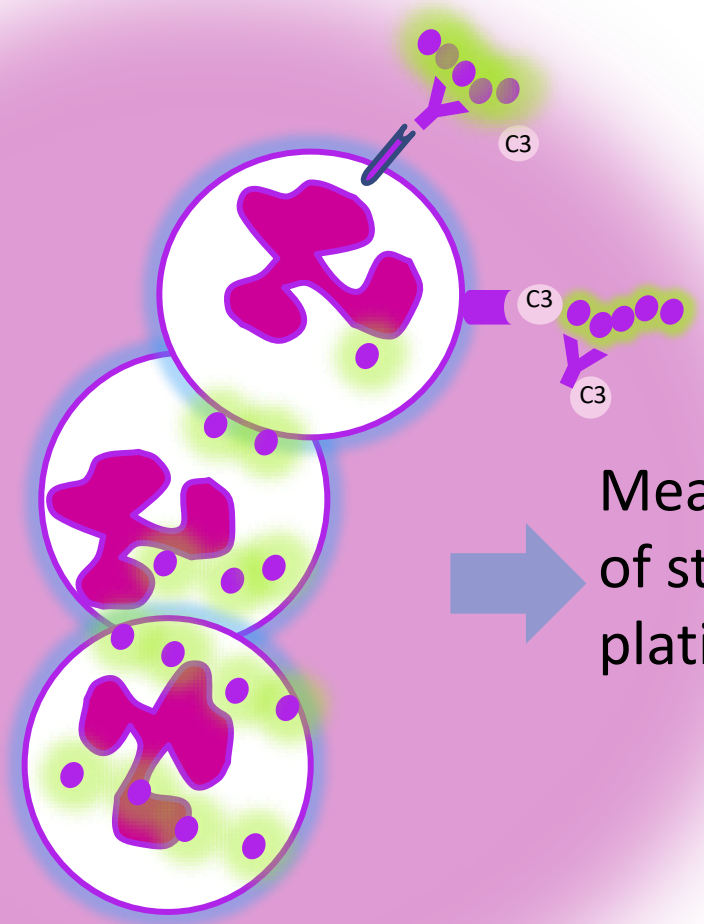
Strep A survival and growth in human blood

