

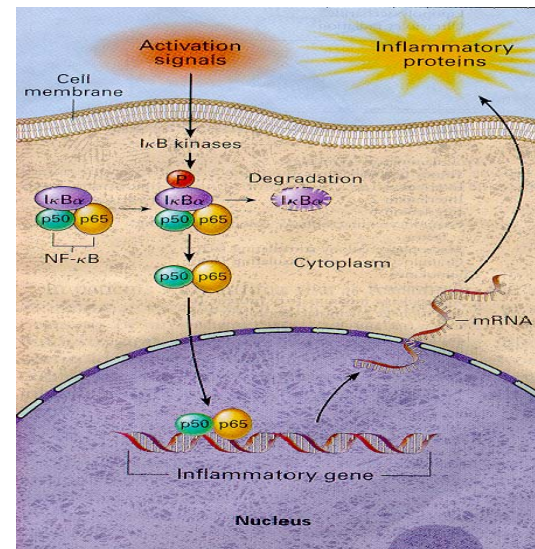
**Gene expression quantitated in PAXgene™ frozen stored blood cells
as compared to freshly Immuno Magnetic Separated (IMS) blood cells.**

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Changes in gene expression pattern in circulating white blood cells may be observed during various disease states



Whole blood



Gene regulation

Accurate quantitation of gene expression by the use of Q-RT-PCR has gained importance as a tool:

- to indicate the presence or prognosis of disease
- to predict or monitor the drug therapy
- to track disease kinetics

The increasing interest has resulted in a need of standardization!

- how to handle the samples
- how to quantitate specific mRNA molecules

The preanalytical aspects

Stabilization of whole blood has been a challenge !

- Degradation of gene transcripts
 - Unintended gene induction caused by sample handling
 - Uncontrolled activation of coagulation
-
- snapshot of the gene expression at a certain time
 - prevent degradation of the nucleic acids
 - Biobanking
 - Epidemiological studies
 - Transport



**PAXgene
Blood RNA System**

- Claim to be: "an integrated and standardized system for the collection, immediate stabilization and purification of RNA"
- Can be handled along with other blood tubes
- Stored for weeks at 4°C

Stabilization of mRNA expression in whole blood samples.

Lynne Rainen et al. *Clinical Chemistry* 48:11 1883- 1890, 2002.

