

BCG-induced immune correlates of protection against tuberculosis

EDCTP meeting, Burkina Faso

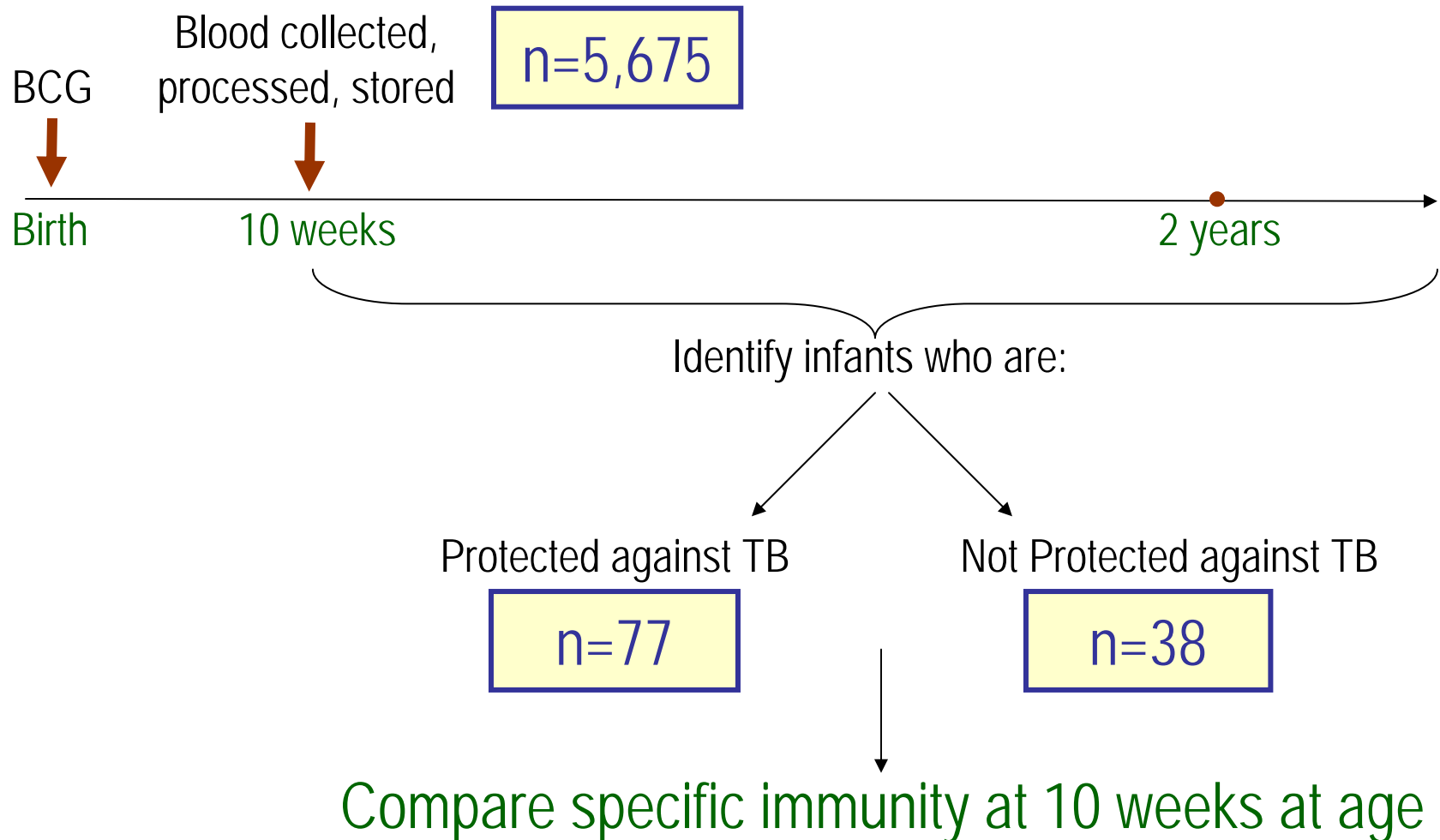


South African TB Vaccine Initiative



Hassan Mahomed, Tony Hawkrige, Greg Hussey

To determine BCG-induced immune correlates of protection against TB



Immune correlates study approach

1. Soluble cytokines

2. T cell-associated cytokines