Whole blood with specific antibody and complement





Measure net growth of strep A by plating

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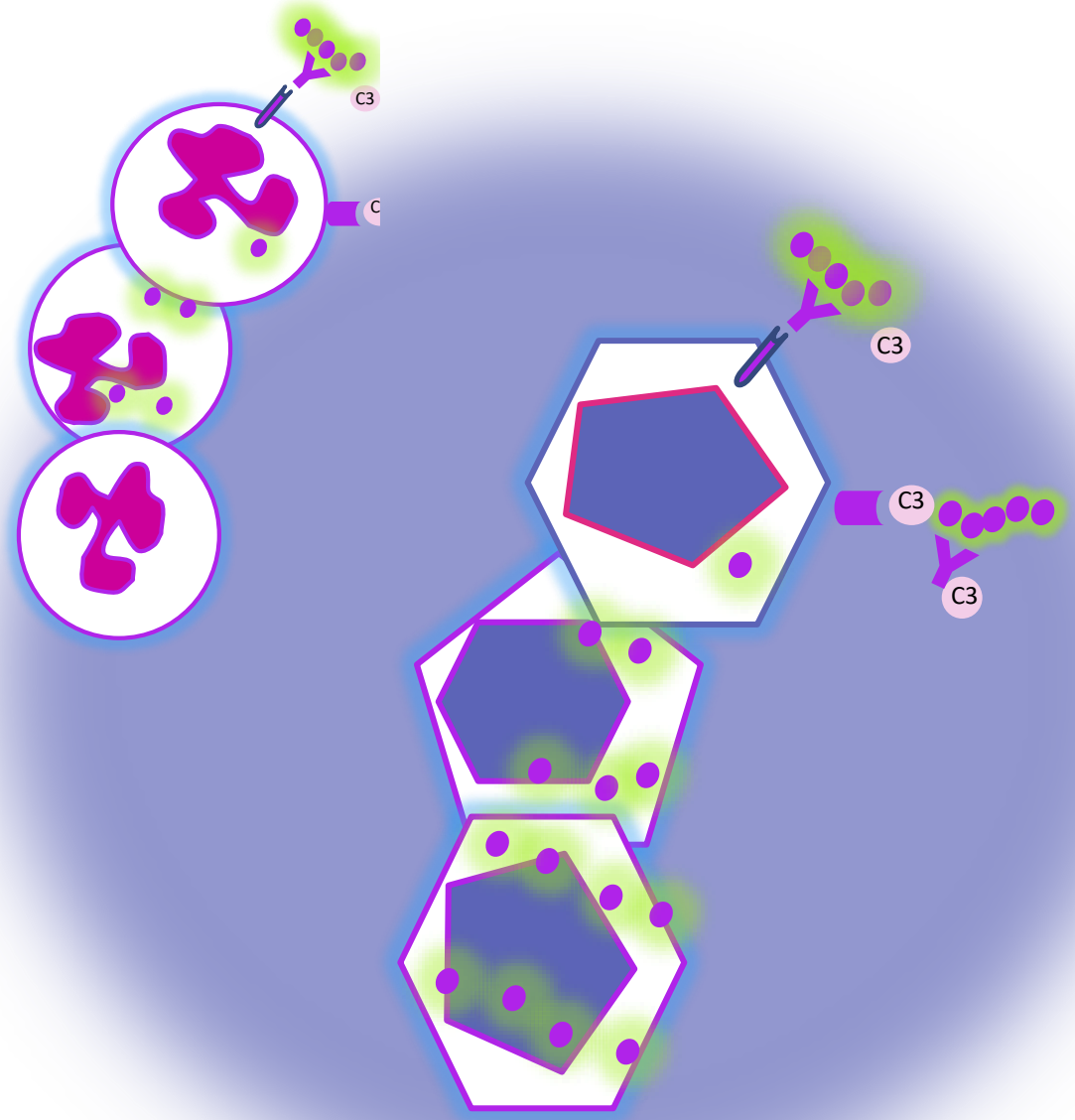
Killing by neutrophils also requires specific antibody



Differentiated HL60 + complement + Strep A + donor serum

Measure killing of strep A by plating

Advantages

- Does not require venupuncture
- Uses frozen bacterial stocks

Jones S, et al. Vaccine. 2018 Jun 18;36(26):3756-3763

Abstract #124 Reuben McGregor -
Abstract #134 Sanaz Salehi

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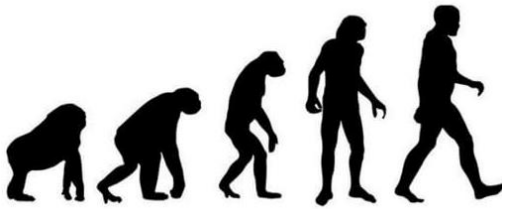
OPK Bioassays of immunity to Strep A



Whole blood assay (+serum)

Neutrophil uptake (+serum)

HL60 assay (+serum)

Reflects plausible mechanism in blood

Needs fresh blood each time

Hard to standardize; requires culture

Dependent on Strep A strain used

OPK assays alone may mislead us

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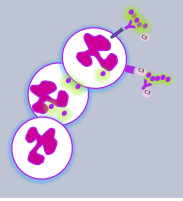
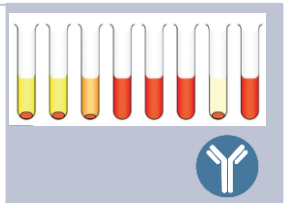
Non-OPK Bio-assays of immunity to Strep A

Not all immunity results in phagocytic killing (both to Strep A and to some vaccine antigens)

