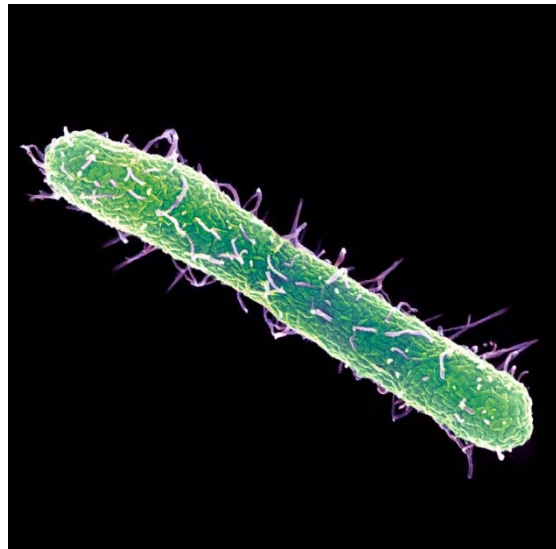




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# Characterising the cellular and humoral immune response to invasive nontyphoidal *Salmonella* (iNTS) disease in west African populations



Dr Sean Elias

# Burden of iNTS & SETA Study



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- Bacteraemia caused by NTS is common in:
  1. Young immune-naïve children
  2. People with HIV
  3. Children infected with malaria
- In such groups particularly in sub-Saharan Africa mortality can be high (~20%).
- As a result this is a key target disease for vaccine development. However vaccines may not provide the same protection in immunocompromised, susceptible individuals as in healthy immunocompetent ones.

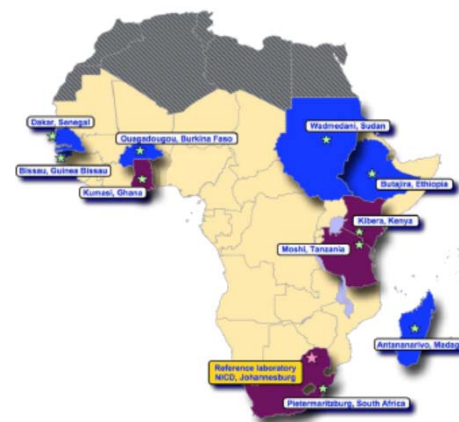
## 'Severe Typhoid in Africa' (SETA)

BILL & MELINDA  
GATES foundation



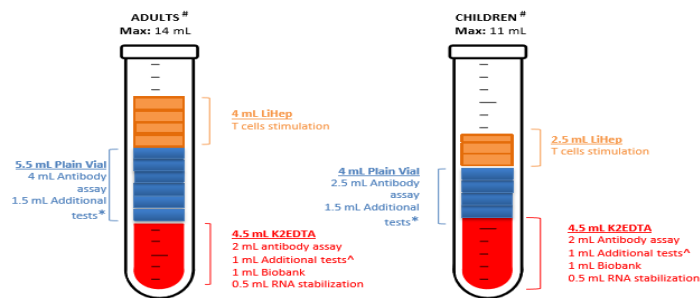
INTERNATIONAL VACCINE INSTITUTE

- Main SETA study led by Florian Marks at IVI.
- Prof Calman MacLennan designed a complementary iNTS study to run alongside the main SETA study.
- The iNTS component is based in both
  - (Schipra Hospital) Ouagadougou, Burkina Faso
  - (KCCR, KNUST) Kumasi, Ghana.



# Study Design and Assays

- Full Immunology bloods will be taken for all confirmed iNTS disease cases at 5 time points
- Neighbourhood (aged matched) and Household controls at time of enrolment only.
- Whole stimulated blood, serum, plasma and RNA collected.
- Blood stimulations done on site, RBCs lysed, Live/Dead stained and fixed. No LN required.
- NTS carriage and malaria co-infections recorded.
- HIV status noted in Ghana only.