3. T cell proliferation, etc.

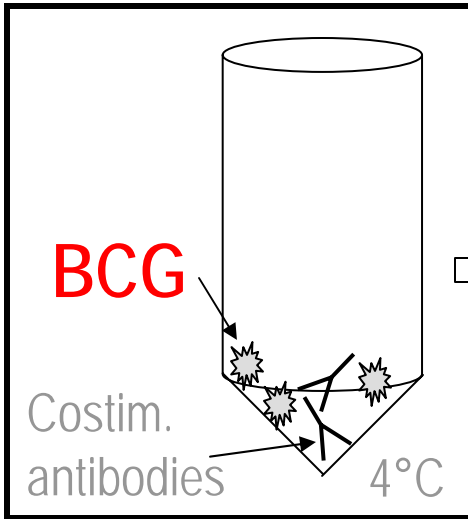
4. T cell antigenic repertoire

5. Antibody responses

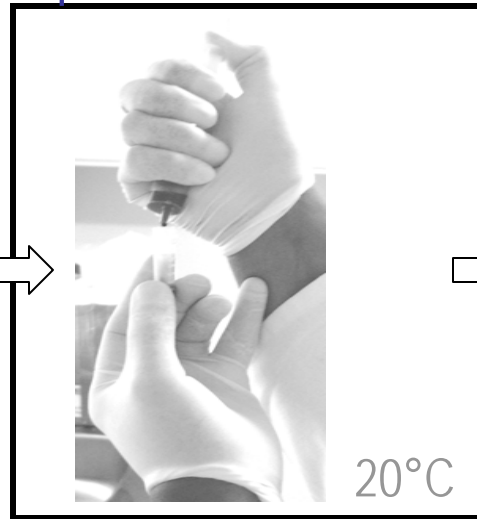
6. mRNA expression

7. Innate immune cells

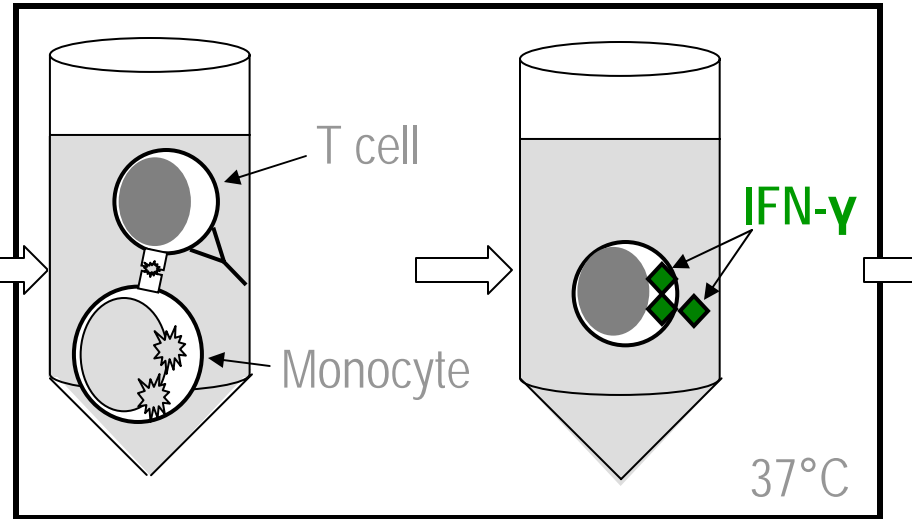
Preparation,
Transport to
Clinic



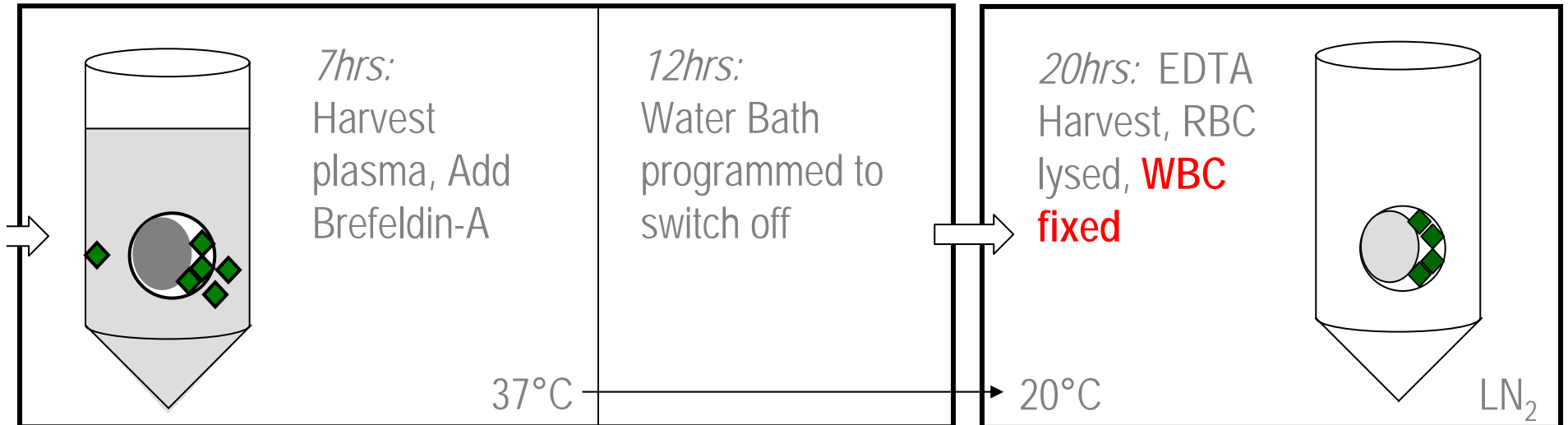
Clinic: Blood
Added to
Opened Tube



Transport to Lab in