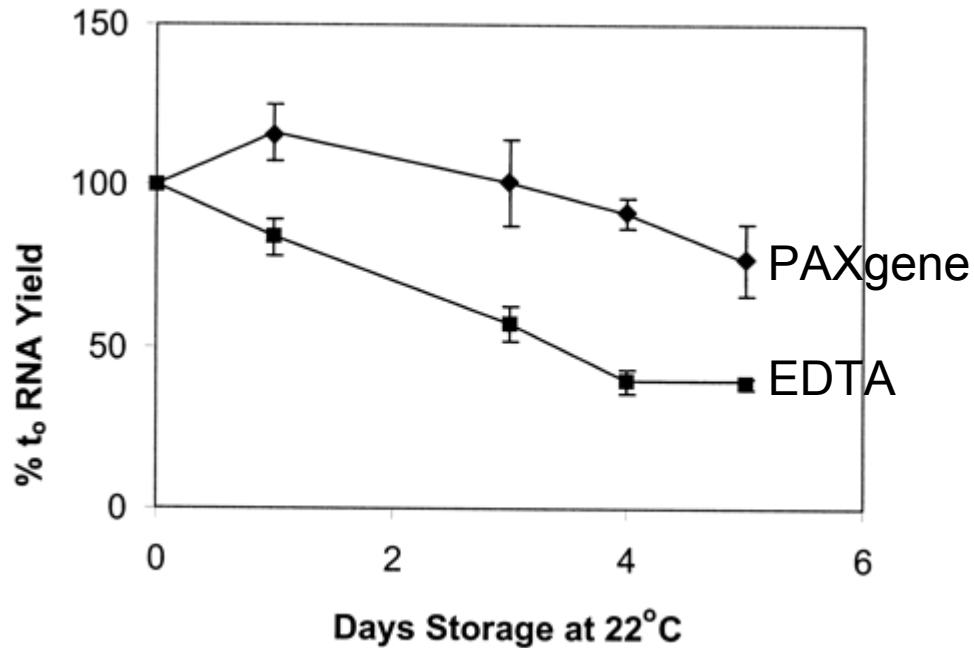
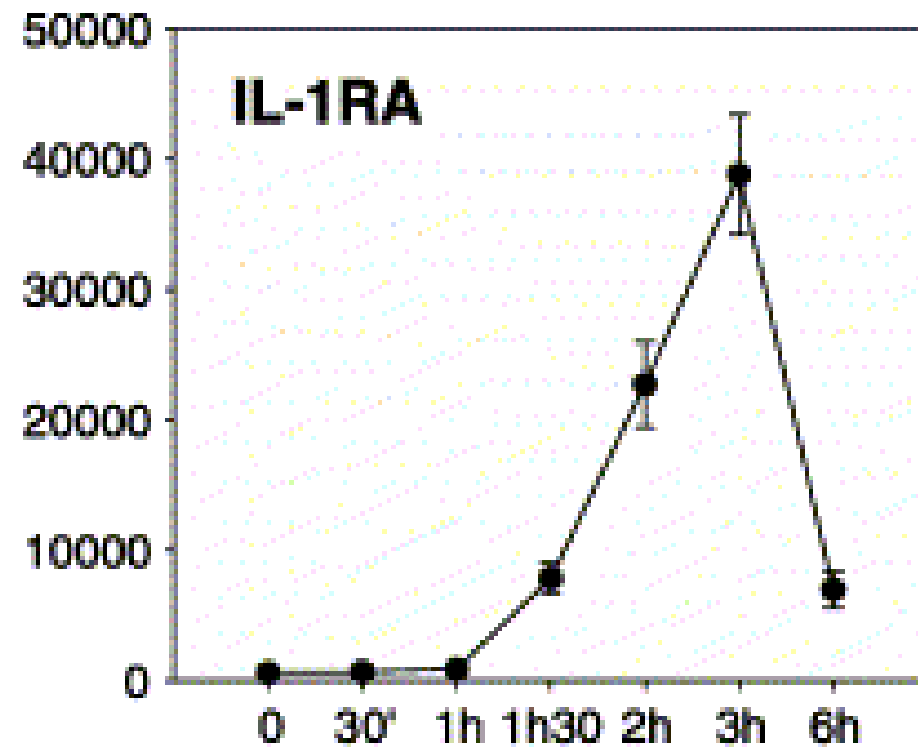
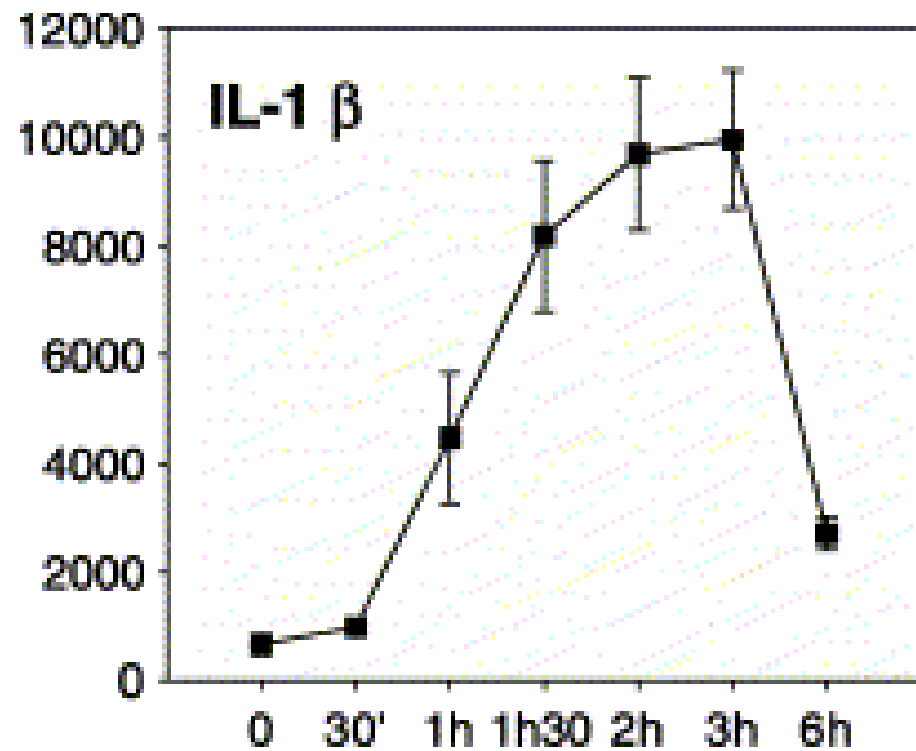


Figure 1. Percentage of t_0 yields of total RNA from blood stored in PAXgene (◆) and EDTA (■) tubes for 5 days at 22 °C.

Immune monitoring in whole blood using real-time PCR.

Patrick Stordeur et al. *Journal of Immunological Methods* 276; 69-77 2003



time after LPS injection \longrightarrow

Overall Aims:

To evaluate the use of PAXgene collection tubes for robotized isolation of high quality RNA from whole blood.

Aim 1:

Quantitate specific mRNA molecules in Paxgene tubes stored at 4°C and -70°C.

Aim 2:

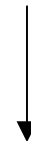
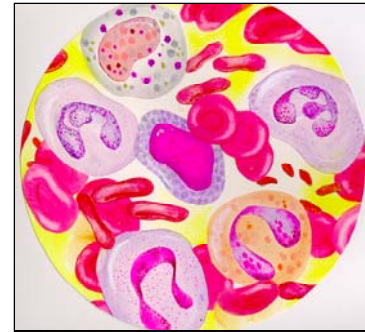
Compare quantitation of specific mRNA molecules in WBC stored in PAXgene tubes at -70 °C and WBC isolated from whole blood using IMS.