**Table 7.** Enrollment and follow-up visit schedule for SETA study participants

Activity day	Window period		Case	Neigh. Control	Household Contact
	Min	Max			
0*	–	–	Enrollment	–	–
3-7**	3	10	Visit 1	Enrollment	Enrollment
28-30	25	33	Visit 2	No visit	No visit
90	83	97	Visit 3	No visit	No visit
180	173	187	Visit 4	Visit 1	Visit 1
360	353	367	Visit 5	Visit 2	Visit 2

\* Reference day correspond with case recruitment

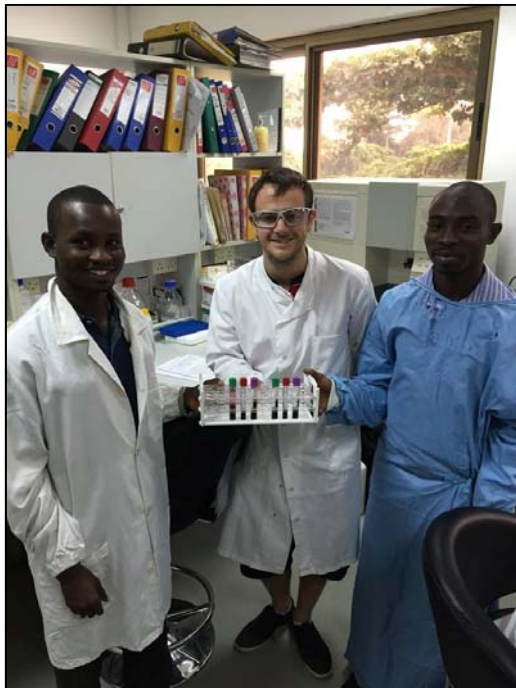
\*\* Corresponds with BC confirmation of a *Salmonella* case

- We have developed a panel of assays to assess NTS specific immunological responses.
  - **Standardised ELISA:** IgG, IgA and IgM ELISA for O:4,5, O:9, LPS and Flagellin for *S. Typhimurium* and *Enteritidis* developed (total 18 different ELISAs).
  - **Flow Cytometry Panels:** T cell and Innate cell panels.
  - **Serum Bactericidal Assay:** To measure complement mediated antibody activity.

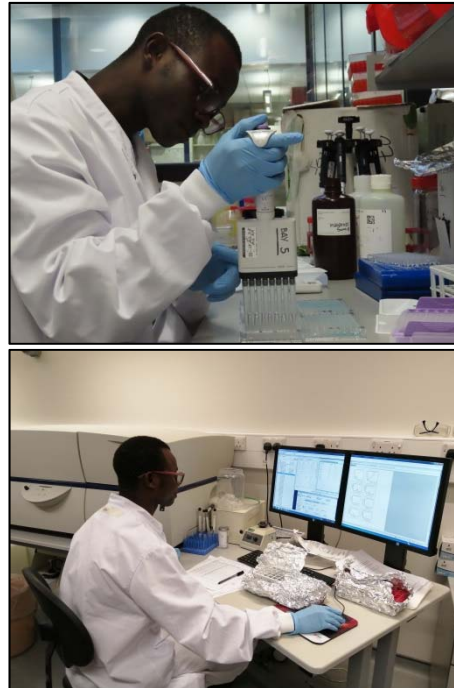
# Capacity Building

- An important part of this study has been capacity building at the field sites.
- At the Schiphra Hospital lab in Burkina Faso we put together an immunology lab from scratch.
- With help from an AfOx (Oxford/Africa Initiative) Travel Grant we arranged for Japhet to visit from Ghana to learn and help run the immunological assays so in future studies we can potentially run the assays in Ghana.

Ghana



Oxford



Burkina Faso

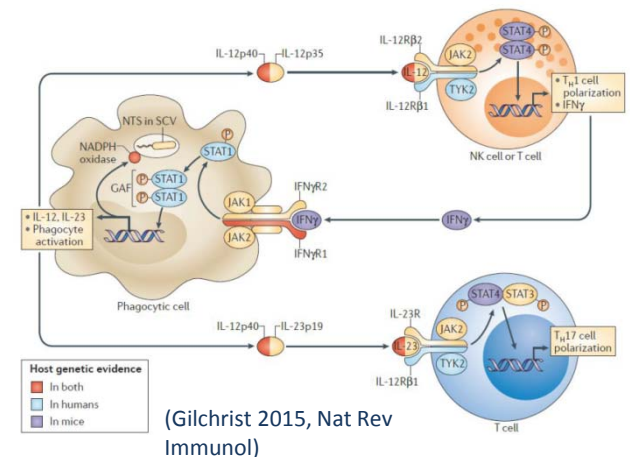
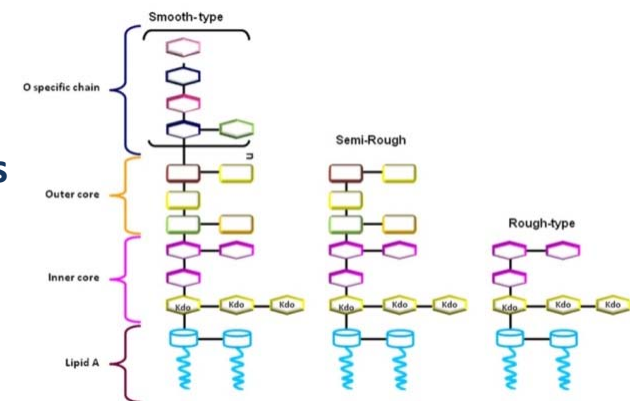


