Use of AQIX® RS-I for lymphocyte culture

- When culturing peripheral blood mononuclear cells (PBMC) the current protocol is carried out in RPMI +10% AB serum.
  - We set out to assess AQIX in this step and whether AQIX was a suitable media for culturing Lymphocytes *ex vivo.*
- For comparison, RPMI alone and AQIX containing 10% AB serum are also used for culturing lymphocytes.
- Parameters measured include
  - Viability pre and post incubation of total cell population
  - % IFNg+ cells following antigen stimulation
  - Number of cells in each condition following incubation

Comparison of 4 different media formulations

- AQIX - Proposed serum free replacement media
- AQIX 10% AB - To assess requirement for serum
- RPMI - To compare serum free current media
- RPMI 10% AB - To compare new media against standard

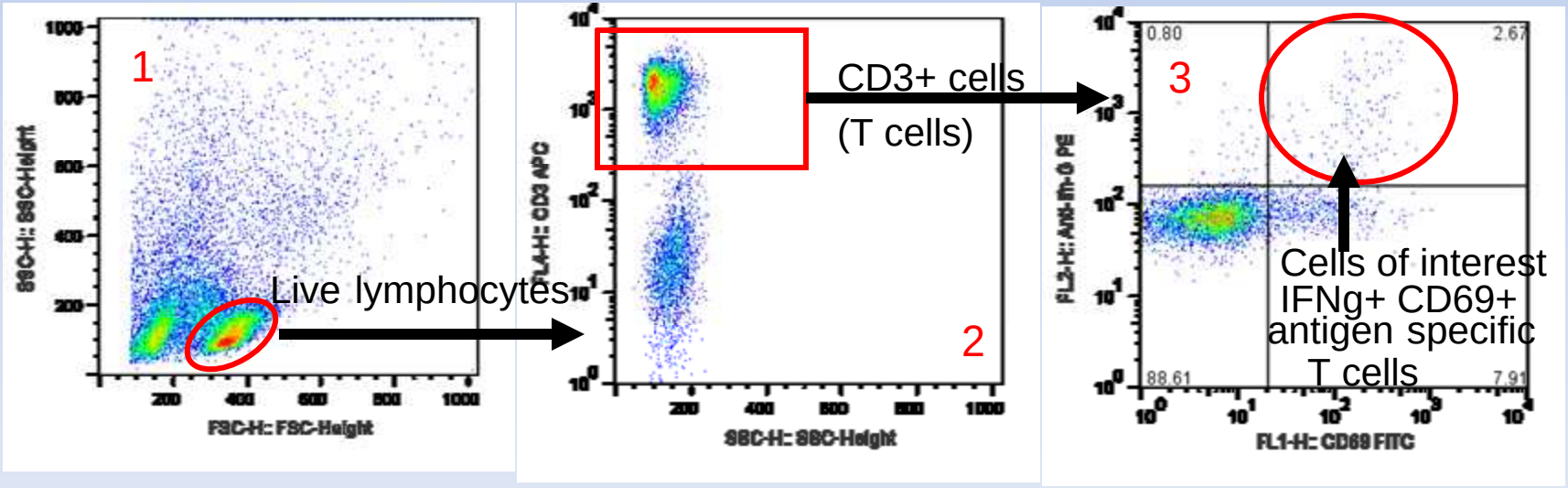
- Experiments conducted on 7 samples
  - 2 patient samples
  - 5 normal donors
- Results presented as individual donor results (FACS plots) showing CD69 vs IFNγ
- Results for viability and yield are shown as graphs of the pooled data from all the donors.

Data analysis

- Cell suspensions post culture with or without antigen are analysed as to their composition by flow cytometry.
  - Cells are identified by their size and granularity (FSC/SSC)
  - Cells are stained with fluorescently labelled antibodies for molecules of interest
    - CD3 T cell marker
    - CD8 T cell subset marker
    - CD69 T cell activation marker
    - IFNg Cytokine of interest
  - Cells are run through the flow cytometer and as each cell passes through the laser the FSC,SSC, and fluorescence of each of the 4 coloured antibodies is measured
  - Each cell is then plotted as a dot on the resulting charts showing the expression profiles for the cell population.

