

**Long-term storage of peripheral blood progenitor cells (PBPC)  
at  $-80\text{ }^{\circ}\text{C}$  after long-term storage below  $-170\text{ }^{\circ}\text{C}$   
leads to a significant loss of cell membrane integrity and clonogenicity**

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## ***Aim of the study:***

To investigate the effect of

- ***long-term storage (approx. 2 years) < - 170 °C***

followed by

- ***long-term storage (approx. 2 years) at***

- a) < - 170 °C (vapour phase over liquid nitrogen)
- b) - 80 °C (mechanical refrigerator)

on the recovery, membrane integrity, and viability of PBSC cryopreserved with 10% Me<sub>2</sub>SO.

# Materials & Methods

#1

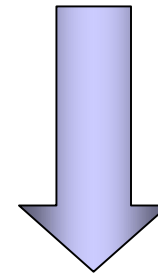
- 48 (= 24 pairs) autologous PBSC concentrates obtained by apheresis from 20 patients with haematological disorders and solid tumours
- Volume/cryoprotectant:  $108 \pm 4$  ml/10% Me<sub>2</sub>SO
- Cooling:
  - controlled rate freezer
  - 0 °C to -45 °C at 1 °C/min
  - -45 °C to -90 °C at 10 °C/min
  - transfer to vapour phase over liquid nitrogen
- Frozen storage: Vapour phase over liquid nitrogen for 26.0 months (range 4.5-51.7), then transfer of one of each pair into a -80 °C refrigerator, continuation of storage at <-170 °C of the other unit for 21.5 months (range 12.6-28.7)
- Thawing: shaking water bath (37 °C) for 3 min;  $t = 15 \pm 3$  °C





**26.0 months (range: 5 - 52)**

**< -170 °C (Group 1 & 2)**



**Liquid nitrogen**

**21.5 months (range: 13 - 29)**

**< -170 °C (Group 1)**

**Mechanical refrigerator**

**-80 °C (Group 2)**

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- **Directly after thawing**

MNC concentration

(Coulter counter)

- **After removal of the cryoprotectant**

(stepwise dilution, centrifugation, removal of supernatant)

Membrane integrity

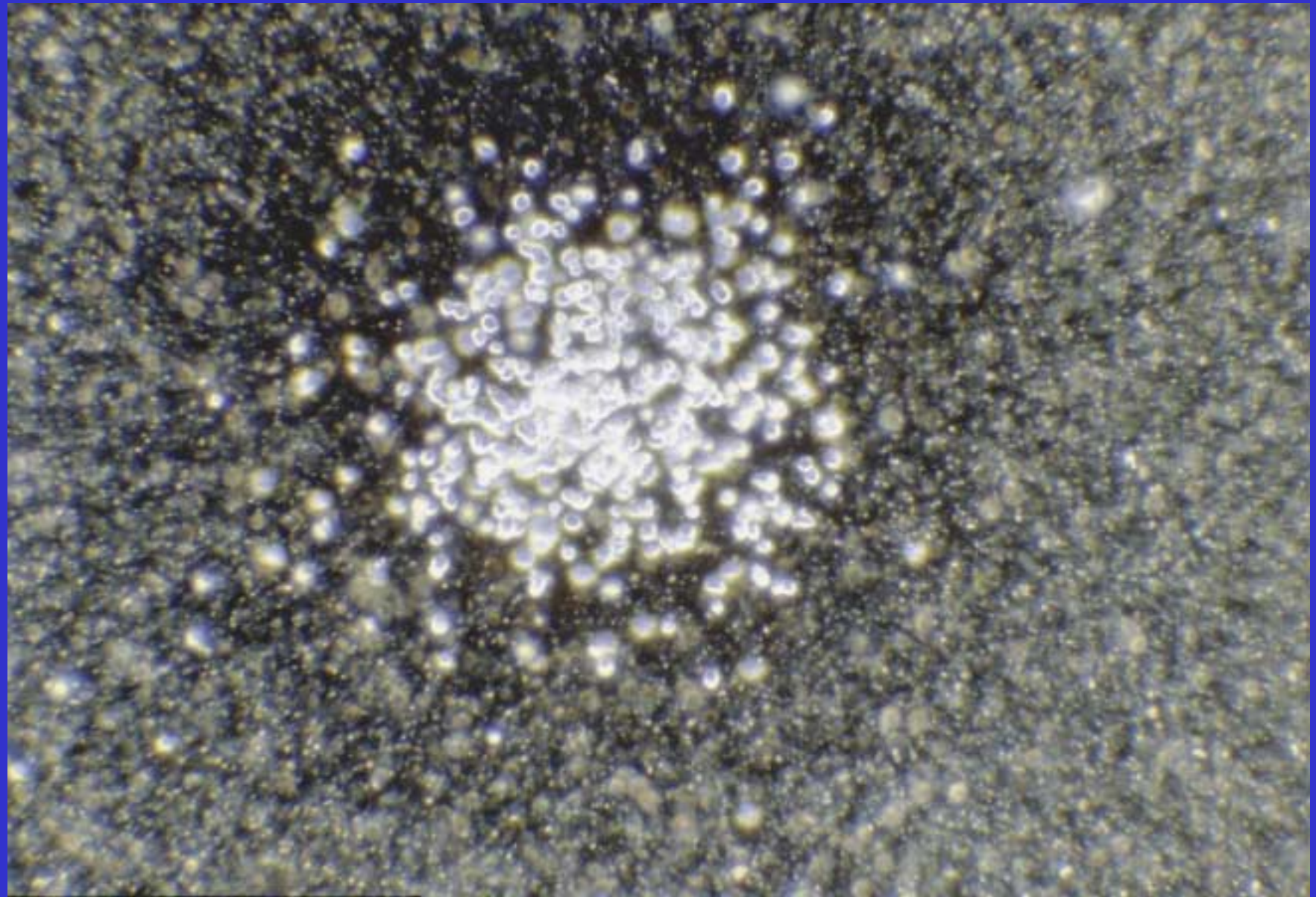
(trypan blue exclusion)