# Immunological Response to iNTS Disease



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- Studies such as those by MacLennan et al in Malawi have provided strong immunoepidemiological evidence for an important role for complement and antibody in protecting individuals, particularly young children against NTS. **Antibodies** against O antigens have been shown to correlate with early acquired immunity in children but not necessarily protection.
- With Salmonella being a facultative intracellular pathogen **CD8+ T cells** are also an important component for killing infected cells and limiting pathogen reservoirs particularly in late stages of disease.
- Acquisition of Salmonella-specific **CD4+ T cells** in early childhood is consistent with early exposure. These play an important role in B cell help but appear insufficient alone in protecting against invasive disease.
- Presence or absence of innate cells, including macrophages, neutrophils, NK cells,  $\gamma\delta$  T cells and MAIT cells also linked with immunity or susceptibility to iNTS disease.

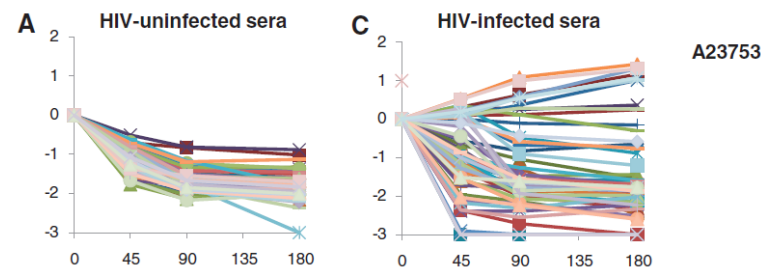


# HIV immune dysregulation and NTS



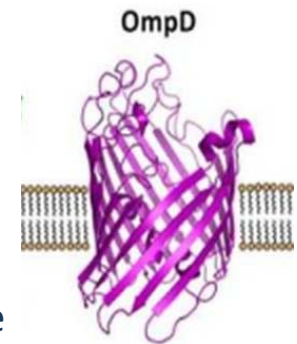
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- High-titre antibodies specific for Salmonella (LPS) were associated with a lack of *S. Typhimurium* killing in some HIV-infected African adults (MacLennan, Science, 2010).



