

# Validation of housekeeping genes for normalising RNA expression

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## **Research projects at the Center for infectious diseases**

- **Clinical trials (Drugs, Vaccine, Diagnostic tests)**
- **Basic science research**
- **Epidemiological studies**

## VACSYS study of Tuberculosis immunology

- **Take a large cohorts of patients with tuberculosis and their contacts**
- **Measure the mRNA expression of genes defining cytokines and apoptotic proteins using real-time RT-PCR**
- **Determine if there are any characteristic expression patterns that are associated with susceptibility to tuberculosis**

**•This project has a large capacity development aspect**

**•We have placed Rotor-Gene 3000 in 4 African sites**



The collaborative role provided by the Center for Infectious Disease includes:

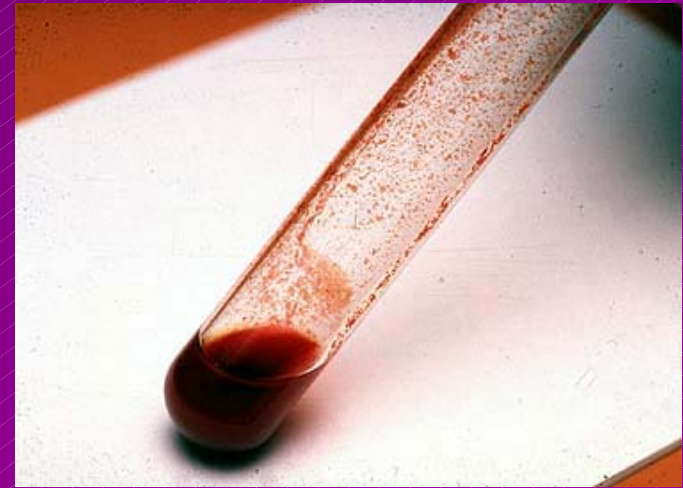
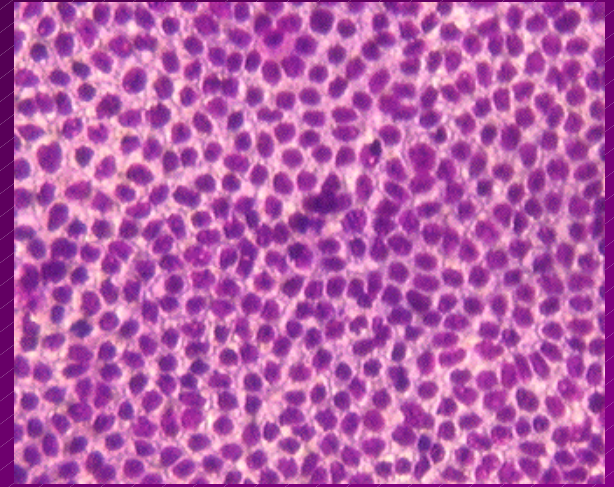
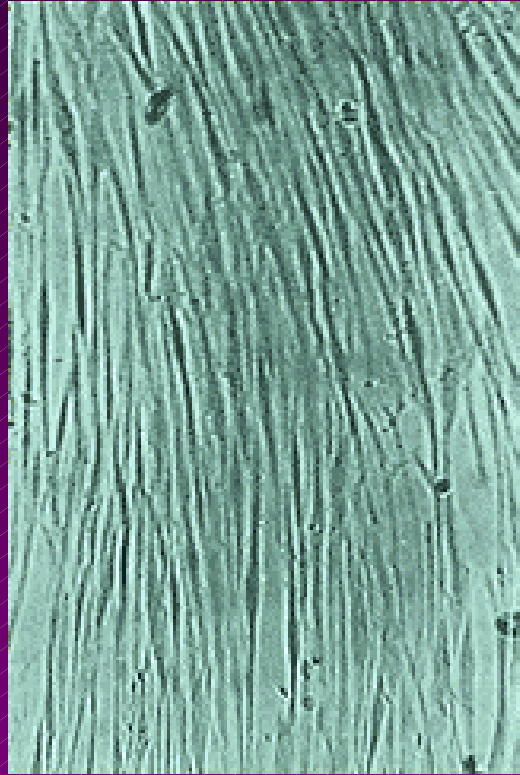
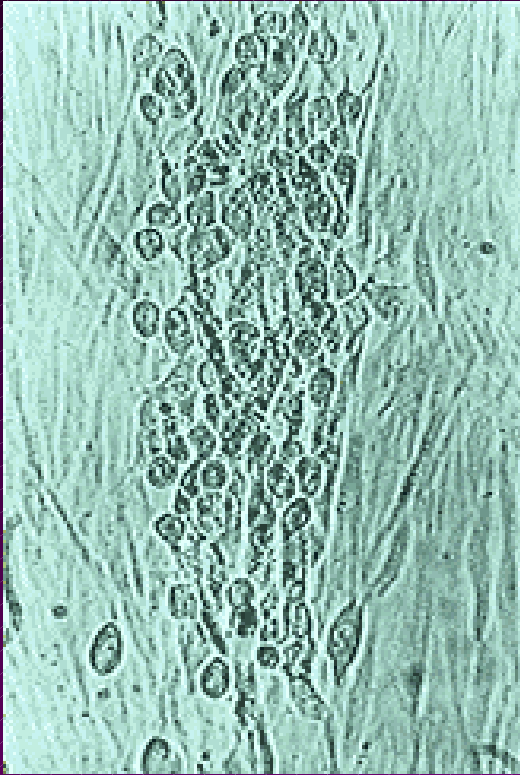
- Assay design and development
- Training
- Trouble shooting