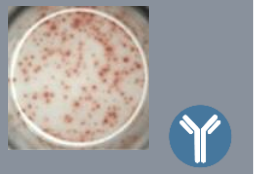


Anti-SpyCEP requires CXCL8 cleavage assay

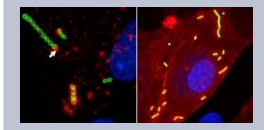
Anti-streptolysin O requires haemolysis assay



Anti-superantigen requires human T cell proliferation or cytokine assay



Anti-adhesin assays require cell co-culture



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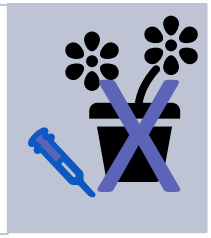
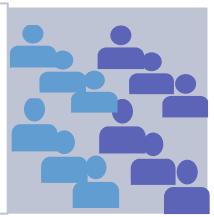
Assays to determine vaccine response and efficacy?

Surrogate indicator of vaccine efficacy (international standards required- portable and reproducible i.e. no culture required!)



Can replace need for clinical end points in trials- licensure (regulatory acceptance)

Allow ongoing surveillance of immunity in target populations

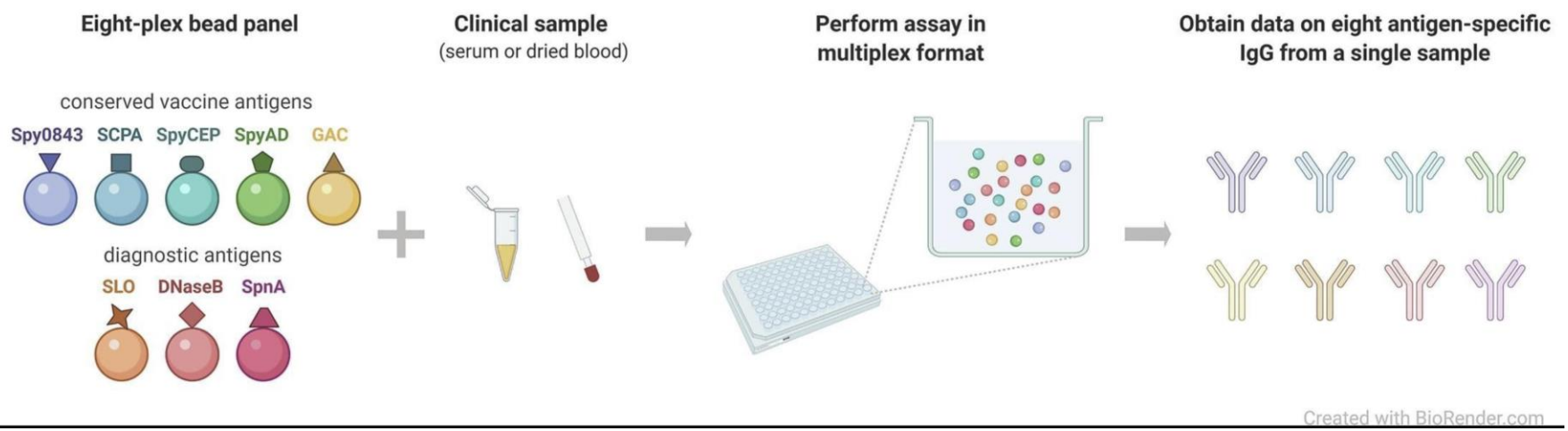


SAVAC Gap: Portable, reproducible correlates of immunity

ELISA? – One plate per single antigen Range not broad. Often requires dilutions

An eight-plex immunoassay for Group A streptococcus serology and vaccine development

A robust and reproducible eight-plex assay, that simultaneously measures IgG antibody responses to eight Group A Streptococcus (GAS) antigens, was developed, optimised and evaluated in clinical samples. Conserved putative vaccine antigens, and antigens involved in rheumatic fever diagnosis were included. The assay principle is demonstrated below.