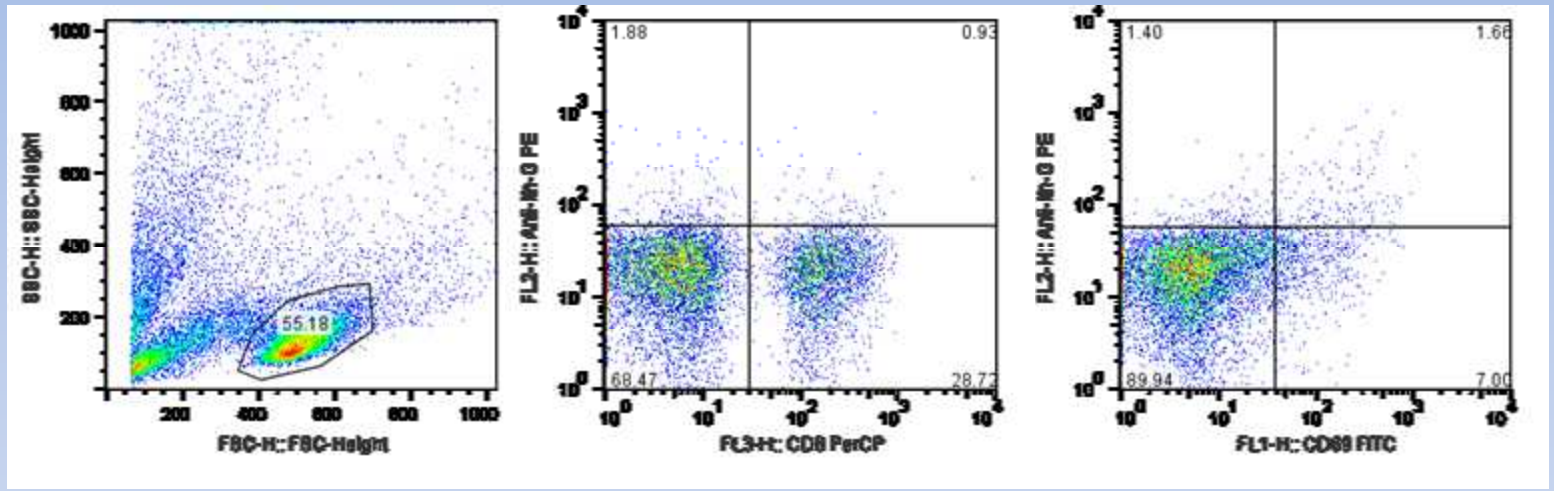
Data display

- Gating strategy
  - Live lymphocytes based on FSC/SSC (1)
  - CD3+ T cells (2)
  - Charts are then plotted to show the phenotype of gated cells
    - CD69 vs IFNg to show the total population of antigen specific cells (activated and secreting IFNg in response to antigen exposure) (3)