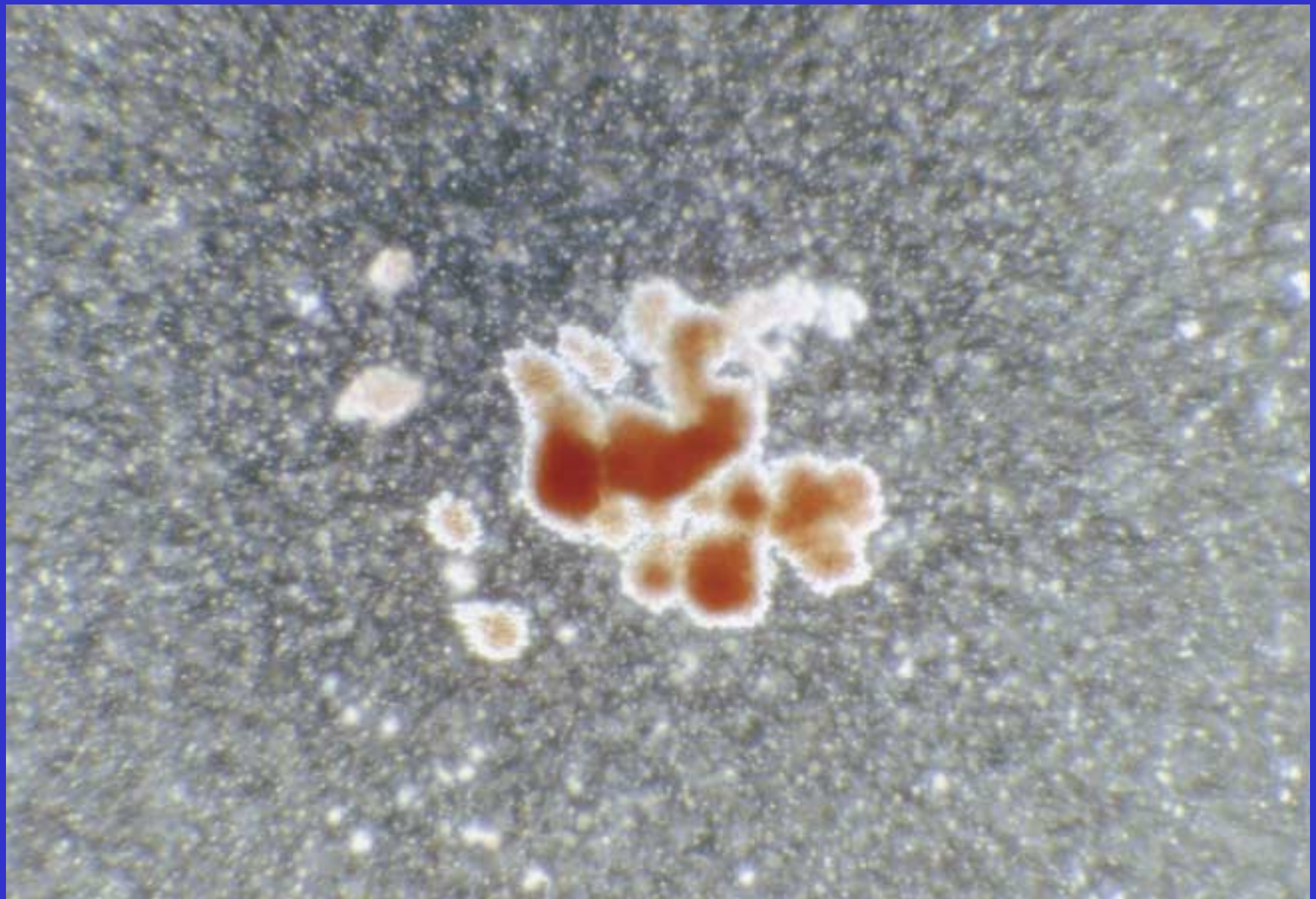
Colony forming potential

(semi-solid cell culture)