- Simultaneous measurement of antibodies to all 8 antigens using 2µl (dried blood spot)
- Luminex principles
- Wide dynamic range

Whitcombe A, et al. J Immunological Methods, 2022

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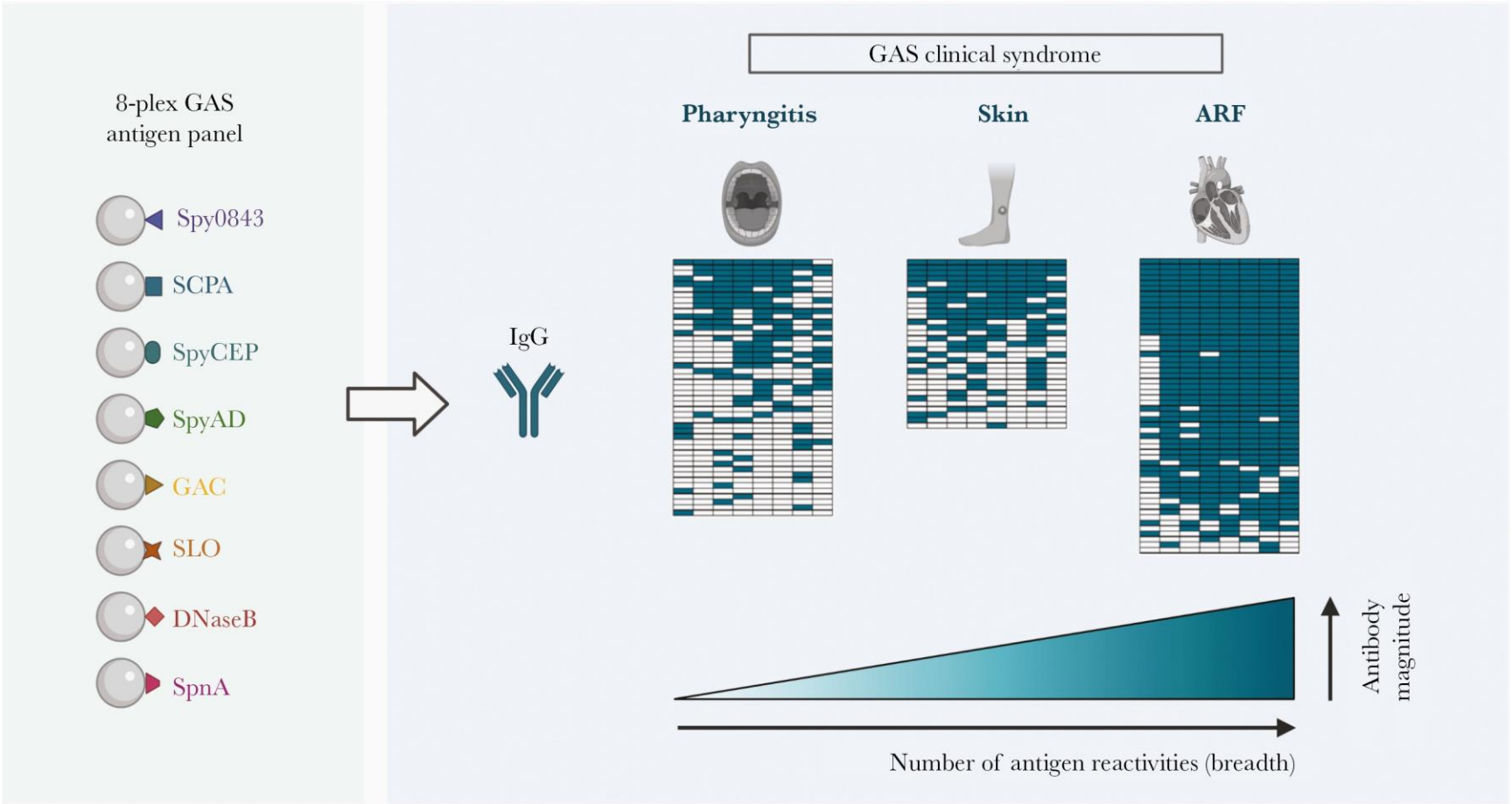
Use of 8-plex assay :antibody prevalence ARF vs Strep A