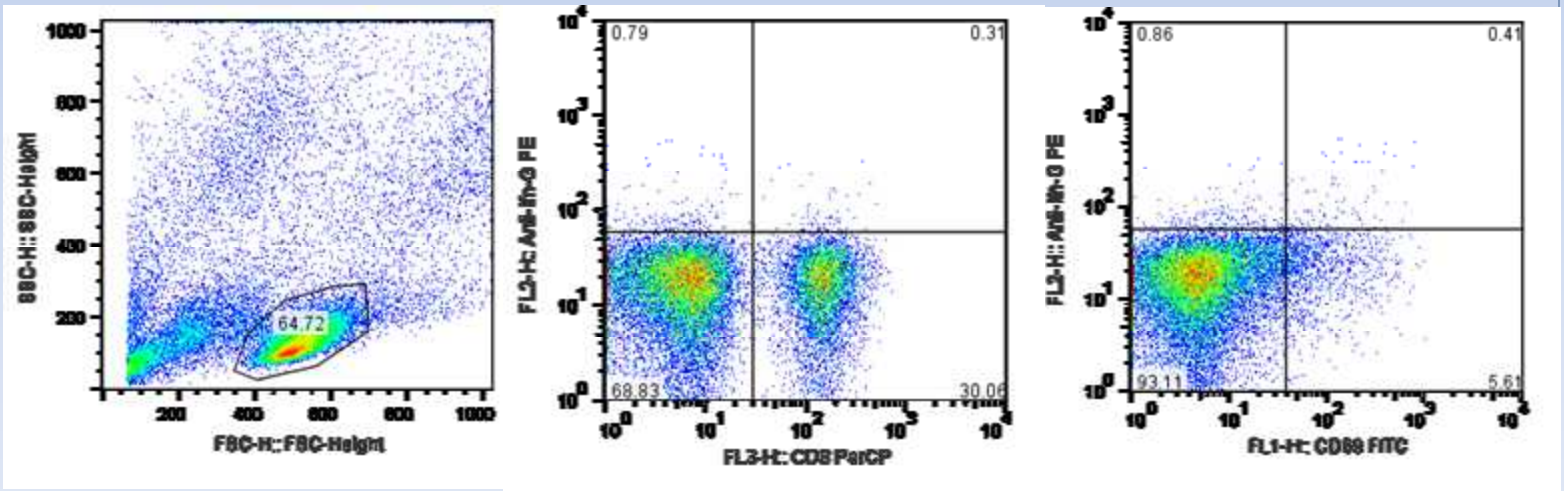


Incubation with antigen (example 1)