## Strategies for normalisation

- Normalising to Cell number/Sample size
- Normalising to [RNA]
- Normalising to an internal reference gene (House keeping gene)

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- **Normalising to [RNA]**
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## Normalising to [RNA]

- **UV ( $OD_{260}$ )**
- **Gel analysis (Agarose, PAGE, Bioanalyser)**
- **Ribo-green**

## Strategies for normalisation

- Normalising to Cell number/Sample size
- Normalising to [RNA]
- **Normalising to an internal reference gene  
(House keeping gene)**

## House Keeping genes

Genes that are assumed to be expressed at a constant level between experimental groups

# Identification of a candidate gene

- Our experimental system compares the gene expression from tuberculosis patients with healthy individuals.
- We are interested in the expression from whole blood and purified peripheral blood mononuclear cells (PBMCs).

- We used the Taqman Human Endogenous Control Plate to investigate 10 potential house keeping genes
- We also chose 3 others from a previous study into T-cell house keeping genes.

RNA was extracted from:

- blood (PAX gene) at the bedside
- cells cultured for 5 days....(RNeasy)

Bioanalyser used to quantified and assessed RNA

400 and 600 ng RNA reversed transcribed (Omniscript) for healthy individuals and tuberculosis patients respectively and 3 ng for cell culture (Sensiscript)

Blood gene expression was measured using 5 ng of reverse transcribed RNA per reaction and cell culture 0.1 ng per reaction

- Candidate gene expression was assessed using the ABI 7000
- The CTs of the samples were compared and fold change assessed assuming 1  $\Delta^{CT}$  is equivalent to a 2 fold change.