Measuring antibody responses to eight group A *streptococcus* (GAS) antigens reveals distinct serological profile in children with acute rheumatic fever (ARF) compared to precursor GAS pharyngitis and skin infections

Whitcombe A, et al. J Infect Dis, jiac043, https://doi.org/10.1093/infdis/jiac043

Created with Biorender.com

Abstract Alana Whitcombe (#149)

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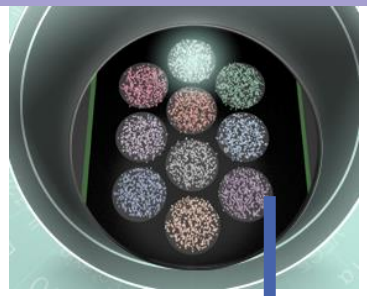
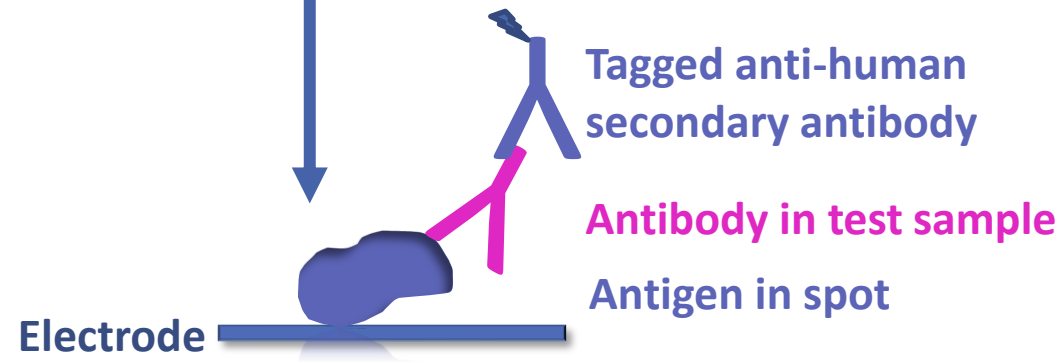
Mesoscale assays (e.g. MSD): learning from COVID-19



- Multiple spots/well
- Single antigen within each spot
- Hence multiple antigens per well
- Signal generated if antibody binds



Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial

Maheshi N Ramasamy, Angela M Minassian*, Katie J Ewer*, Amy L Flaxman*, Pedro M Folegatti*, Daniel R Owens*, Merryn Voysey*, Parvinder K Aley, Brian Angus, Gavin Babbage, Sandra Belij-Rammerstorfer, Lisa Berry, Sagida Bibi, Mustapha Bittaye, Katrina Cathie, Harry Chappell, Sue Charlton, Paola Cicconi, Elizabeth A Clutterbuck, Rachel Colin-Jones, Christina Dold, Katherine R W Emary, Sofiya Fedosyuk, Michelle Fuskova, Diane Gbesemete, Catherine Green, Bassam Hallis, Mimi M Hou, Daniel Jenkin, Carina C D Joe, Elizabeth J Kelly, Simon Kerridge, Alison M Lawrie, Alice Lelliott, May N Lwin, Rebecca Makinson, Natalie G Marchevsky, Yama Mujadidi, Alasdair P S Munro, Mihaela Pacurar, Emma Plested, Jade Rand, Thomas Rawlinson, Sarah Rhead, Hannah Robinson, Adam J Ritchie, Amy L Ross-Russell, Stephen Saich, Nisha Singh, Catherine C Smith, Matthew D Snape, Rinn Song, Richard Tarrant, Yrene Themistocleous, Kelly M Thomas, Tonya L Villafana, Sarah C Warren, Marion E E Watson, Alexander D Douglas*, Adrian V S Hill*, Teresa Lambe*, Sarah C Gilbert*, Saul N Faust*, Andrew J Pollard*, and the Oxford COVID Vaccine Trial Group*