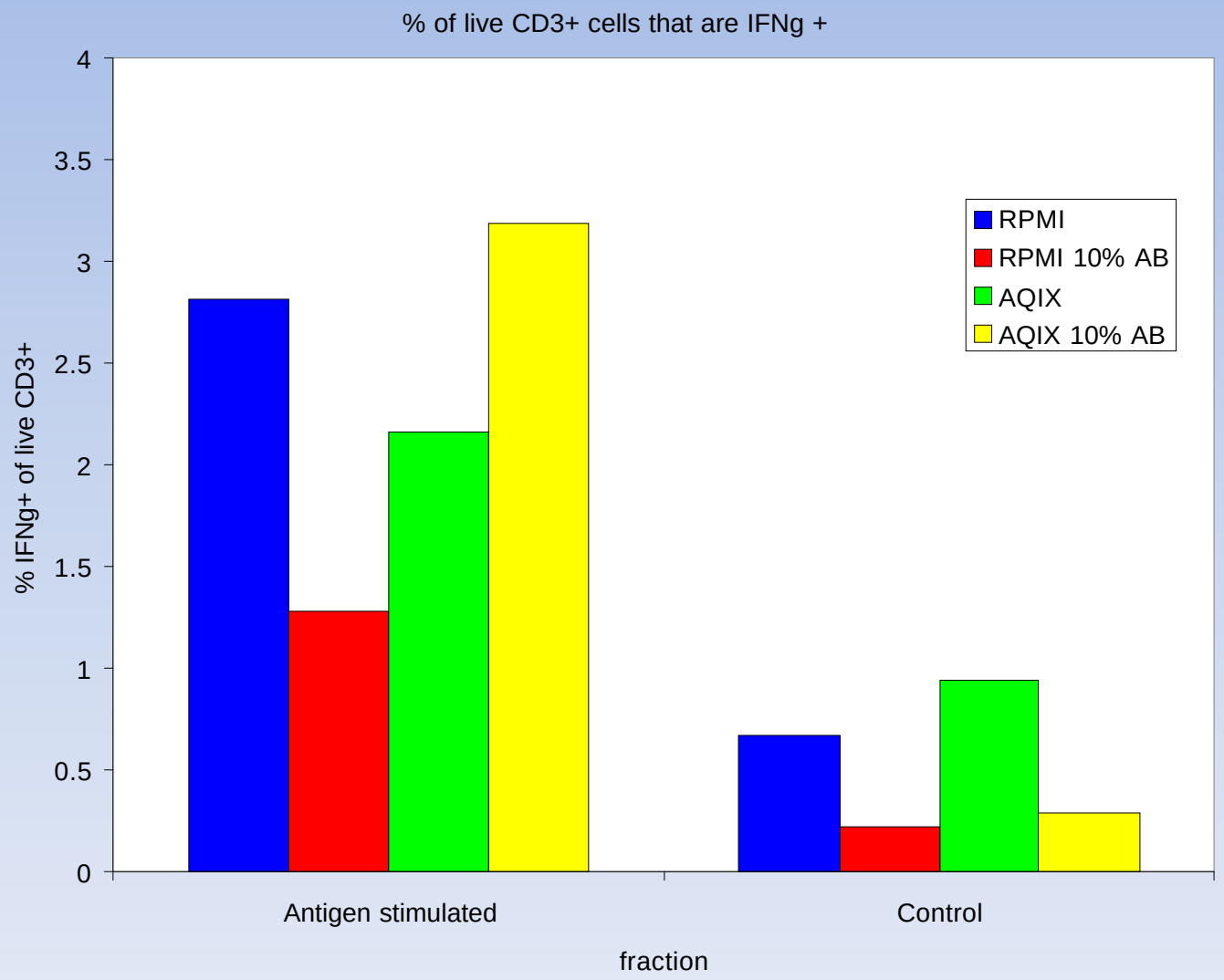
AQIX



RPMI
10% AB

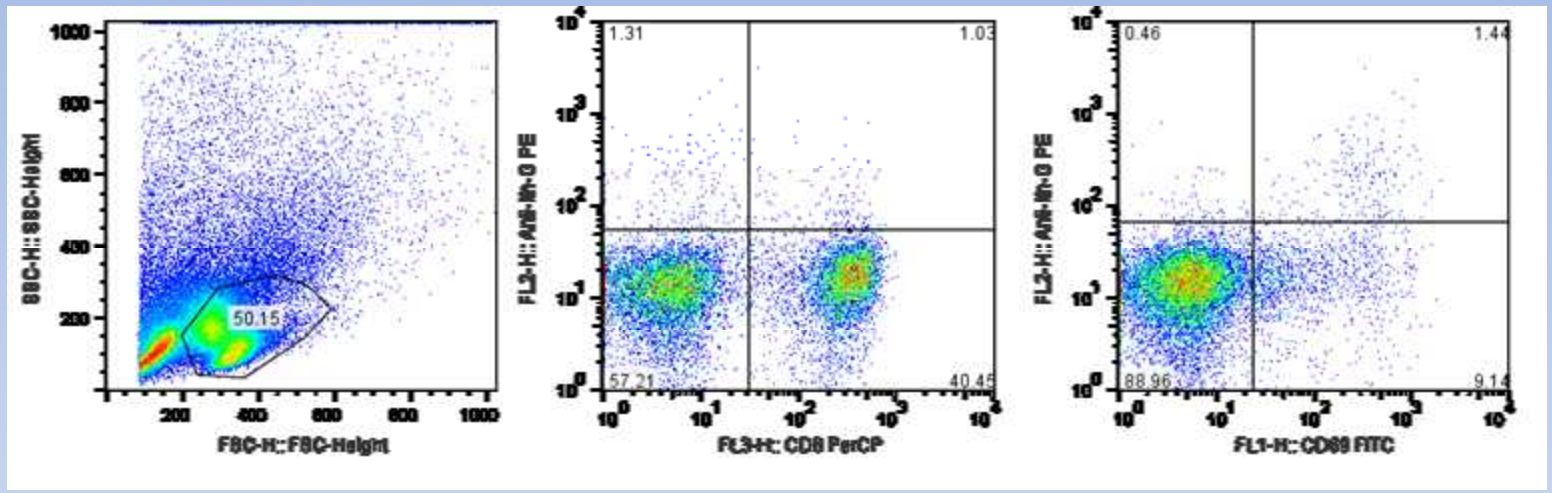


Ability to stimulate cells (example 1)