Wholeblood model

Compare gene expression: Paxgene / IMS



Paxgene tubes:
all WBC



IMS CD15/45 :
isolate all WBC

Methods

Modellsystem

Heparinized blood \pm LPS (0, 100 pg 1000 pg/ml) \rightarrow Upregulation of distinct genes
4 hours in incubator



IMS

PaxGene



+ 4°C
- 70°C

Cell-lysates of WBC

mRNA isolation BioSoftM4

BioSoftM4



Reverse transcription

PCR



What happens in a PAXgene tube ?



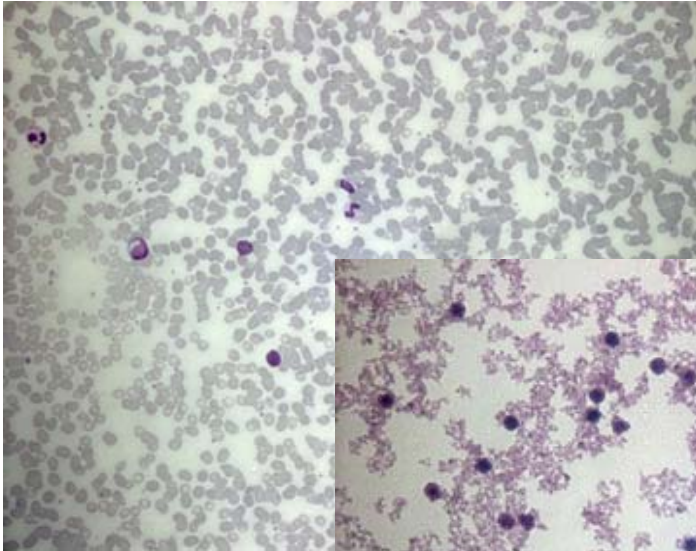
**PAXgene
Blood RNA System**

2,5 ml whole blood is added to 6,9 ml stabilizer reagent

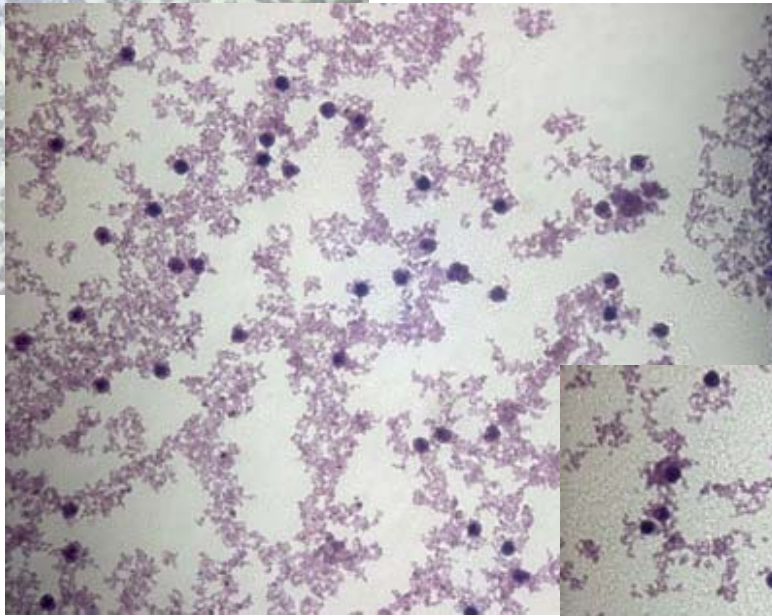
- RBC are lysed
- WBC stay intact as cells
- a precipitation occurs

Paxgene blood:

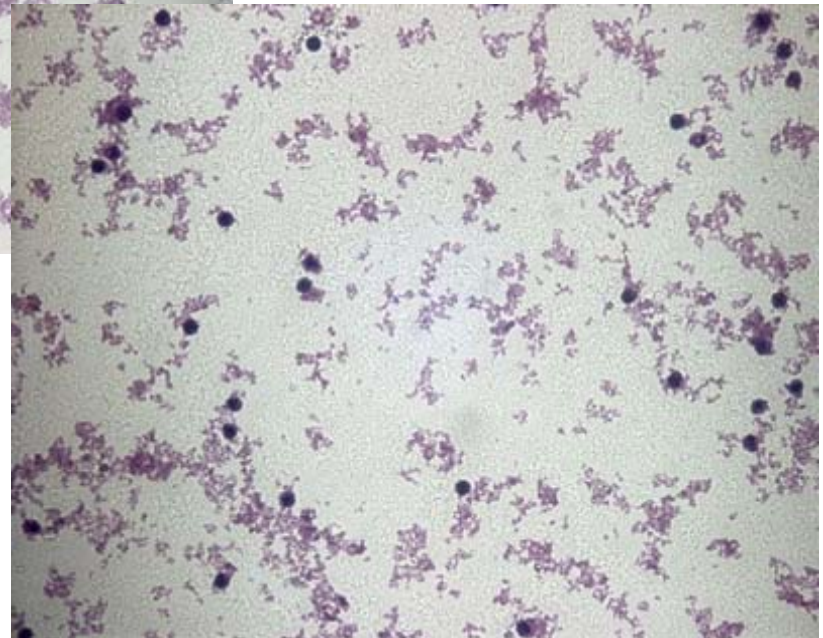
- will the stabilization reagent maintain the gene expression? ?
- What about freezing?