"We found here that anti-spike IgG levels correlate with neutralising antibody titres for all age groups. This finding suggests that, should neutralising antibodies be shown to be protective in humans, routine serological assays could be used for the standardised evaluation of functional antibody by vaccine candidates in clinical trials." Lancet, 2020

ASAVI

Australian Strep A Vaccine Initiative

LISSSD Symposium 1

Weds June 8th (J Carapetis)

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Ongoing questions for high throughput assays

Positive control

- Pooled IVIG?
- Standard high titre anti-serum (Goldblatt et al, Clin Vacc Imm, 2011. '007sp'. 278 volunteers, 23-valent vax, 2 units each, 15,333 vials= 25y supply)

Negative control

- IgG-depleted human serum (commercial supply)
- IgG-cleaved human serum (IdeS)
- Screen large pools of donors for least immune?

Prevalence of systemic immunity in adults (Neutrophil OP assay)

IVIG	Complete control of Strep A
5%	Immunity similar to IVIG
90%	Variable- Medium immunity
5%	No detectable immunity

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Ongoing questions for high throughput assays

What antigens to include

- Vaccine antigens
- Pathogen exposure antigens
- DIVA capability (differentiation of infected versus vaccinated)

Non-whole blood samples

- Fingerprick/blood spot
- Mucosal/crevicular fluid

Validation using bioassays?

- Extrapolate viruses to bacteria?
- Do they need to correlate?

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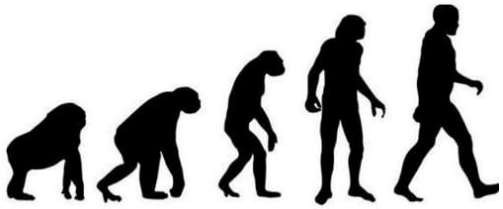
Bioassays of immunity vs. Correlates of immunity?



Whole blood assay

Neutrophil uptake

HL60 assay

Virulence inhibition (single Ag)

ELISA + single antigen

Bead multiplex assays

MSD-multi plex

Reflects true mechanism of immunity ?

Dependent on Strep A strain used

Needs fresh blood each time

Cell culture or fiddly assay

Hard to standardize and replicate or scale up

Easy to standardise

Irrelevant to immunity?

Serum: Are we looking in the right place?

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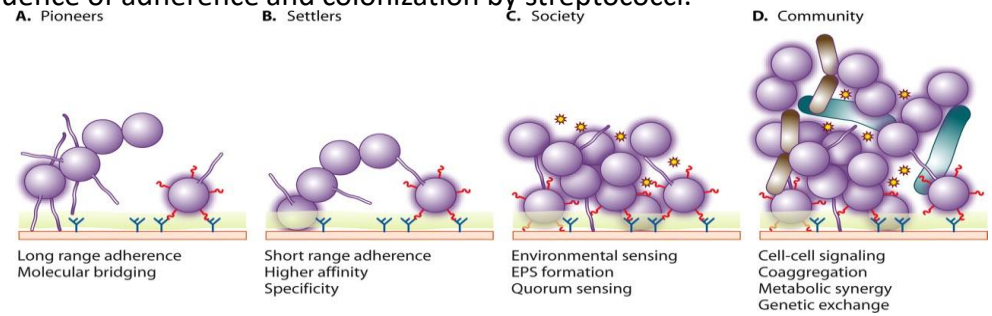
What are we trying to prevent with a Strep A vaccine?

Temporal sequence of adherence and colonization by streptococci.



Strep A adhesins
M protein; Pilus; ScpA
Fibronectin binding proteins
Collagen like proteins

Strep A virulence factors
Streptolysin O/nga;
Superantigens; SpyCEP;
HA capsule

Immune Response
What is required to combat this process
????