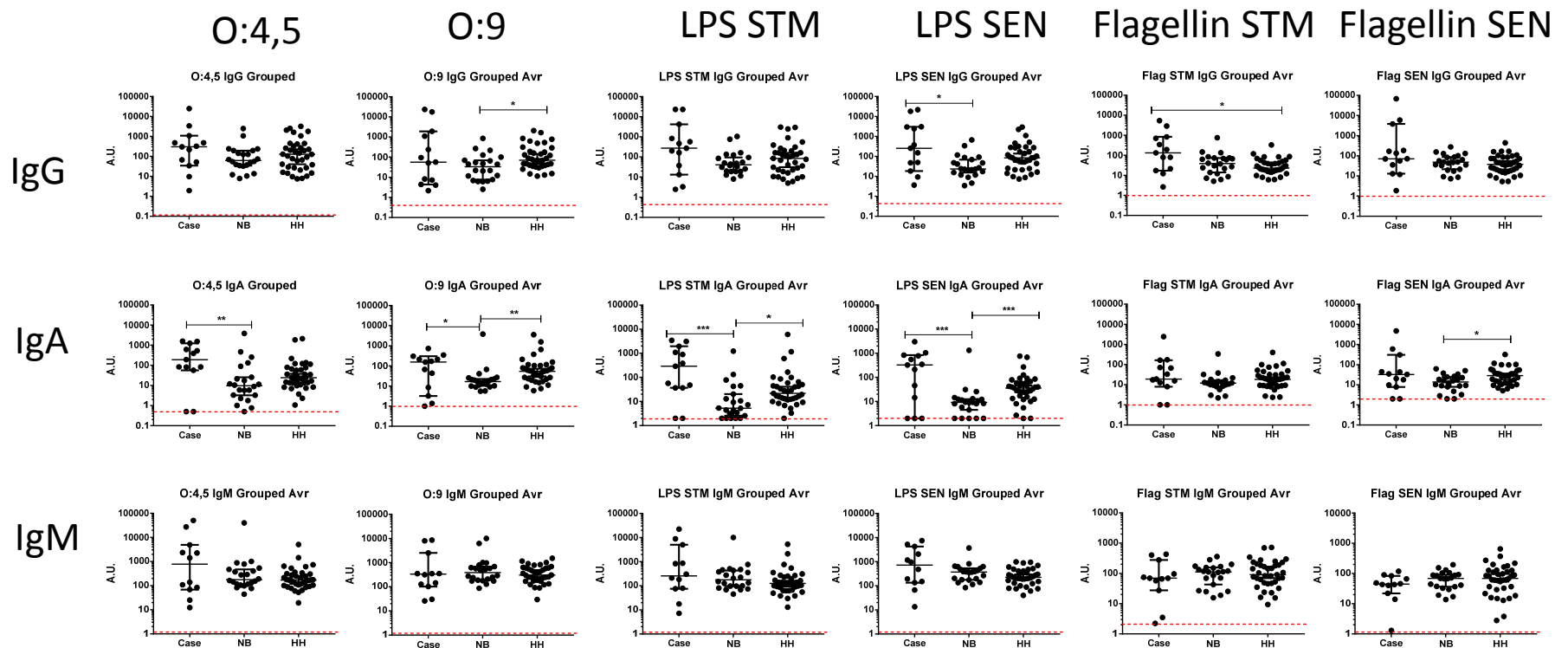
- LPS-specific antibodies are found at a lower level in healthy and HIV+ individuals whose sera can induce killing.
- Removal of LPS-specific antibody from the sera by absorption with STM LPS (but not SEN LPS) restores killing activity of HIV+ inhibitory sera.
- HIV+ inhibitory sera can also kill STM with an O antigen KO.
- Therefore HIV+ inhibitory sera has the inherent ability to kill Salmonella.

- Follow up work has shown the target of killing antibodies were outer membrane proteins such as porins (e.g. OmpF, OmpC & OmpD).
- It has also shown that inhibition was highly associated with IgA and IgG2 antibodies (Goh 2016 Plos NTD).



# Standardised ELISA Results

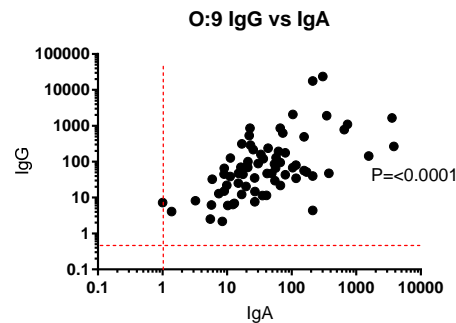
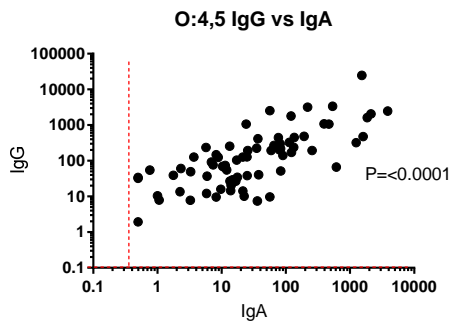
- **IgG** and **IgM** to all iNTS antigens tested were present among all cases and controls
- **IgA** was absent in some infants (particularly <1 year old).
- iNTS IgG and IgA median antibody levels were often higher in cases compared with controls, and up to 1-3 logs higher for some individual cases.