## GROUP HOUSE KEEPING GENES

Taqman Human Endogenous Control Plate

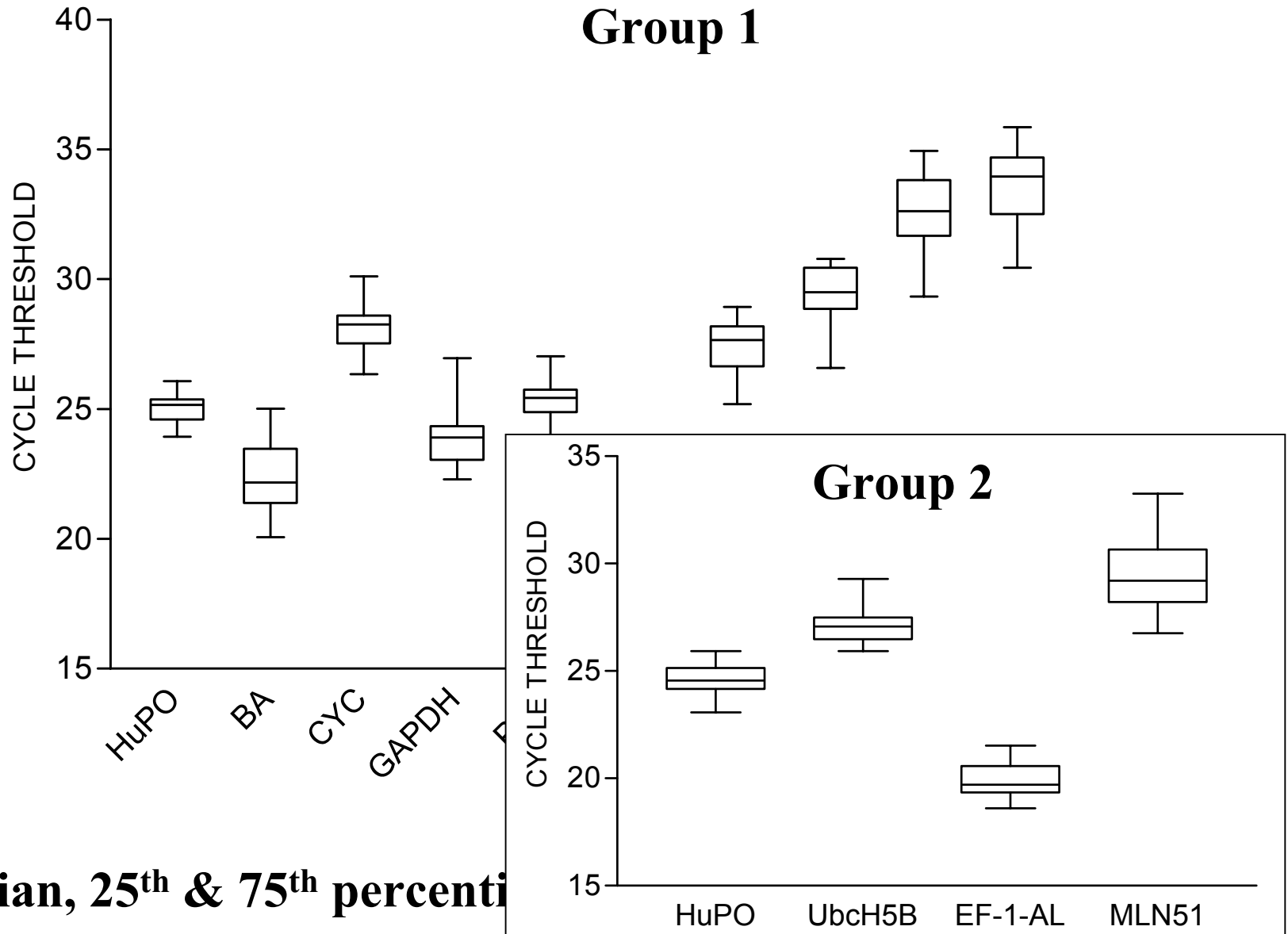
- 1) Human acidic ribosomal protein (HuPO)
- 2)  $\beta$ -Actin (BA)
- 3) Cyclophylin (CYC)
- 4) Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)
- 5) Phosphoglycerokinase (PGK)
- 6)  $\beta$ -Microglobulin (B M)
- 7)  $\beta$ -Glucuronidase (GUS)
- 8) Hypoxanthine ribosyl transferase (HPRT)
- 9) Transcription factor IID TATA binding protein (TBP)
- 0) Transferrin receptor (TfR).