Nobbs et al. Microbiol. Mol. Biol. Rev. 2009

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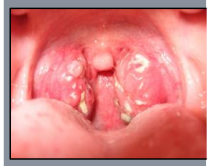
Observational studies for mucosal immunity: CHIM

"CHIVAS-M75" Controlled human challenge model of Strep A pharyngitis

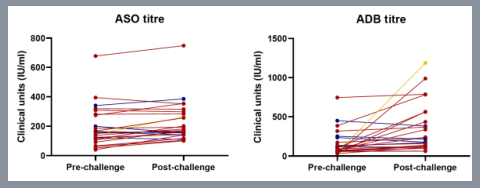
Osowicki et al, Lancet Microbe, 2021

Emm75 Strep A administered to volunteers.
17/20 developed clinical pharyngitis



Antibody responses to multiple strep A antigens
Serum and saliva. (see LISSSD)

Pros: compare pharyngitis vs none
Cons: early treatment, M75, Adults



0915: LISSSD Symposium 1
Weds June 8th

3pm-4pm Weds June 8th
Abstract #19 Alice Halliday mucosal gene expression
Abstract #21 Hannah Frost Salivary IgA

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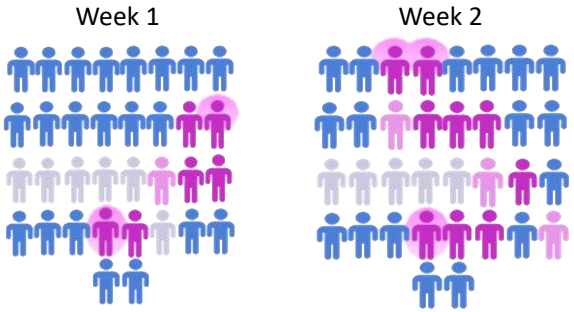
Observational studies for mucosal immunity: Children



Blue=swab negative
Pink= swab positive for S. pyogenes (dark pink=identical)
Grey=unavailable that day

- What is the basis of hetero geneity despite similar exposure?
- What does 'colonization' mean?

Resistant

'Colonised'

Colonised & heavy shedding

Pharyngitis or tonsillitis

Scarlet fever

Cordery et al, Lancet Microbe 2022

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Are there different layers of immunity that prevail?

Antibody	Resistant	Colonised	Shedder	Pharyngitis	Scarlet Fever
Attachment	+	-	-	-	-
Opsonic	[+]	+	-	-	-
Anti-virulence	[+]	[+]	+	-	-
Anti-SF toxin	[+]	[+]	+	+	-
Host genetics	?	?	?	?	?

Abstract #20, Alex Keeley, 3pm-4pm Wed June 8th
Serological responses following colonisation in children in Gambia