Portable Incubator

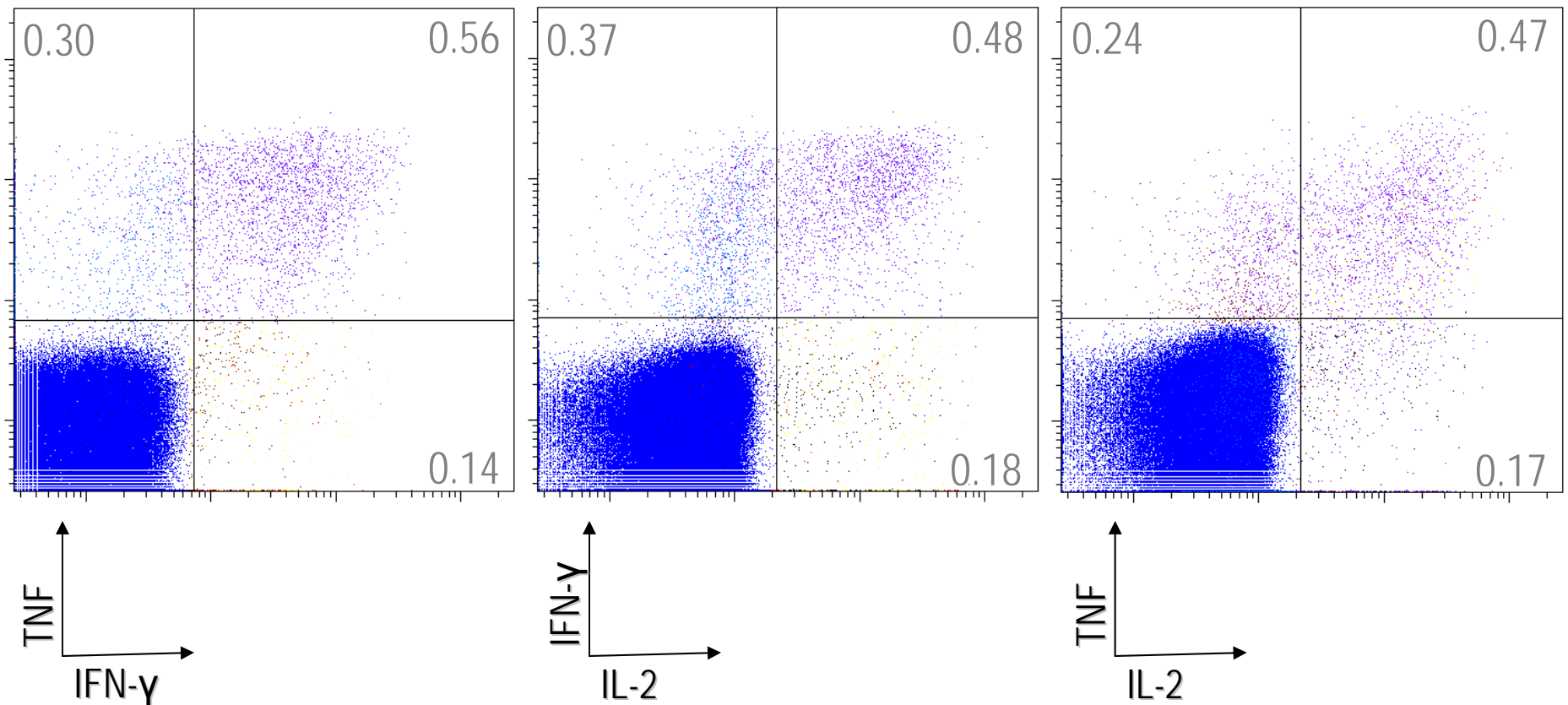


Lab: Transfer to Water Bath



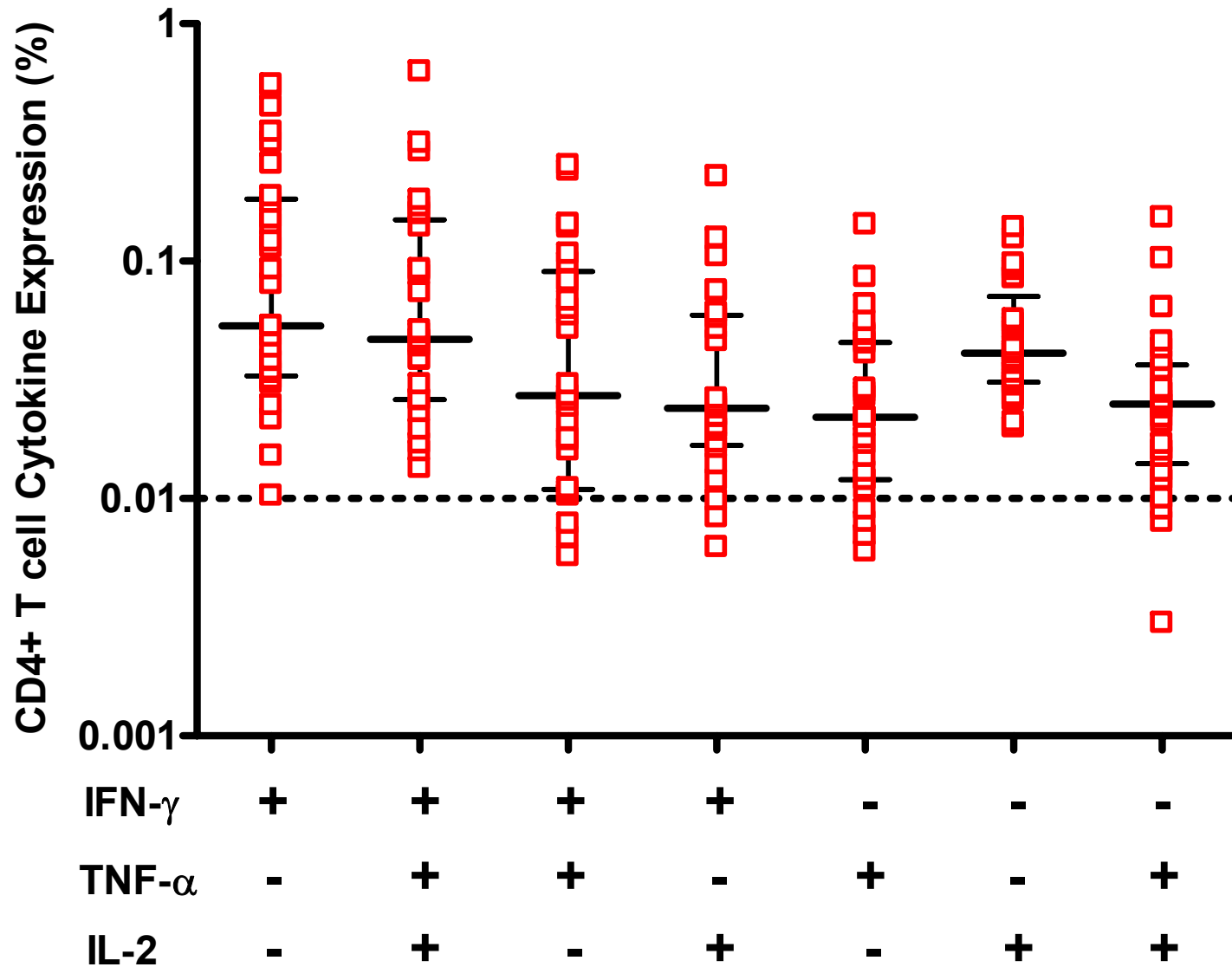
BCG-induced T cell cytokine expression

Unstimulated levels < 0.05%



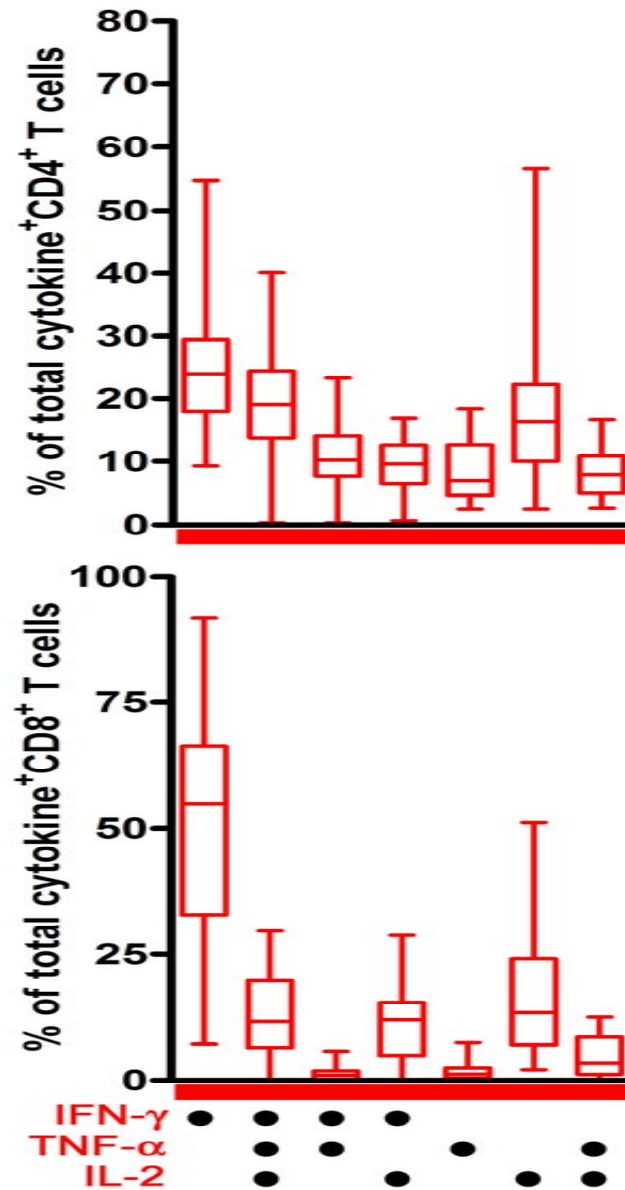
*Gated on CD4+ T cells. 12 hours incubation of whole blood with BCG, from a 10-week old infant, vaccinated with BCG at birth.