## GROUP HOUSE KEEPING GENES

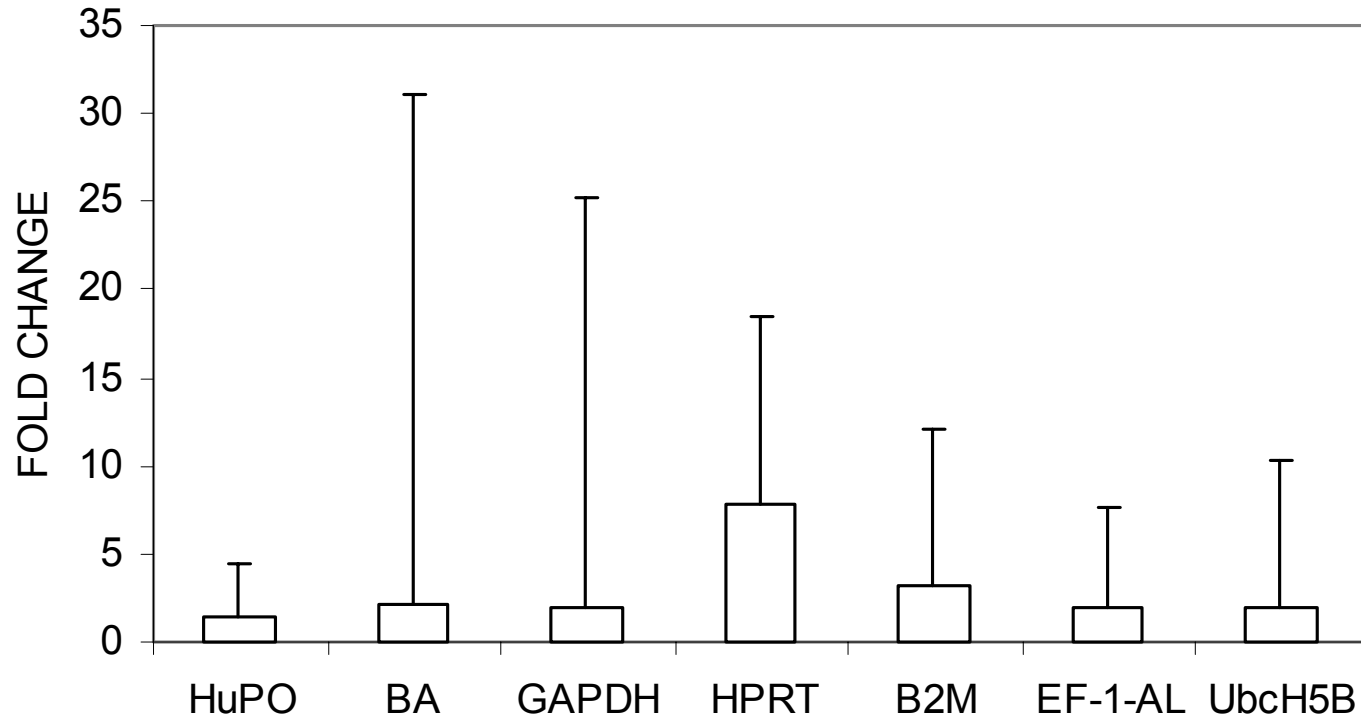
(Hamalainen, HK. 2002 *Anal Biochem.*, 99, 63-70)

- 1) Human acidic ribosomal protein (HuPO)
- 2) Elongation Factor-  $\alpha$  (EF-  $\alpha$ )
- 3) MLN 5
- 4) Ubiquitin conjugating enzyme (UbcH5B)