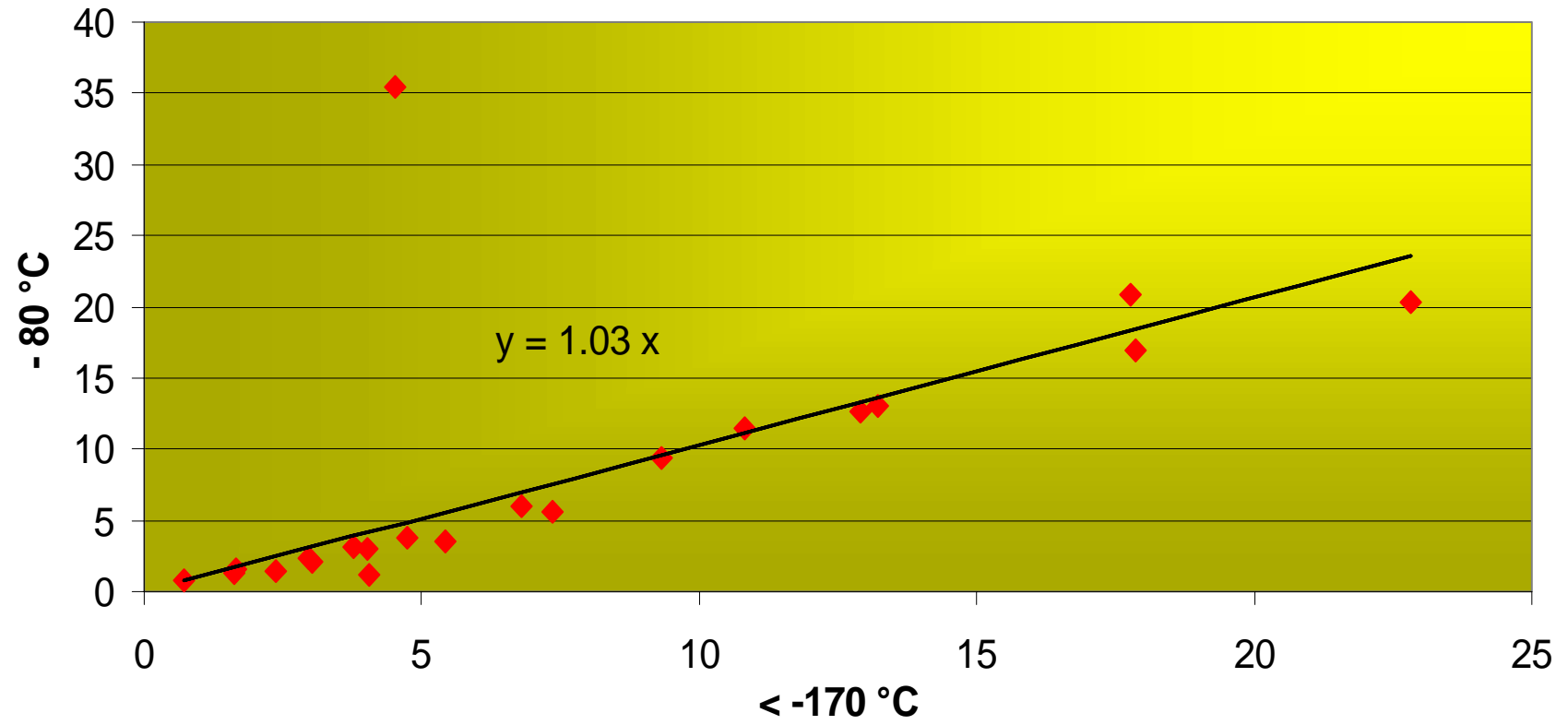
- **Statistics**

(Student's t-Test;  
significantly different  
if  $p < 0.05$ )