BCG-induced T cell cytokine expression



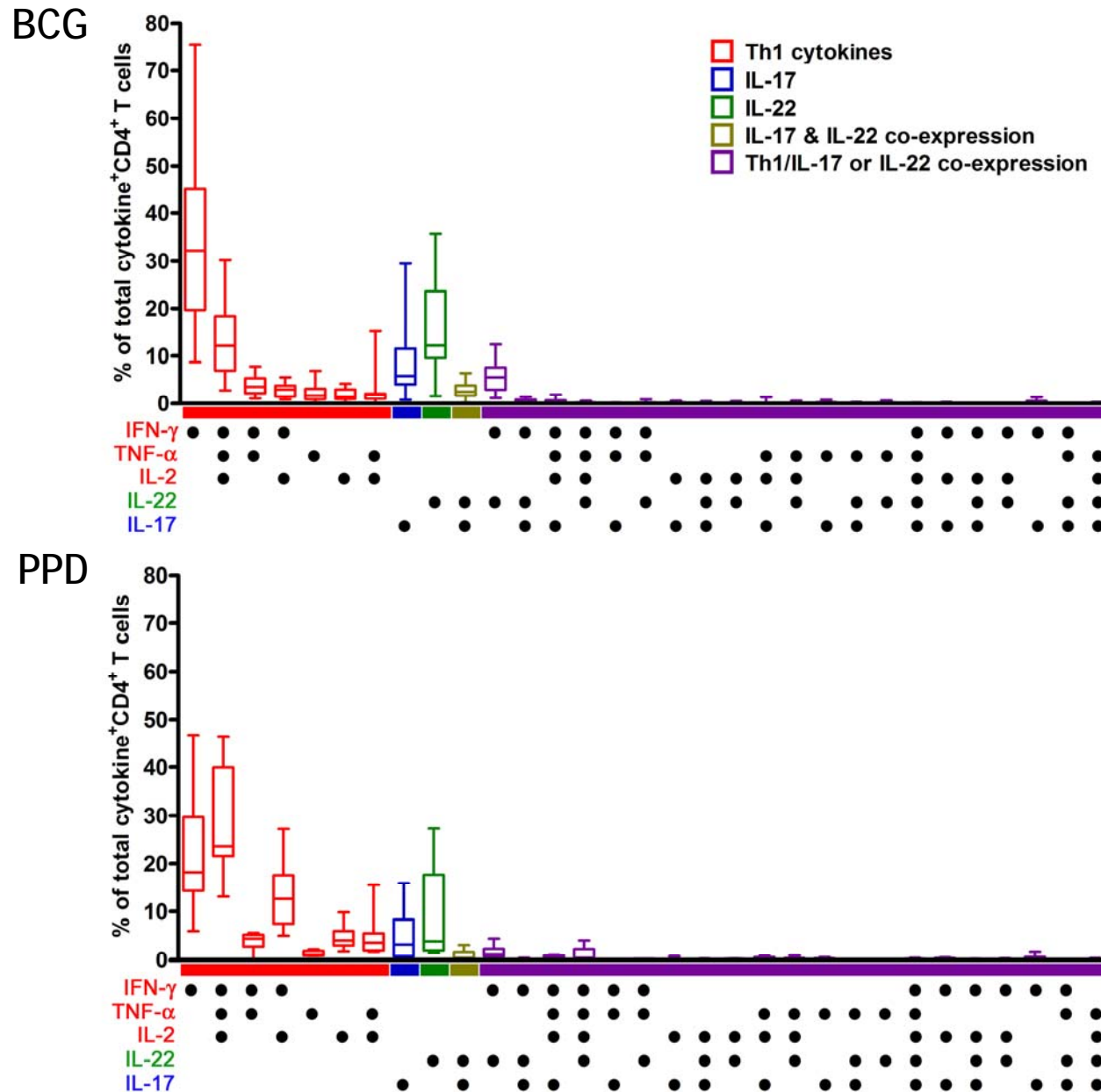
*12 hours incubation of whole blood with BCG, in 29 10-week old infants, vaccinated with BCG at birth. Medians and interquartile ranges shown.

BCG-induced T cell cytokine expression



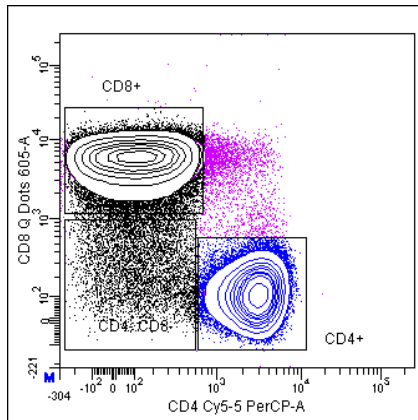
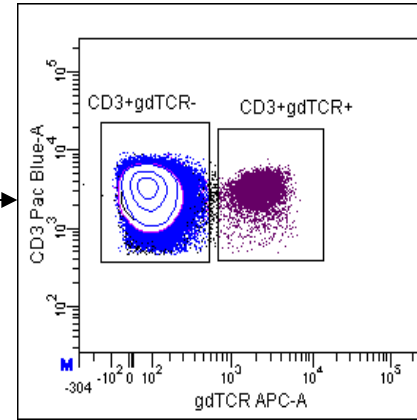
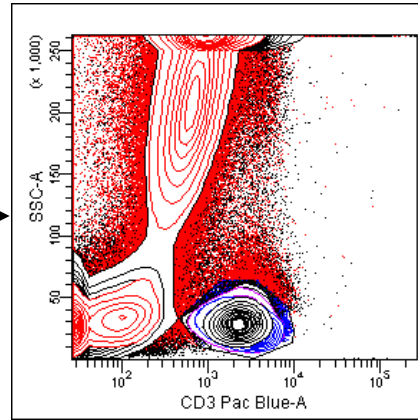
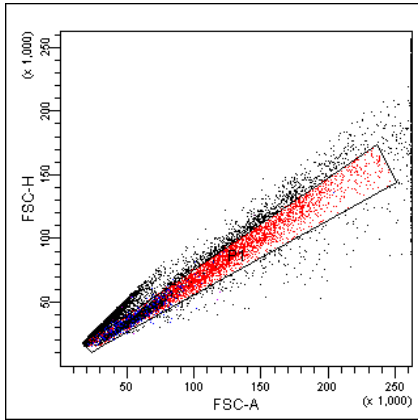
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Specific T cell cytokines in adults

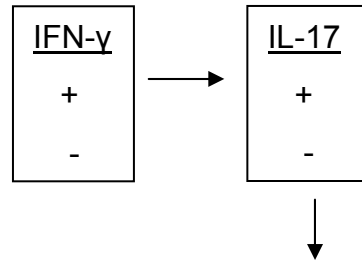


*12 hours incubation of whole blood from mycobacteria-exposed adults with antigen.