# CT Variation of house keeping genes from human blood



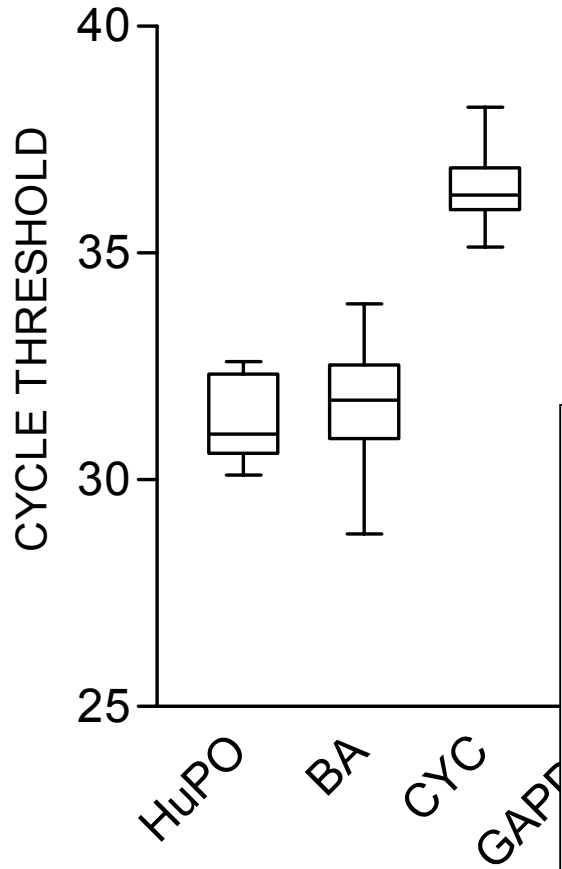
# Fold change variation of house keeping genes from human blood



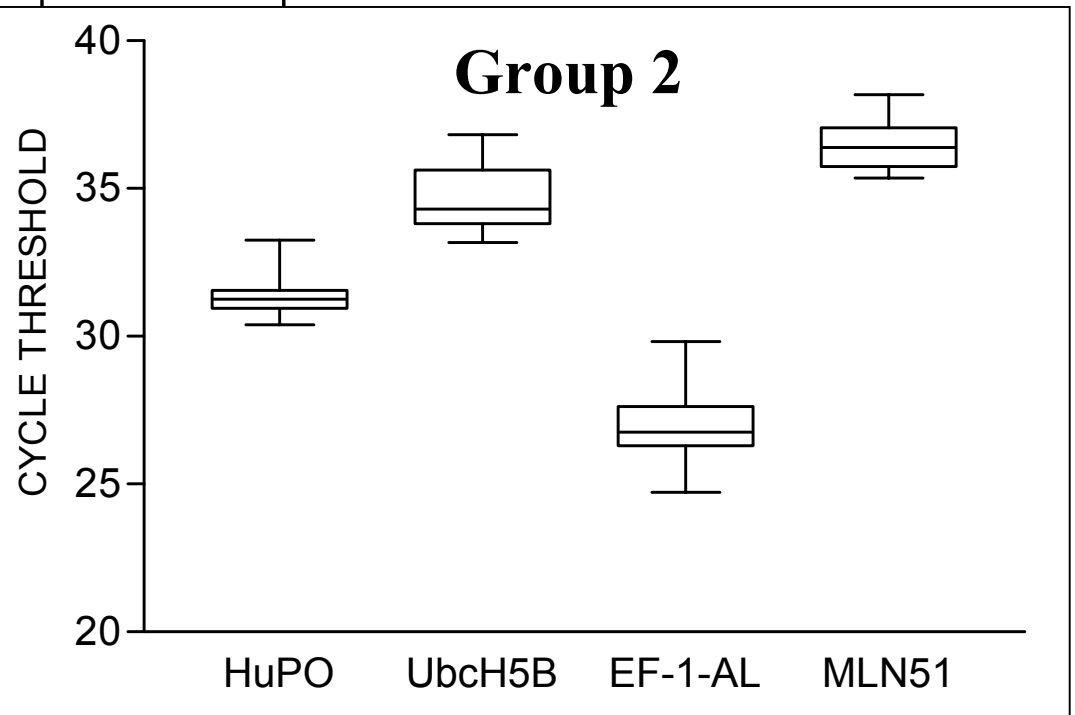
**Standard deviation and range from the mean (n = 16)**