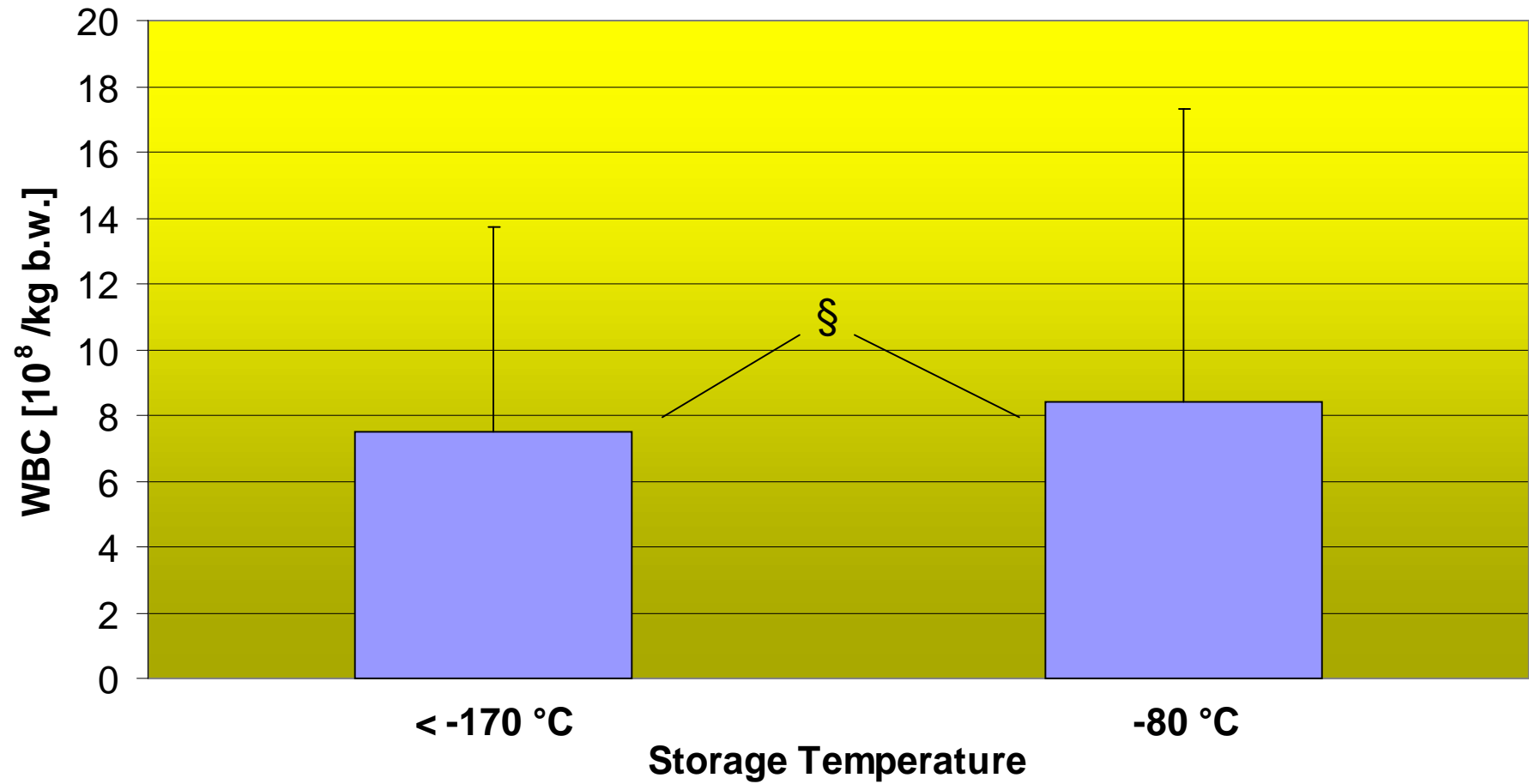




# Numerical WBC Recovery (after Thawing) [10<sup>8</sup> /kg b.w.]