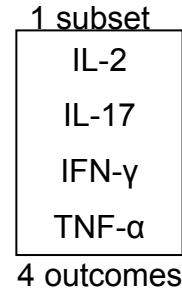
Immune correlates study analysis



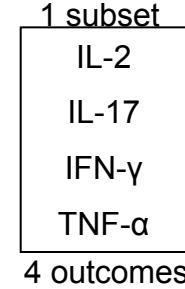
CD4+ or CD8+ $\gamma\delta$ - T cells



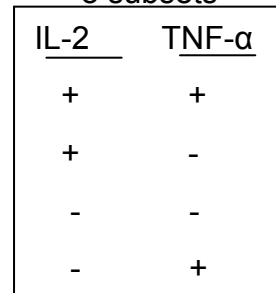
$\gamma\delta$ + T cells



CD4-CD8- $\gamma\delta$ - T cells



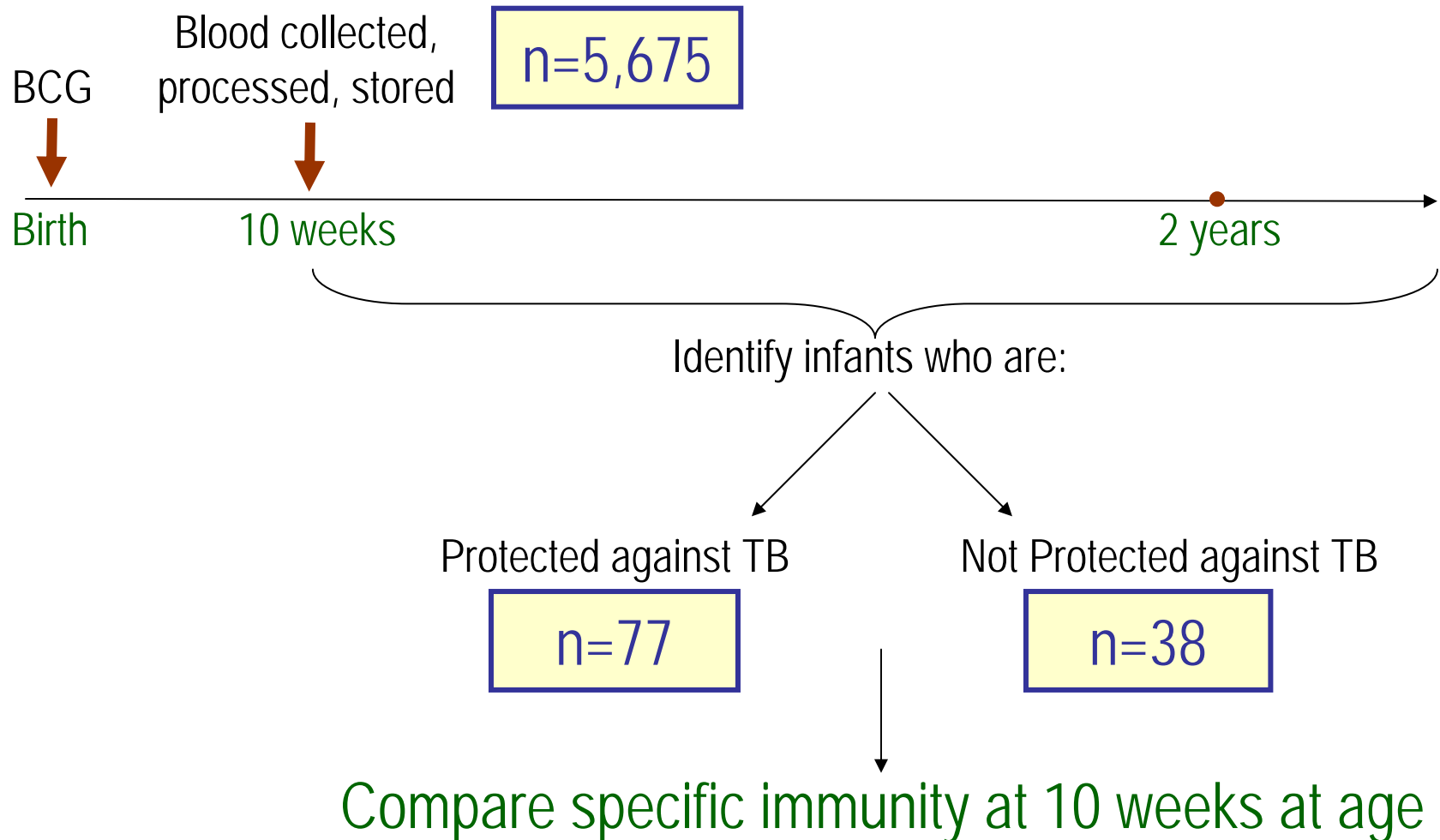
8 subsets



64 outcomes for each

136 outcomes

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Immune correlates study approach

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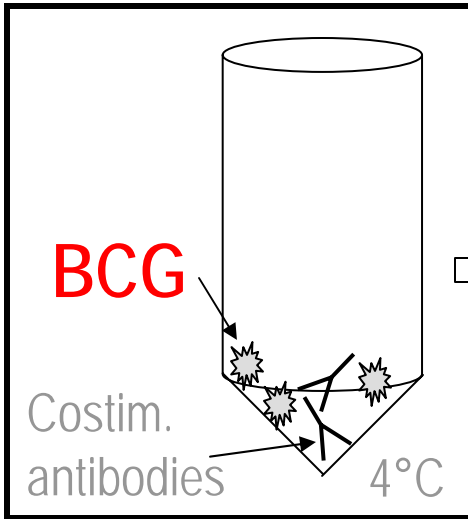