# CT Variation of house keeping genes from PBMC cell culture

## Group 1

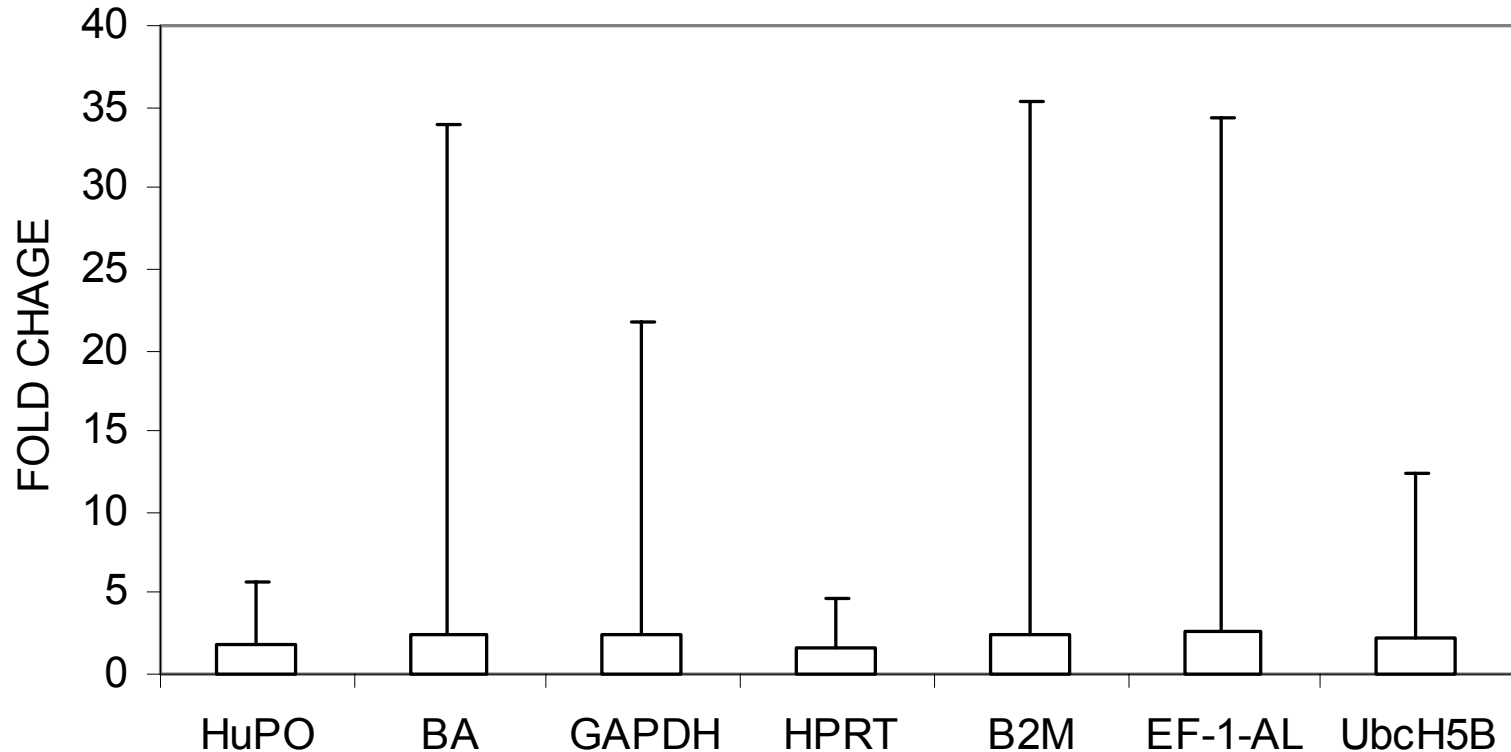


## Group 2



Median, 25<sup>th</sup> & 75<sup>th</sup> percentiles

# Fold change variation of house keeping genes PBMC cell culture



**Standard deviation and range from the mean (n = 16)**

# Conclusion

- We have identified HuPO as a reference gene with a standard deviation of  $<2$  fold and range of  $\sim 5$  fold
- The “classic” reference genes standard deviation was  $<5$  fold however range varied by as much as  $\sim 35$  fold
- We feel that using reference genes is an ideal method of normalisation provided they are first validated

Thankyou to....