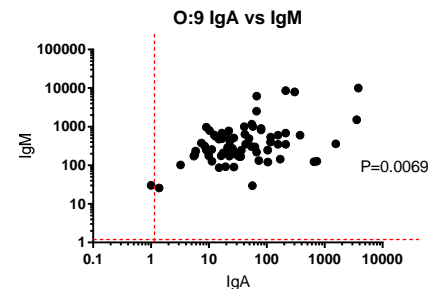
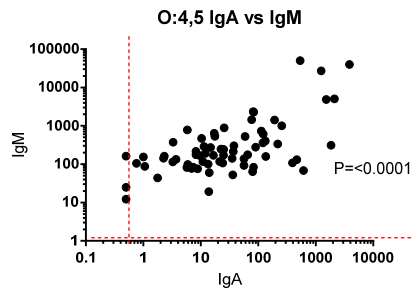
For cases, A.U. shown is average across multiple time points. NB= Neighbourhood Controls, HH= Household Controls

# Standardised ELISA Results

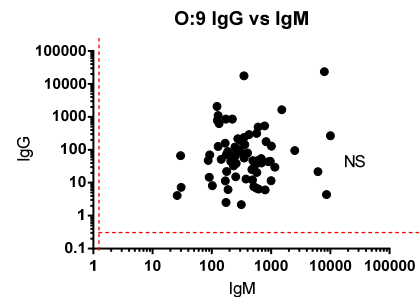
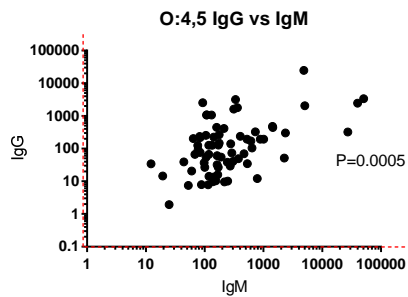
- iNTS IgA correlated with iNTS IgG and IgM.
- iNTS IgG and IgM only correlated for O:4,5 and STM LPS.



IgG vs IgA



IgM vs IgA



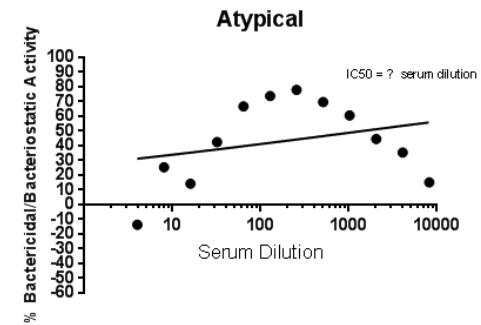
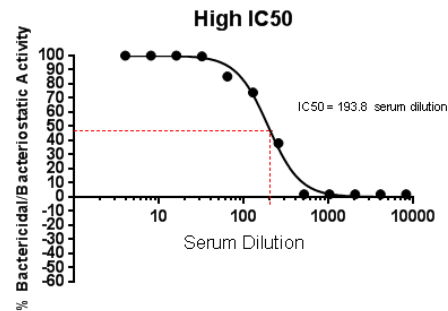
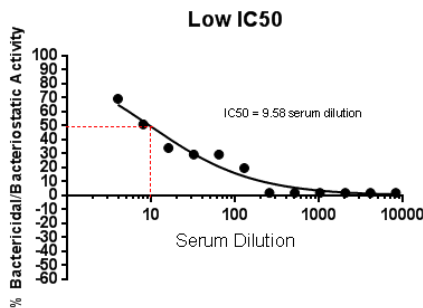
IgG vs IgM



# Serum Bactericidal Assay (SBA)

## IC50 SBA

- **Heat treated (HT) serum** and differing concentration is incubated with a exogenous source of human complement and STM bacteria over a 1 hour incubation, before plating on agar overnight to grow colonies of replication competent bacteria.
- Activity of serum is compared by calculating serum **IC50**, the dilution of HT serum that gives 50% **Bactericidal** (killing)/**Bacteriostatic** activity (growth inhibitory), i.e. half of the initial starting bacterial CFU are rendered incapable of replication by either mechanism.
- 3 activity profiles have been observed at this point. More data are required to validate observations so far.



- Link with antibody titres yet to be determined. Increase from low to medium titres doesn't always increase IC50. A lot of variables to dissect.
- Confirmed in 3 Individuals with highest IgA/IgG O antigen/LPS titres, but not HIV positive.