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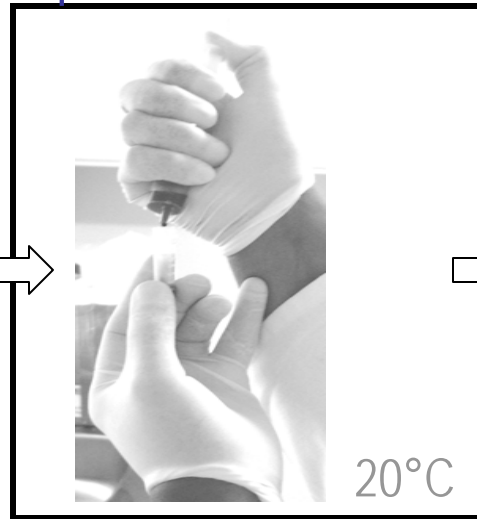
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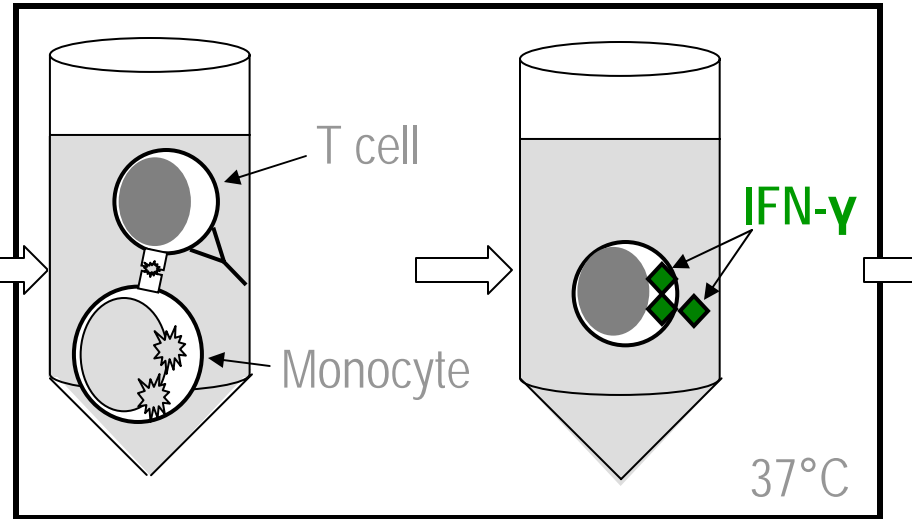
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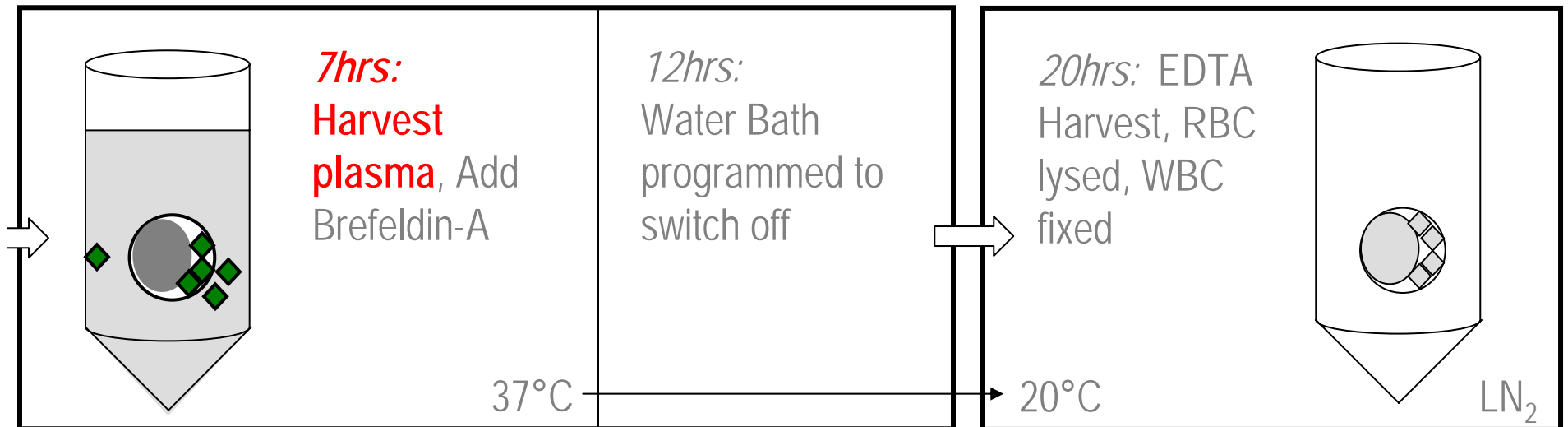
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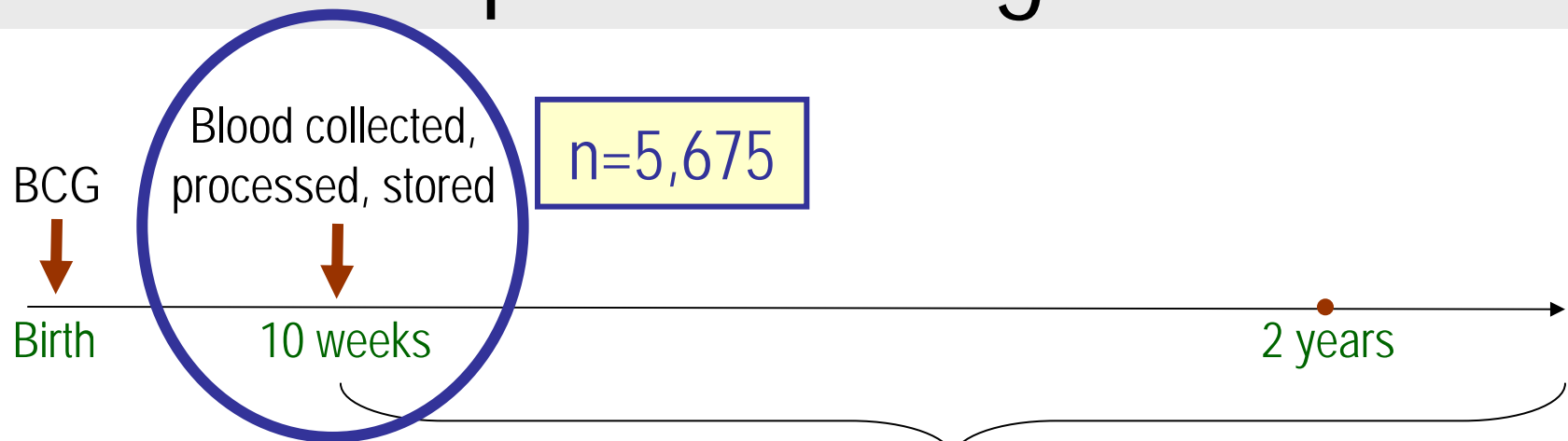
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Portable Incubator