EDTA blood

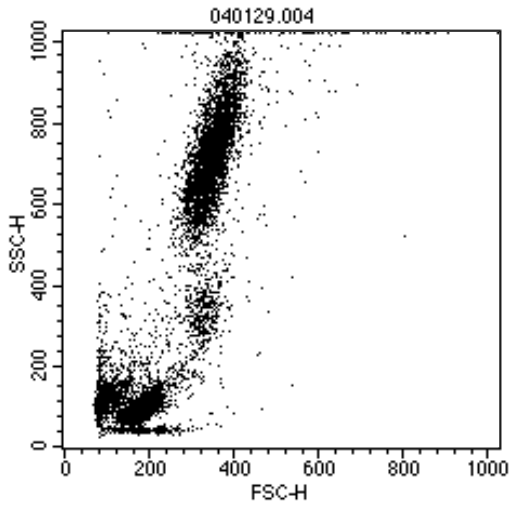


Paxgene blood 4 °C

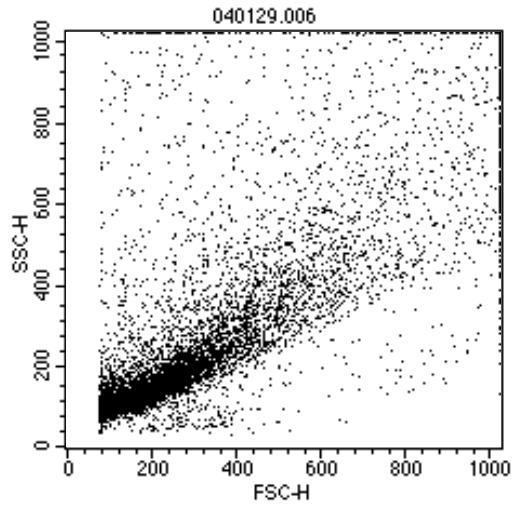


Paxgene blood -70 °C

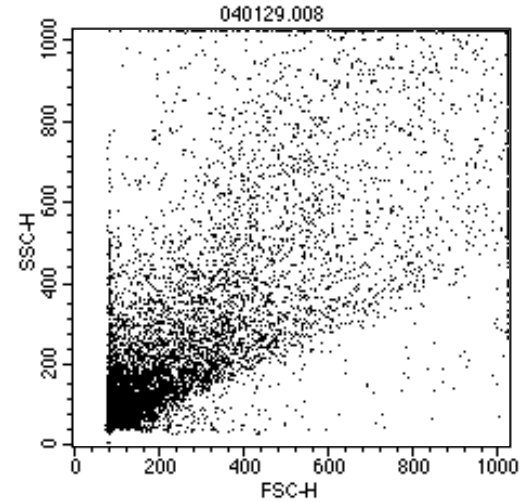
PAXgene cells in FCM



Whole blood



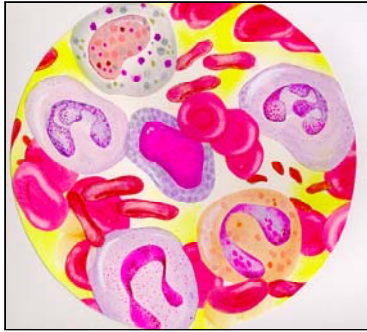
Paxgene 4°C



Paxgene -70°C

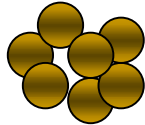
IMS

Immuno
Magnetic
Separation

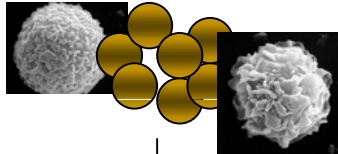


+

CD 45/15
magnetic beads



10 min incubation
4°C

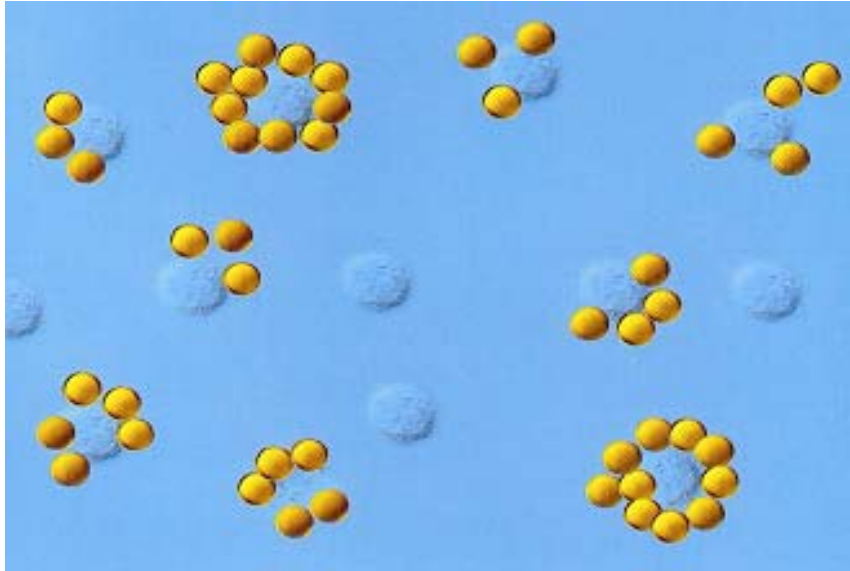


Cell lysate

IMS

- will all the WBC be isolated?
- will the isolation method affect the gene expression?