# Flow Cytometry Results

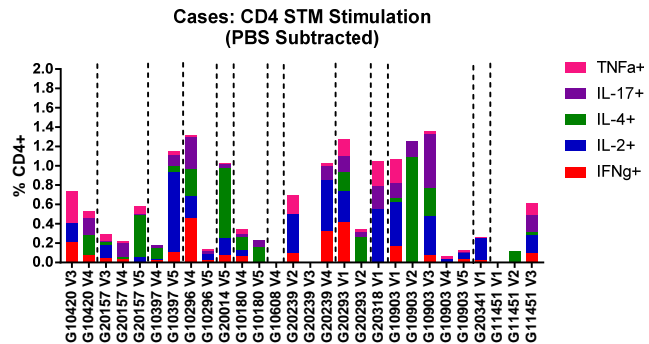


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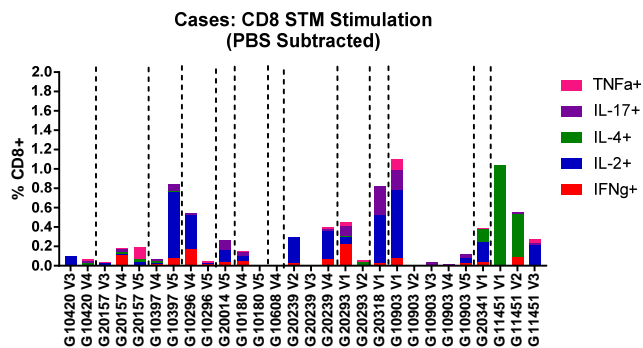
## T cell Panel

- CD4+ and CD8+ T cells secreting: IL-2, IL-4, IL-17, TNF-a and IFN-g.
- Cells stimulated with beaten (BB) Salmonella Typhimurium (STM) homogenate.
- Cases: CD4+ T cells producing multiple cytokines dominate.
- Controls: Monofunctional CD4+ and CD8+ T cell populations. Some very high in frequency.

### Cases

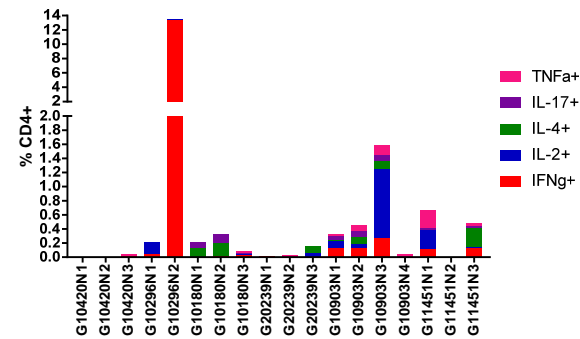


### CD4



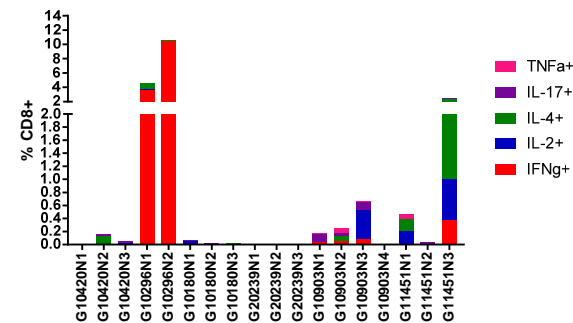
### CD8

Controls: CD4 STM Stimulation (PBS Subtracted)



### Controls

Controls: CD8 STM Stimulation (PBS Subtracted)



# Conclusions and Future Work



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## Conclusions

- Antibody to NTS antigens are nearly universally present in west Africans (Ghana).
- Serum IgG and IgA is acquired in parallel from a very young age and are markers of exposure.
- Serum Ab observations appear to hold true in east Africans (Kenya) in other concurrent work.
- Very high titres of NTS antibodies may however be problematic, and not just in HIV-infected African adults as previously shown by MacLennan et al (Science, 2010). Vaccine concern?
- iNTS disease drives acquisition of multifunctional CD4+ T cells.
- Controls show mixed CD4 and CD8 responses that are typically monofunctional.

Further studies are required to understand the precise role of these cellular and humoral responses in preventing and clearing iNTS infection.

## Future Work on the SETA iNTS study

- Add data from Burkina Faso site
- Vi + OmpD ELISA
- Innate cell flow cytometry panels
- Expand SBA dataset
- Run samples from known HIV positive cases
- Investigate the effects of malaria co-infection on immunogenicity
- Correlate immunological data with serotype and bacterial carriage data

# Acknowledgements

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