Lab: Transfer to Water Bath



To determine BCG-induced immune correlates of protection against TB



Identify infants who are:

Protected against TB

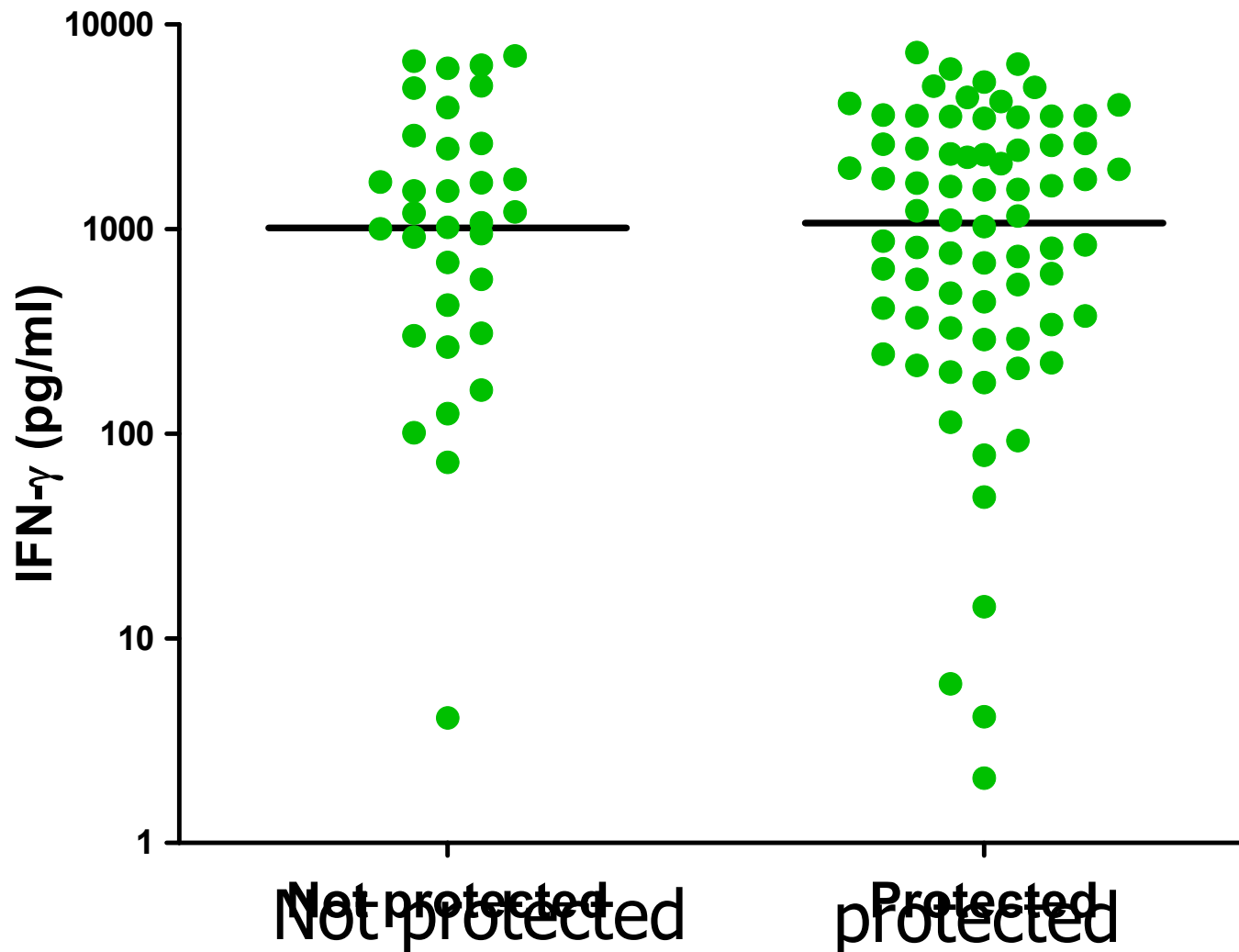
n=77

Not Protected against TB

n=38

Compare specific immunity at 10 weeks at age

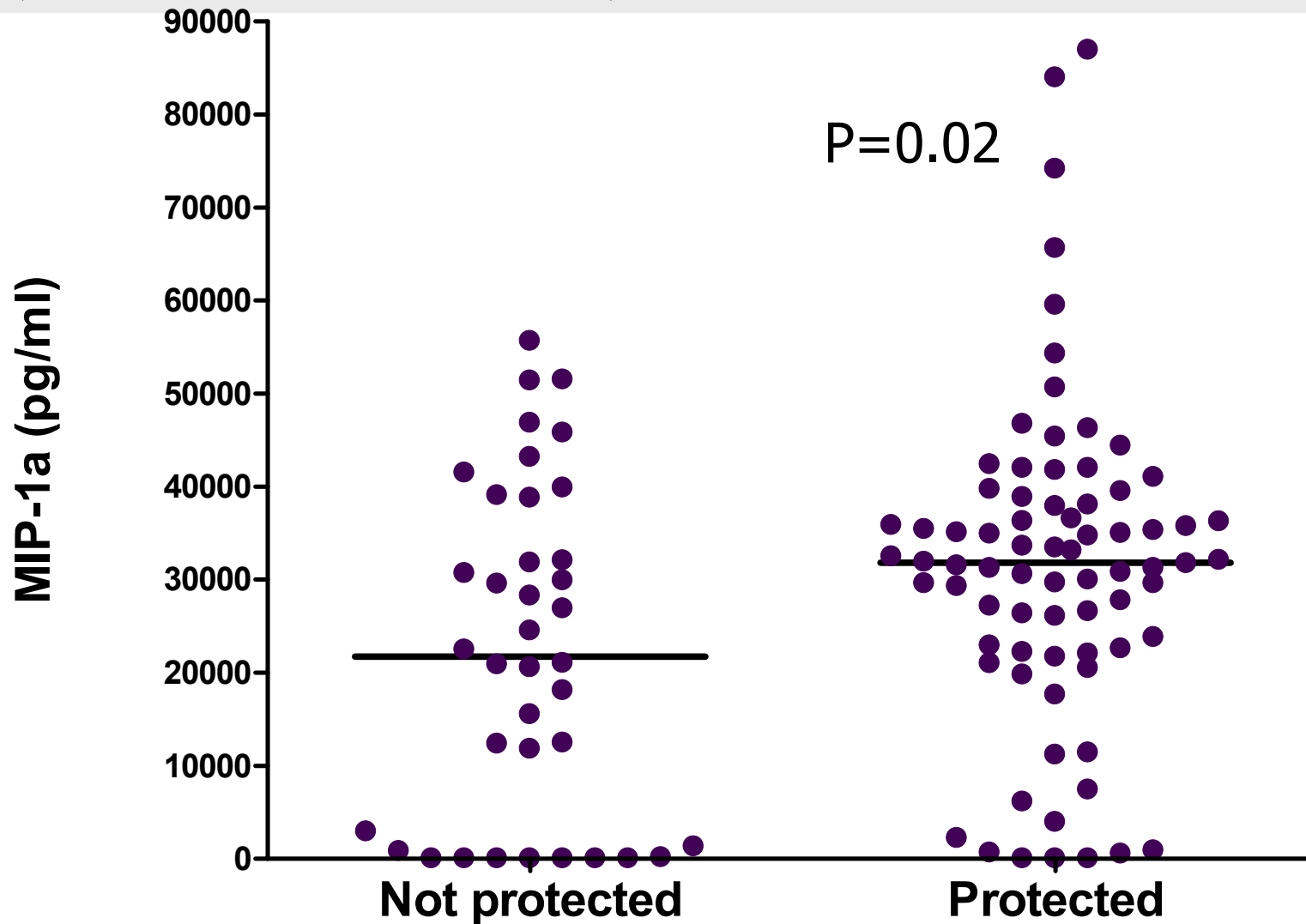
IFN- γ is not a BCG-induced immune correlate of protection against TB



IL-1 β , IL-2, IL1RA, IL-4, IL-5, EGF, IL-6, IL-7, TGF- α , Fraktalkine, IL-8, IL-10, IL-12p70, IL-13, IL-15, IL-17, IL-1 α , G-CSF, GM-CSF, TNF- α , Eotaxin, IL-12p40, MIP-1 β , IP-10, VEGF, sCD40

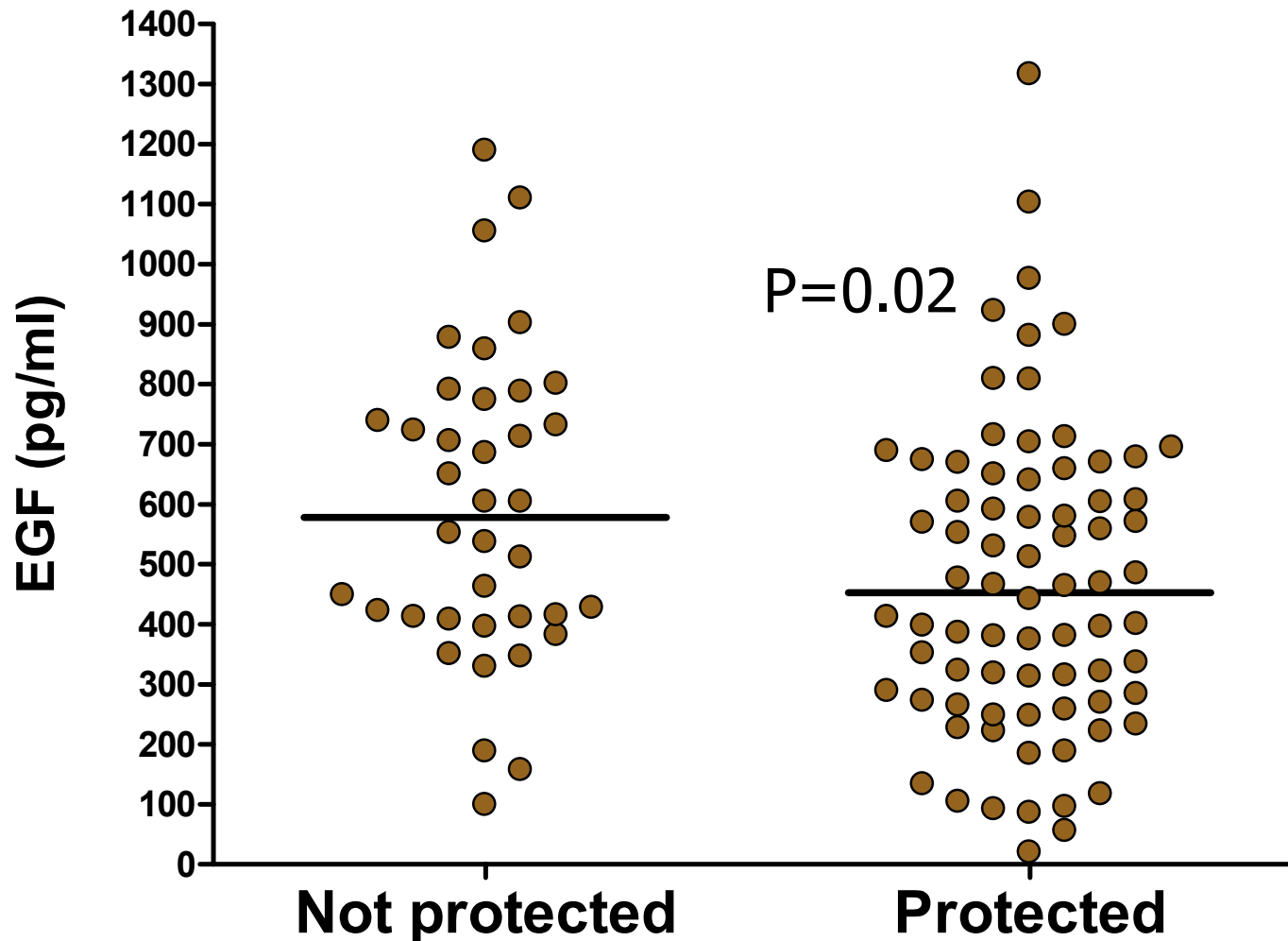
*Measured in plasma collected 7 hours after incubation of whole blood with BCG; Linco beads, Luminex.