Specific cell - Dynabeads[®] interaction



LM: cells bound to Dynabeads[®]



EM: cell bound to 2 Dynabeads[®]

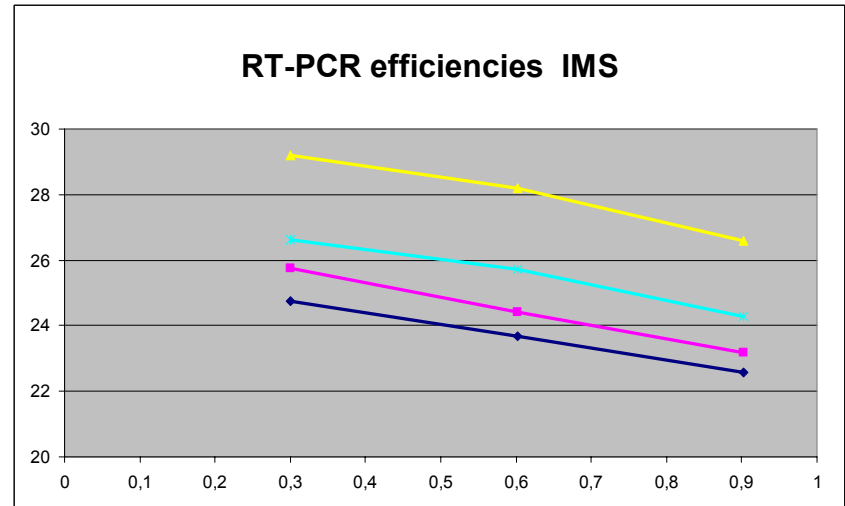
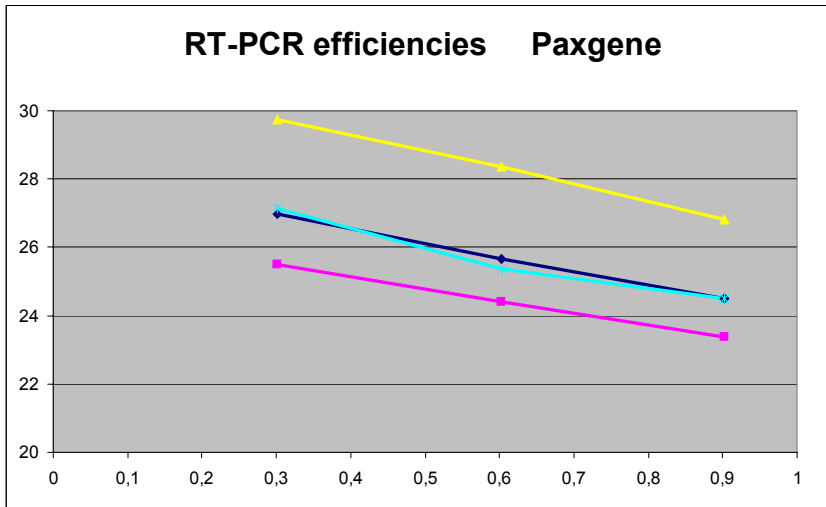
Quantitation of specific mRNA molecules

- Reverse Transcription:
 - oligod(T) primers
 - Omniscript
- reproducible standardcurves generated from PCR products
- SYBR green detection
- results expressed as a ratio between the target gene and a housekeeping gene