Alimuddin Zumla

Keertan Dheda

Stephen Bustin

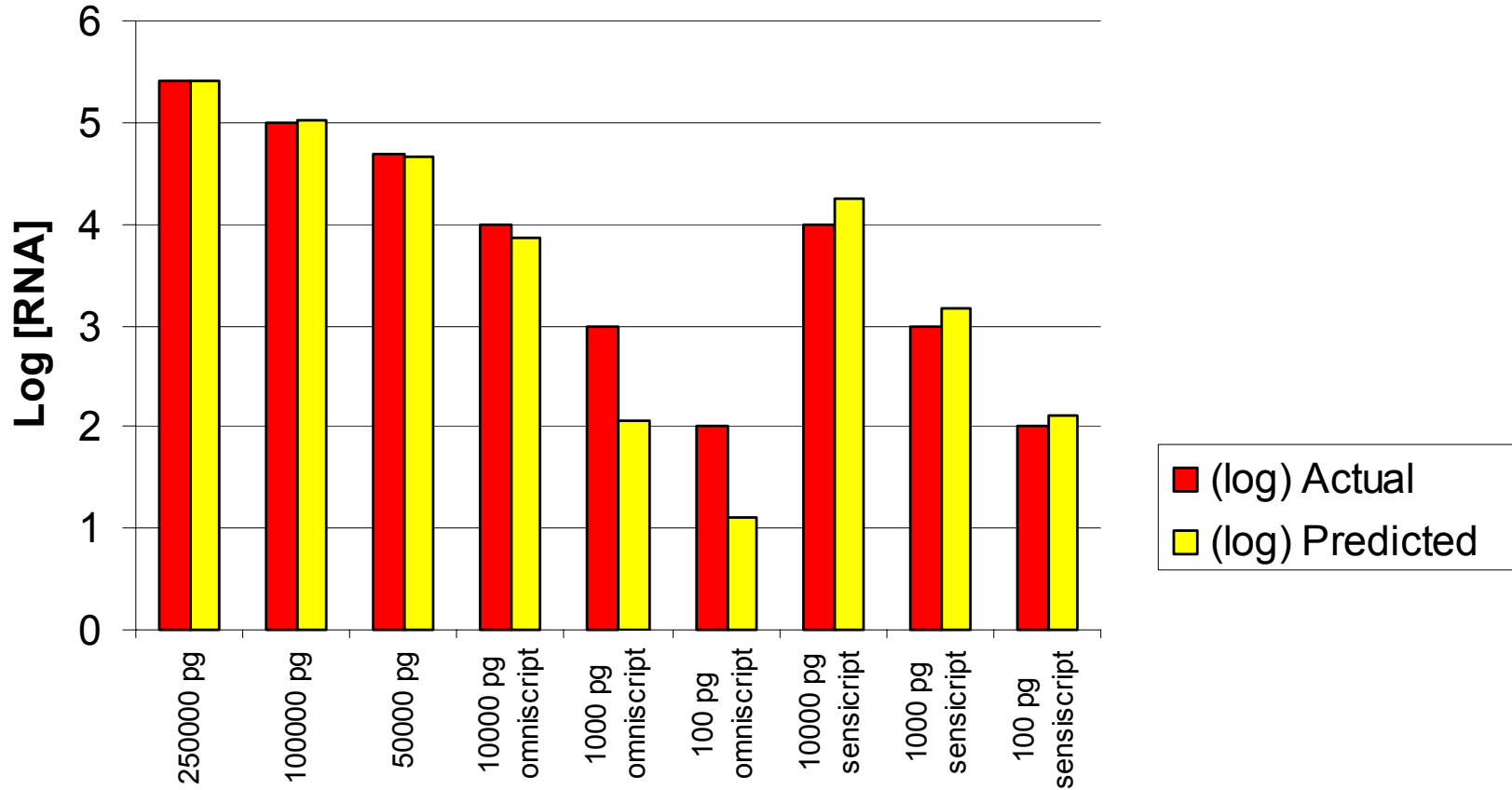
Molecular group (CID)

British Lung Foundation

Department for International Development

European Union

## Difference between actual [RNA] and real time calculated [RNA]



## Differences in fold change measurements with PCR efficiency