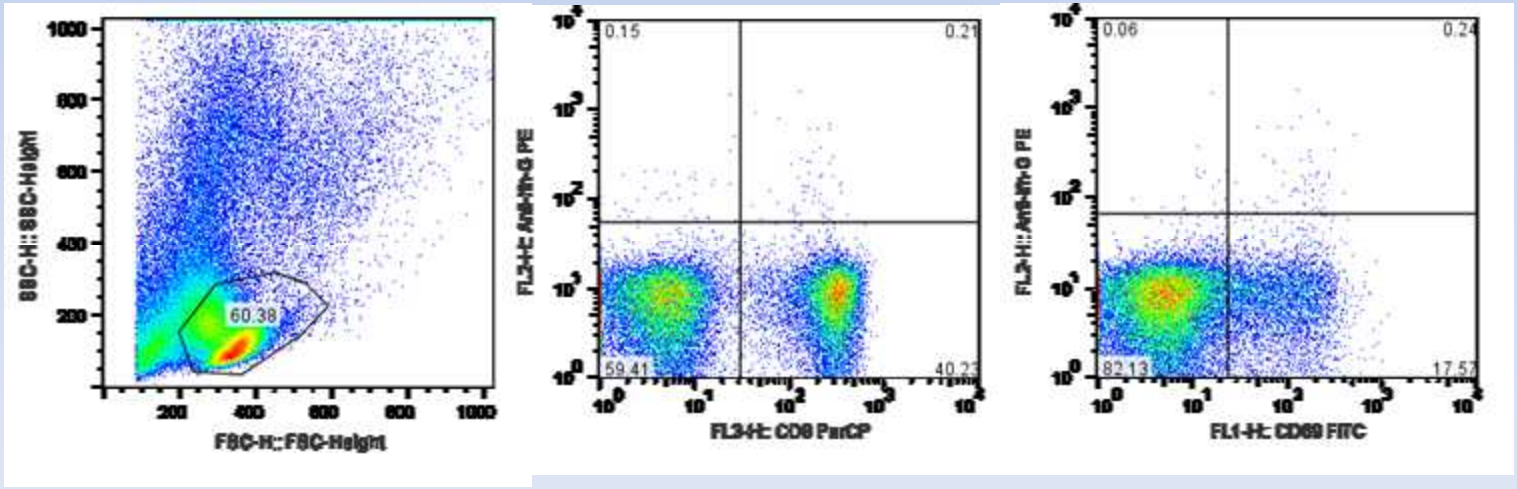


Incubation with antigen (example 2)

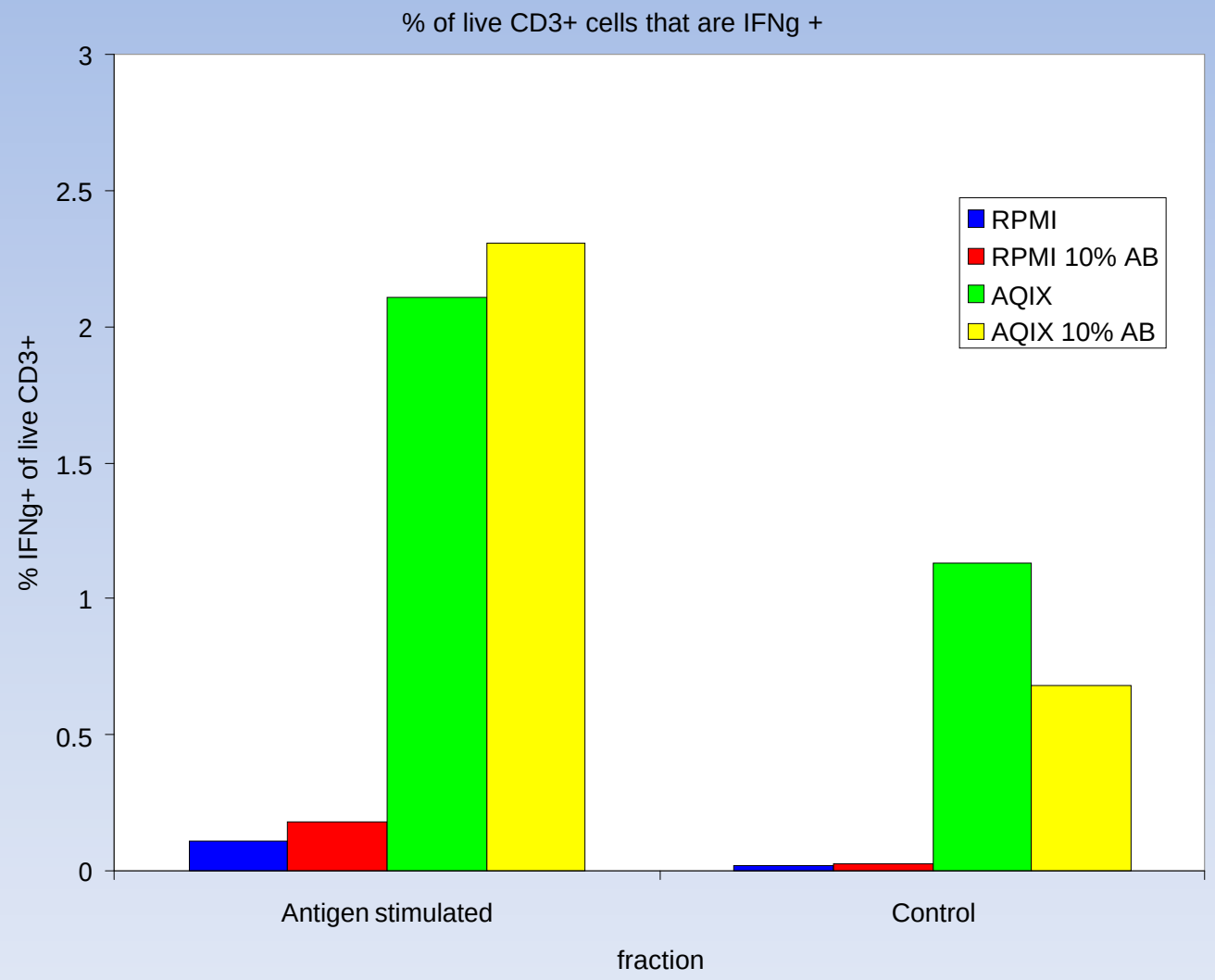
AQIX



RPMI
10% AB



Ability to stimulate cells (example 2)