Validation experiments

RT-PCR inhibition

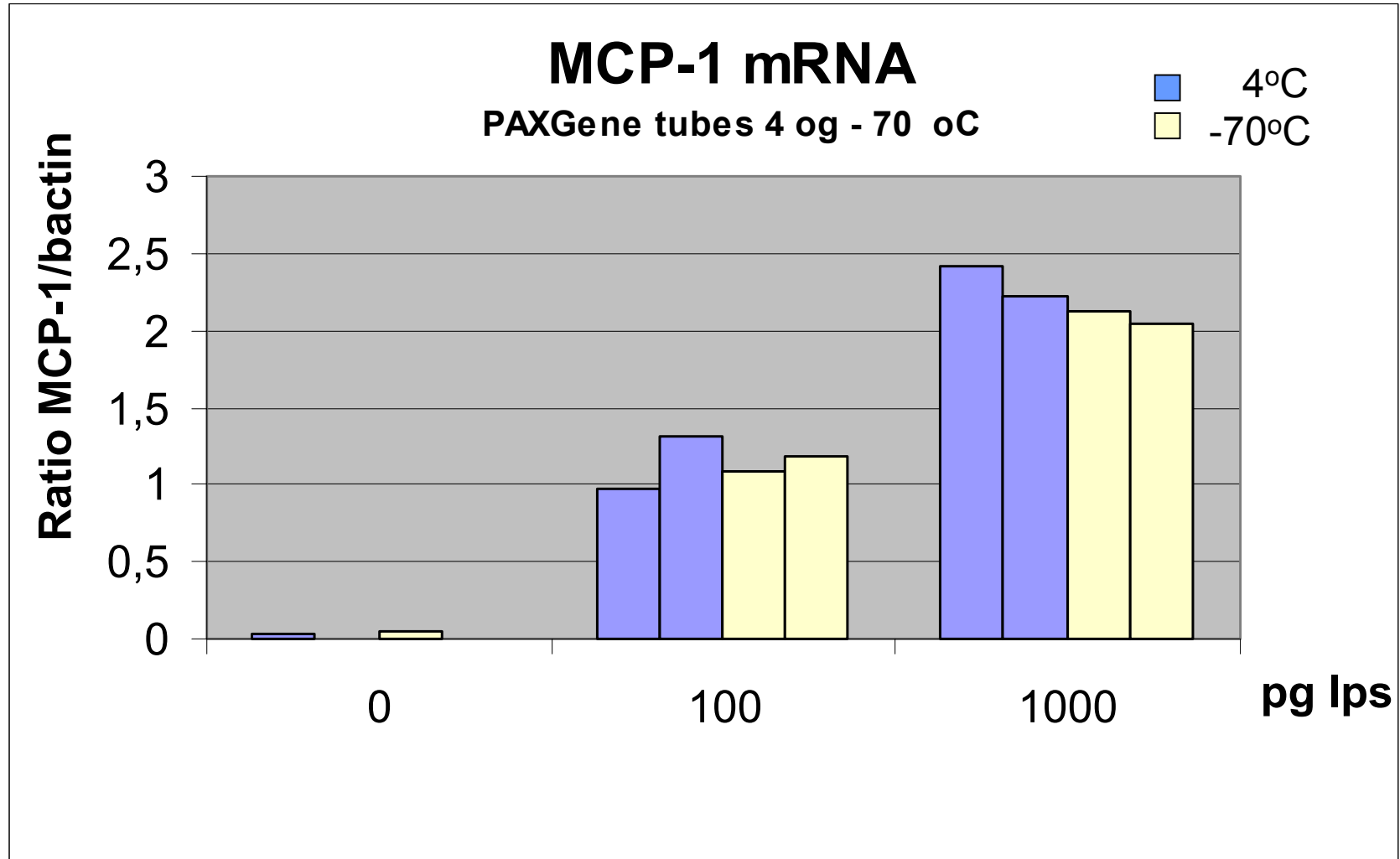
Cp



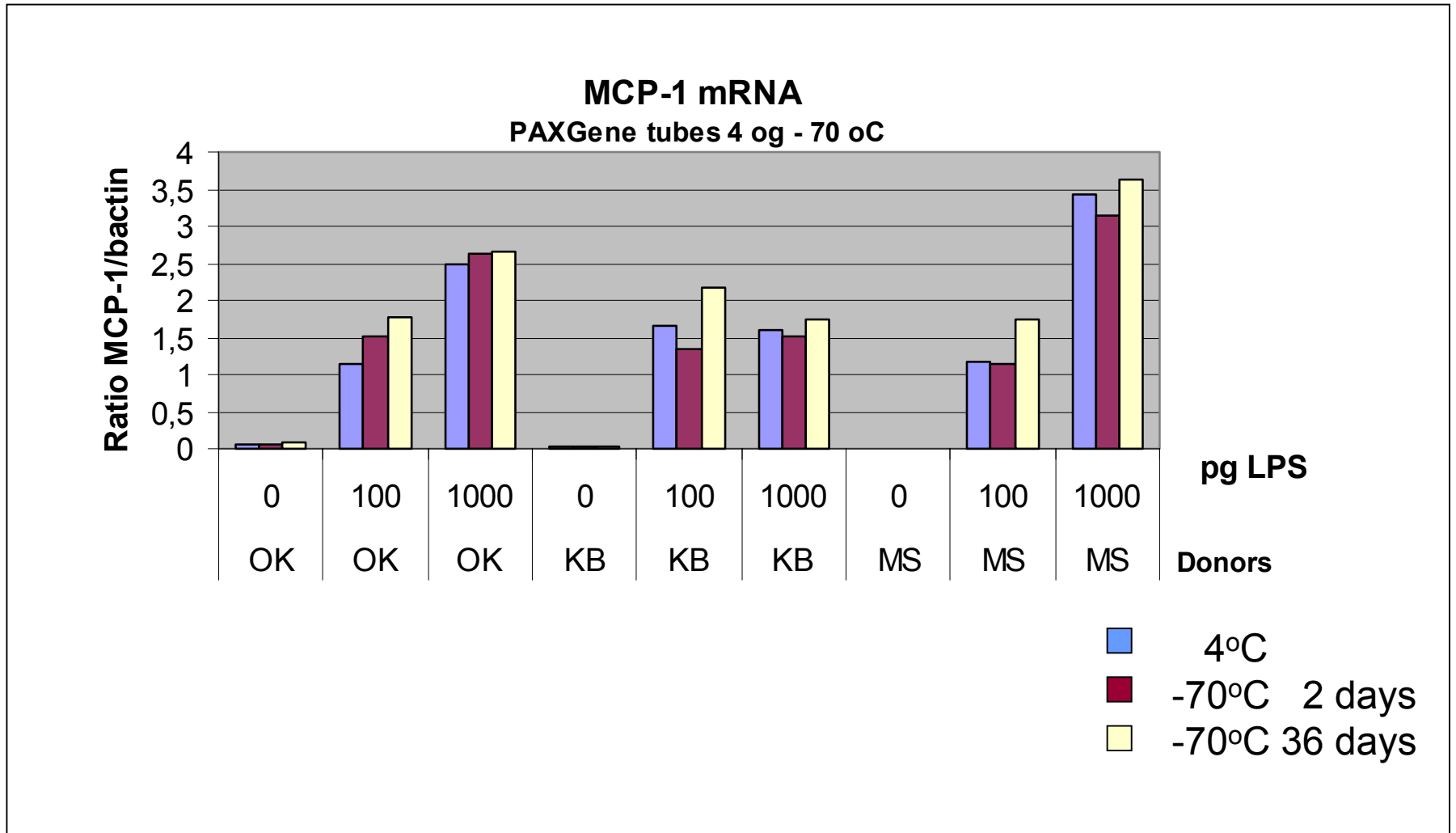
Log concentrations

- MCP-1
- TNF α
- β_2 -microglobulin
- β -actin

Maintained gene expression after freezing ?