MIP-1 α levels were higher when protected (BCG stimulated)



*Measured in plasma collected 7 hours after incubation of whole blood with BCG; Linco beads, Luminex.

Plasma from UNstimulated blood: EGF levels were lower when protected



*Measured in plasma collected 7 hours after incubation of whole blood with nothing; Linco beads, Luminex.

UNstimulated plasma: Differences detected by univariate analysis

	<i>When protected</i>	<i>p value</i>
IL-13	Higher	0.03
EGF	Lower	0.02
Fraktalkine	Lower	0.04

*Measured in plasma collected 7 hours after incubation of whole blood with nothing; Linco beads, Luminex.

Multivariate logistic regression analysis of data from UNstimulated blood

MIP1- β

TNF- α

IL-4

IL-17

EGF

IL-13

Eotaxin

TGF- α

Fraktalkine

GM-CSF

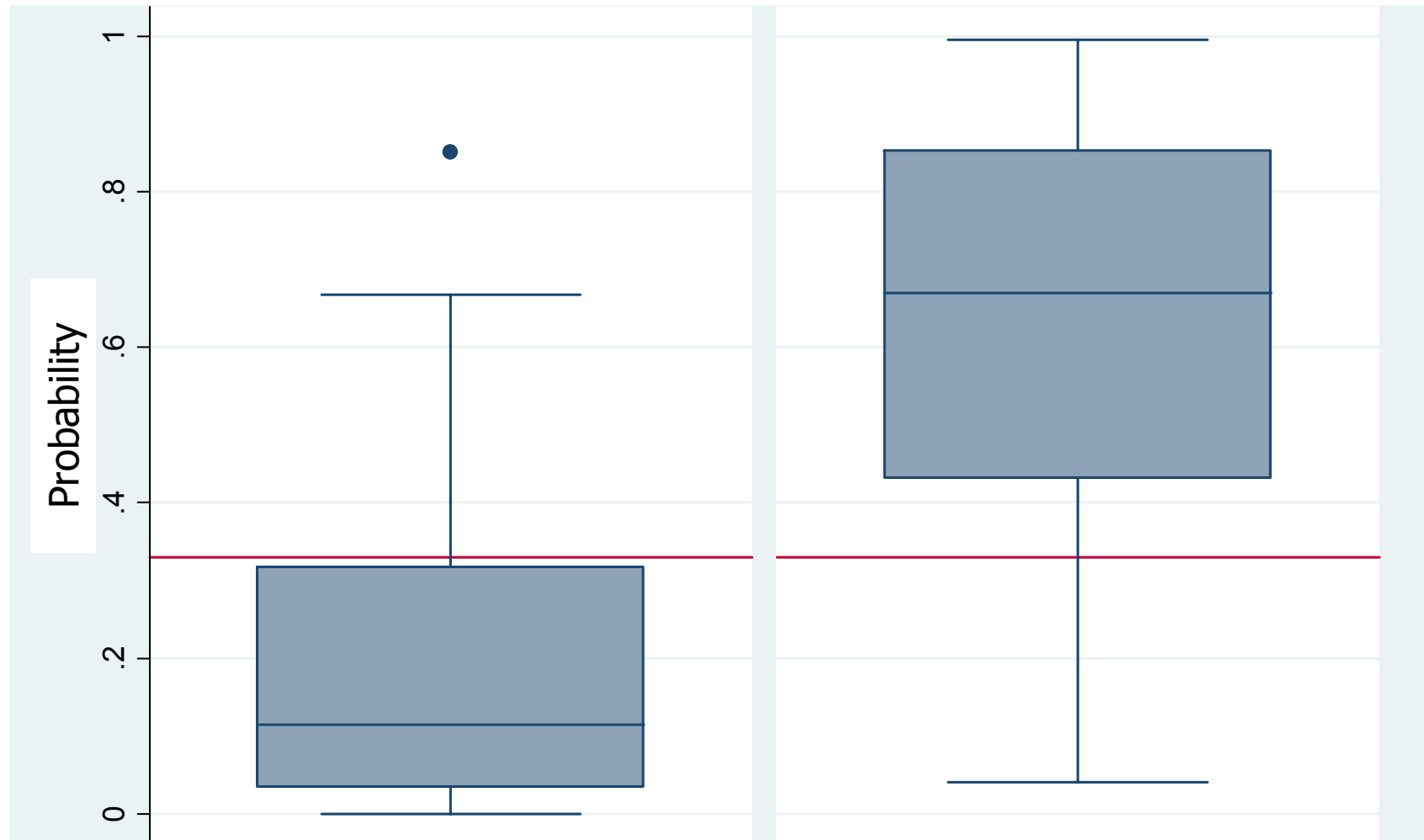
$$\eta = -1.31 + 1.70 * \text{MIP1beta} - 1.97 * \text{TNFa} - 0.76 * \text{IL4} + 0.89 * \text{IL17} + 1.02 * \text{EGF} - 1.53 * \text{IL13} - 1.24 * \text{Eotaxin} + 1.05 * \text{TGFa} + 2.19 * \text{Fractalkine} - 1.16 * \text{GMCSF}$$

$$\hat{p} = \exp\left(\frac{\eta}{1 + \eta}\right)$$

Model: data from UNstimulated blood

Not protected

Protected



Model: UNstimulated blood

MIP1- β

TNF- α

IL-4

IL-17

EGF

IL-13

Eotaxin

TGF- α

Fraktalkine

GM-CSF

Sensitivity 84.21%

Specificity 78.95%

Positive predictive value 66.67%

Negative predictive value 90.91%

False + rate for classified+ 33.33%

False - rate for classified- 9.09%

Correctly classified 80.70%

Model: BCG-stimulated blood

MIP1- α
Fraktalkine
IL-12p70

Sensitivity	65.79%
Specificity	71.05%
Positive predictive value	53.19%
Negative predictive value	80.60%

False + rate for classified +46.81%
False - rate for classified - 19.40%

Correctly classified	69.30%
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Conclusions

1. IFN- γ alone measured at 7hrs in a WBA is not a BCG-induced immune correlate of protection against TB
2. Combined analysis of multiple measures may allow differentiation
3. Analysis of unstimulated blood may yield answers
4. Critical determinants of protection \neq Immune correlates of protection?



Other acknowledgements

Greg Hussey, Willem Hanekom, Tony Hawkrige, Hassan Mahomed,
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Sarah Joseph

Holden Maecker

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