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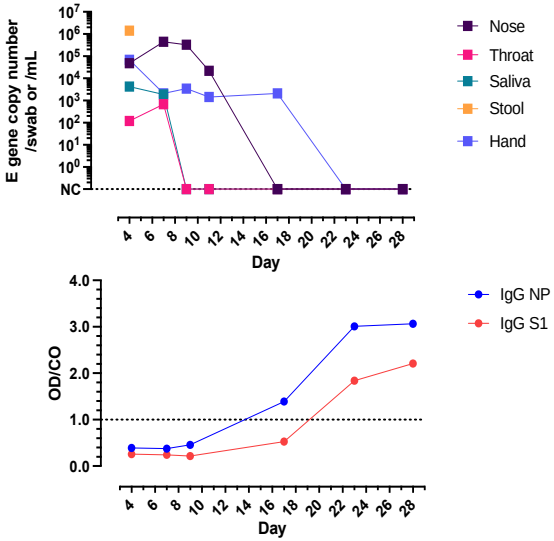
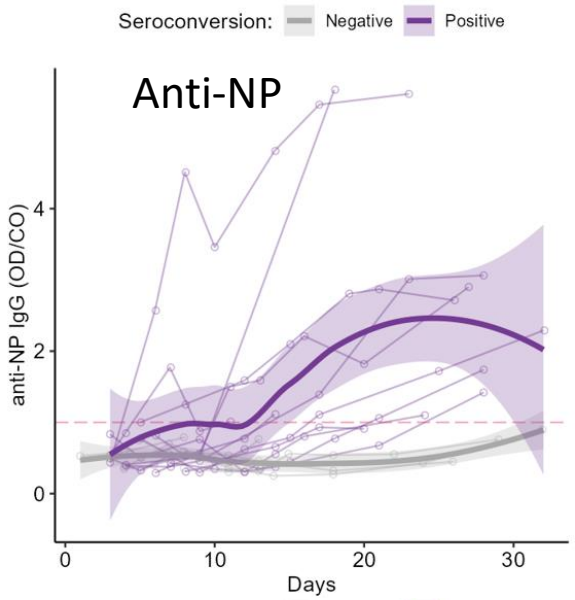
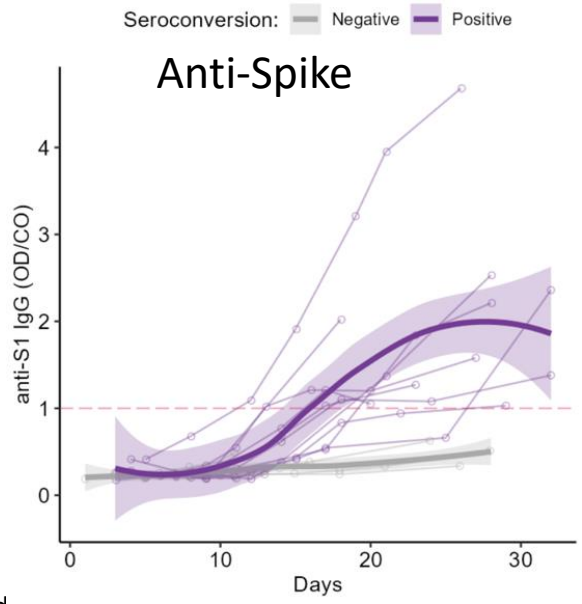
Learning from COVID-19: mucosal responses in children

SARS CoV2 viral shedding



IgG responses:
No blood tests required





N=20 children

Cordery et al, Lancet Microbe 2022, in press; Taylor G, et al unpublished

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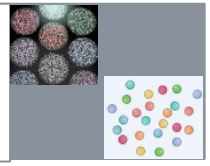
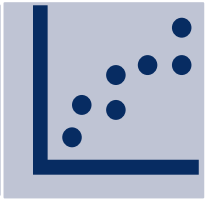
The way forwards….identify the gaps

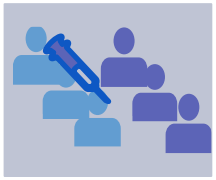
High throughput assays now in use/developed
?qualification



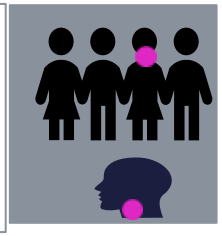
Compare and correlate with established assays
(OPK and virulence inhibition)



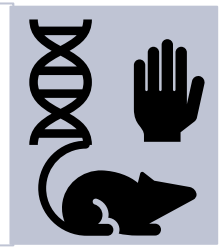
Use in (i) population surveillance;
disease samples (ii) vaccinated cohorts



Mucosal immunity in pharyngitis- children.
Mucosal IgG and sIgA assays ?saliva or oral fluid



T cell and innate immunity/B cell memory, tonsils
Mouse models; CHIM; immunity in skin;
genetic susceptibility ; i.n. vs i.m. vaccination



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Thank you

wellcome

SAVAC
Strep A Vaccine Global Consortium

Jean-Louis Excler (IVI)
Jerome Kim (IVI)
SAVAC Executive members
CoP team
All those whose work I have mentioned!