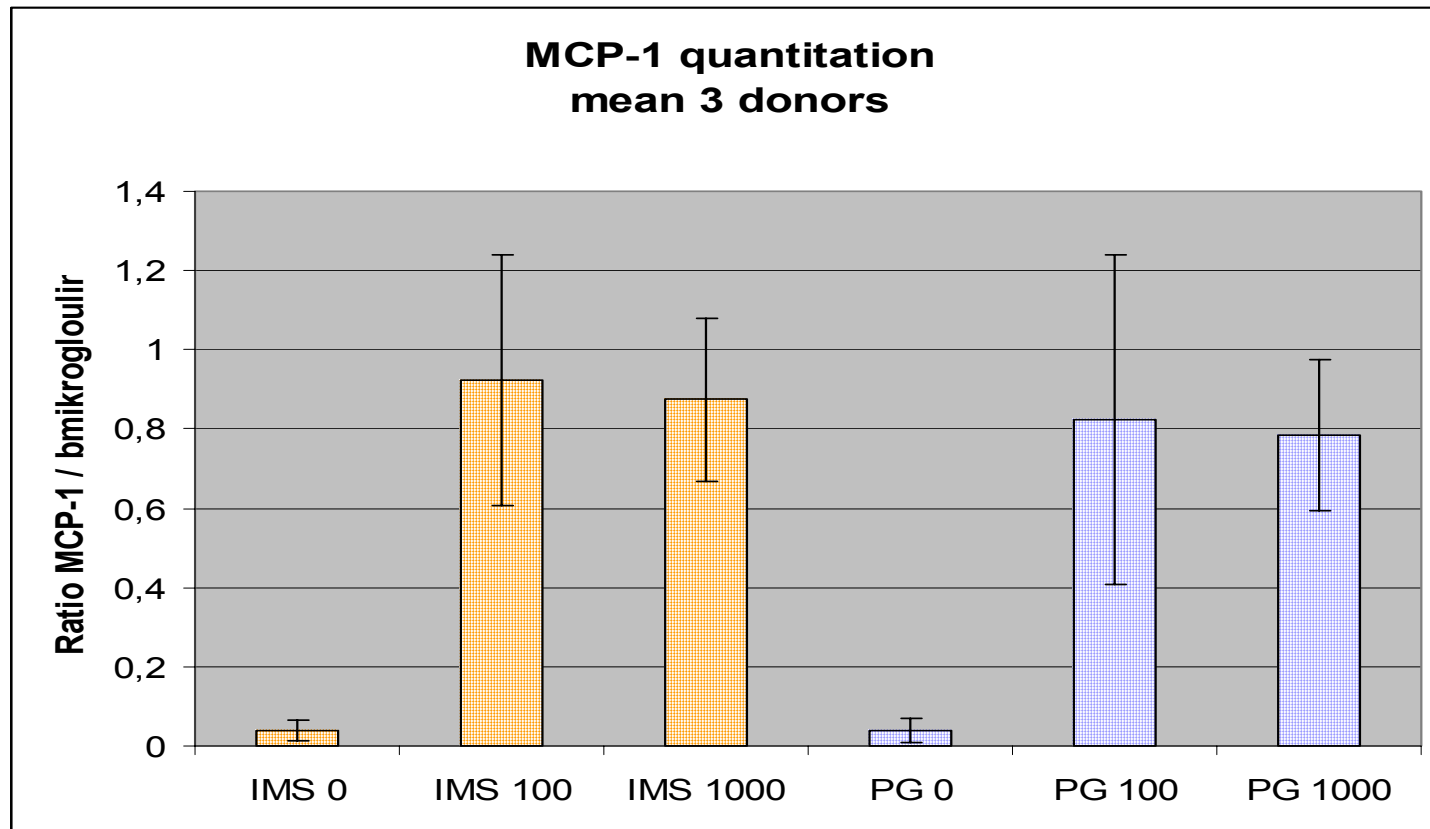


Increased storage time Different donors



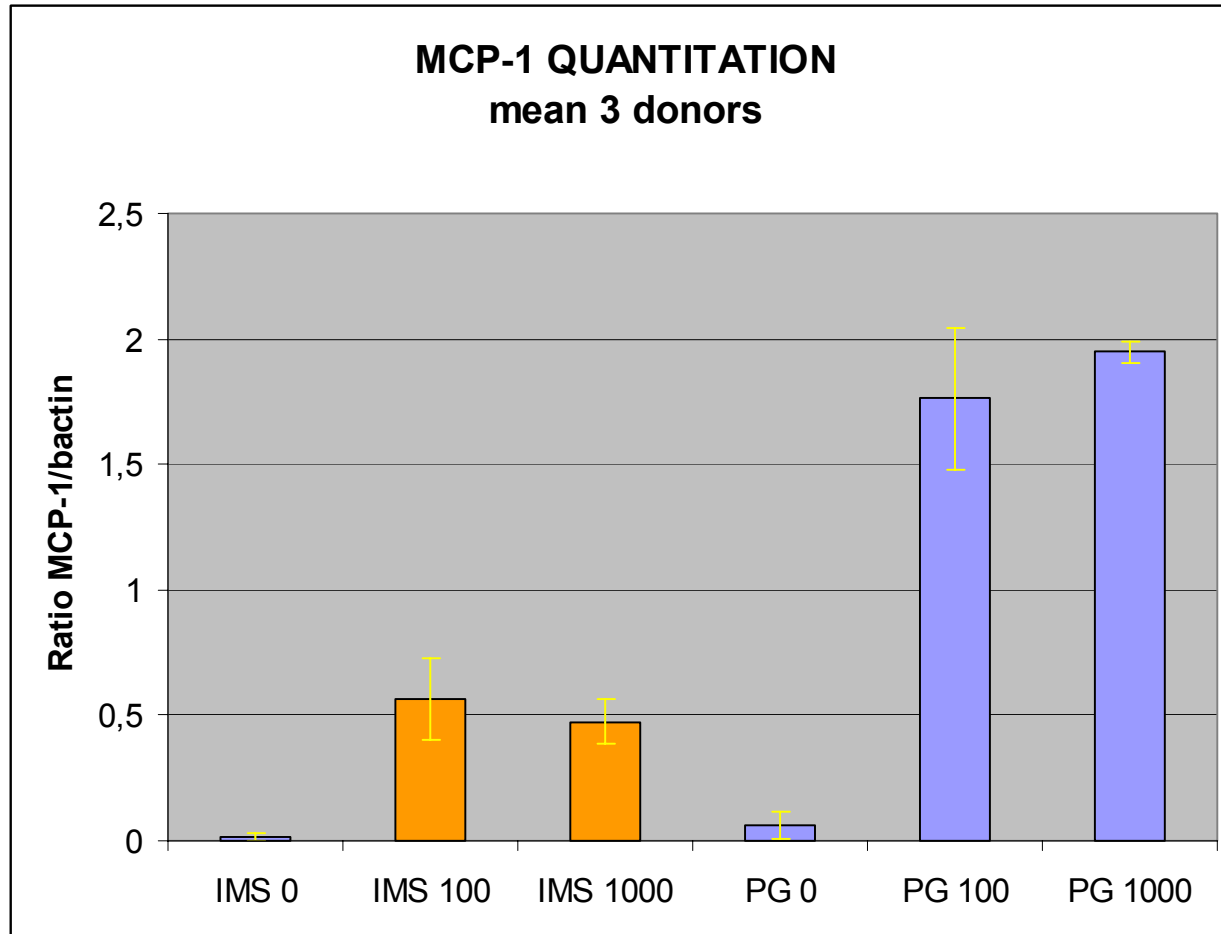
Paxgene versus IMS- 1

β_2 -microglobulin



Paxgene versus IMS- 2

β -actin



Conclusions:

- RNA isolated from frozen PAXgene™ tubes appears to be satisfactory compared to 4°C.
- The gene expression from frozen and IMS-isolated blood cells appears to be comparable if using β_2 -microglobulin as housekeeping gene.
- The quantity of β -actin transcripts seems to be reduced in Paxgene tubes. Further investigation is needed.