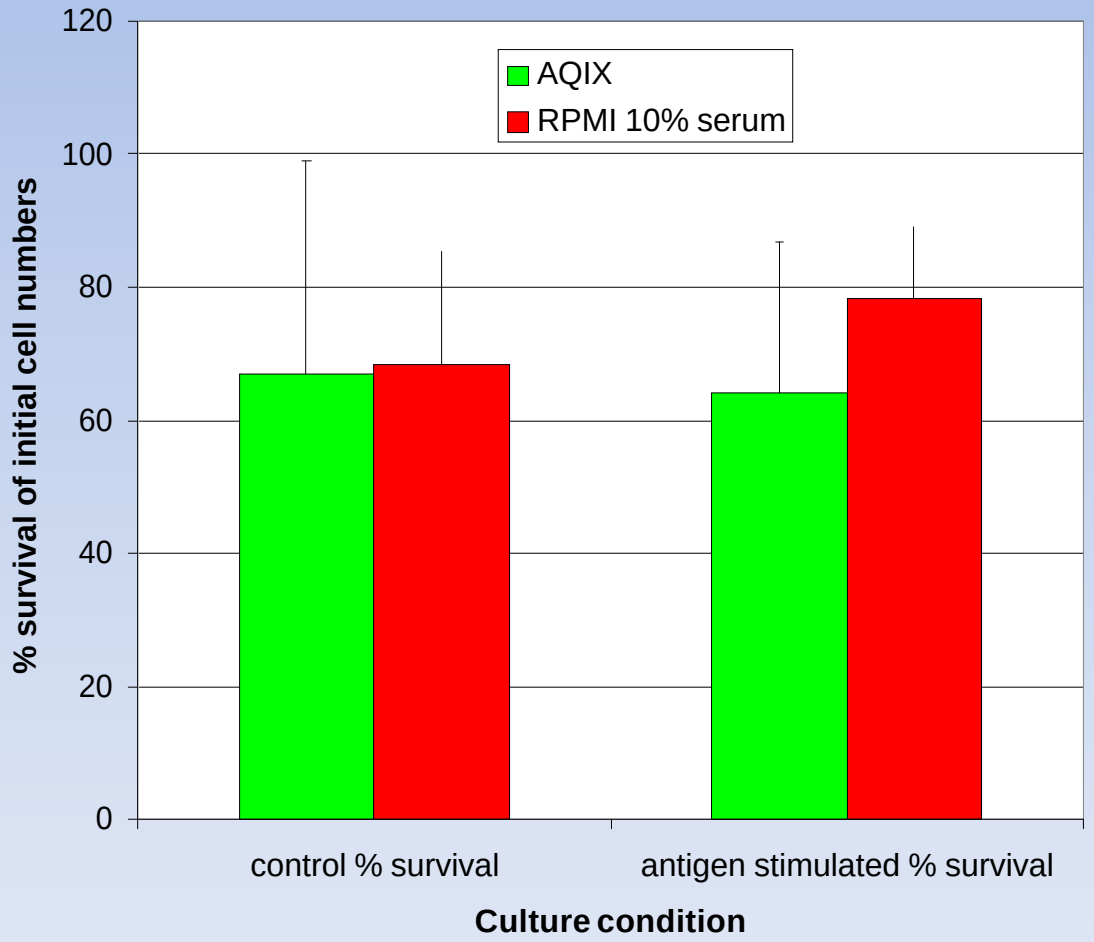


Preliminary conclusions

- AQIX performed as well as if not better than RPMI with 10% Human AB serum in both the patient samples and the control individuals.
  - Based on comparison of the percentage of IFNg+ cells in samples stimulated with antigen vs control
  - However of note is that background production of IFNg is higher in AQIX cultured cells.

- Other parameters measured.
  - Number of cells post incubation in each condition

Cell survival



There is no significant difference between the number of cells surviving overnight culture in each of the media. (n=6)

Conclusions

- There is no significant difference in % cell survival/recovery post incubation however a trend towards higher survival in RPMI in the stimulated conditions may warrant further investigation.
- There is no significant difference in the viability of the cells following overnight incubation with or without antigen when comparing AQIX with RPMI 10% AB as assessed by trypan blue staining (data not shown).