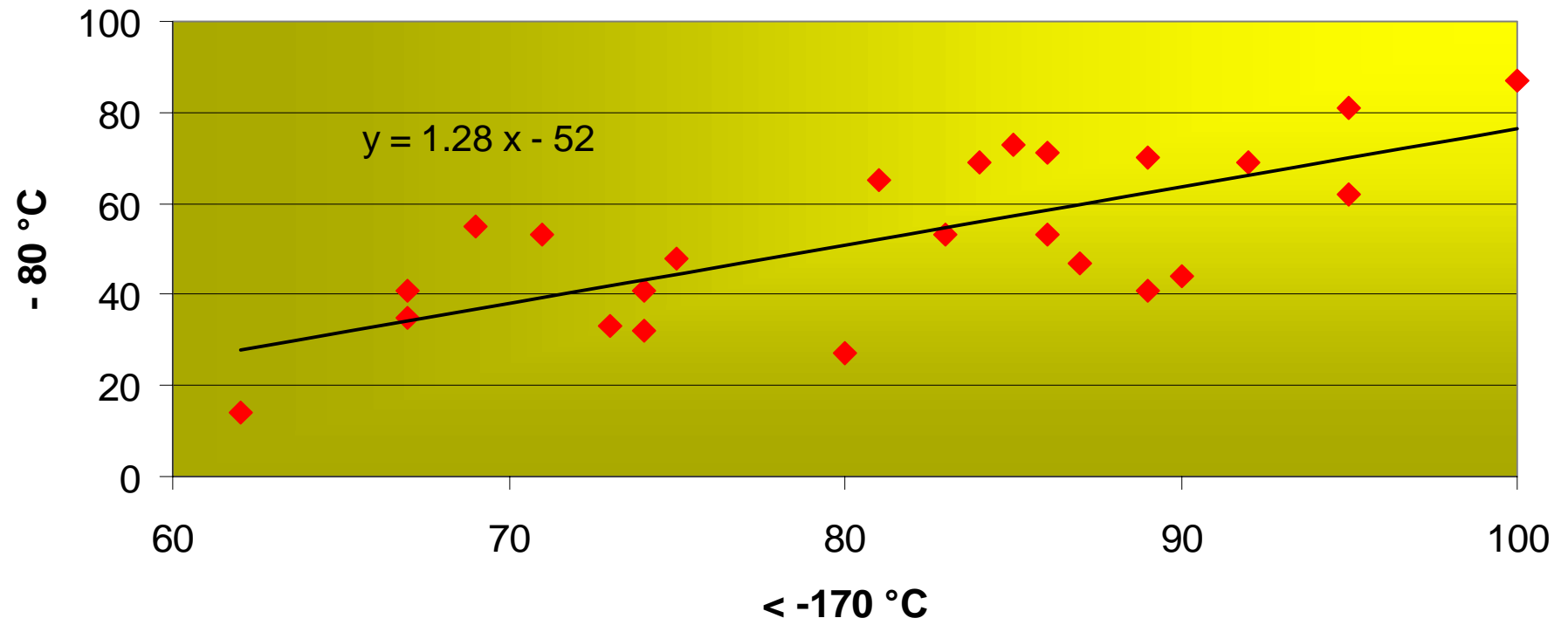


## Numerical WBC Recovery (after Thawing)

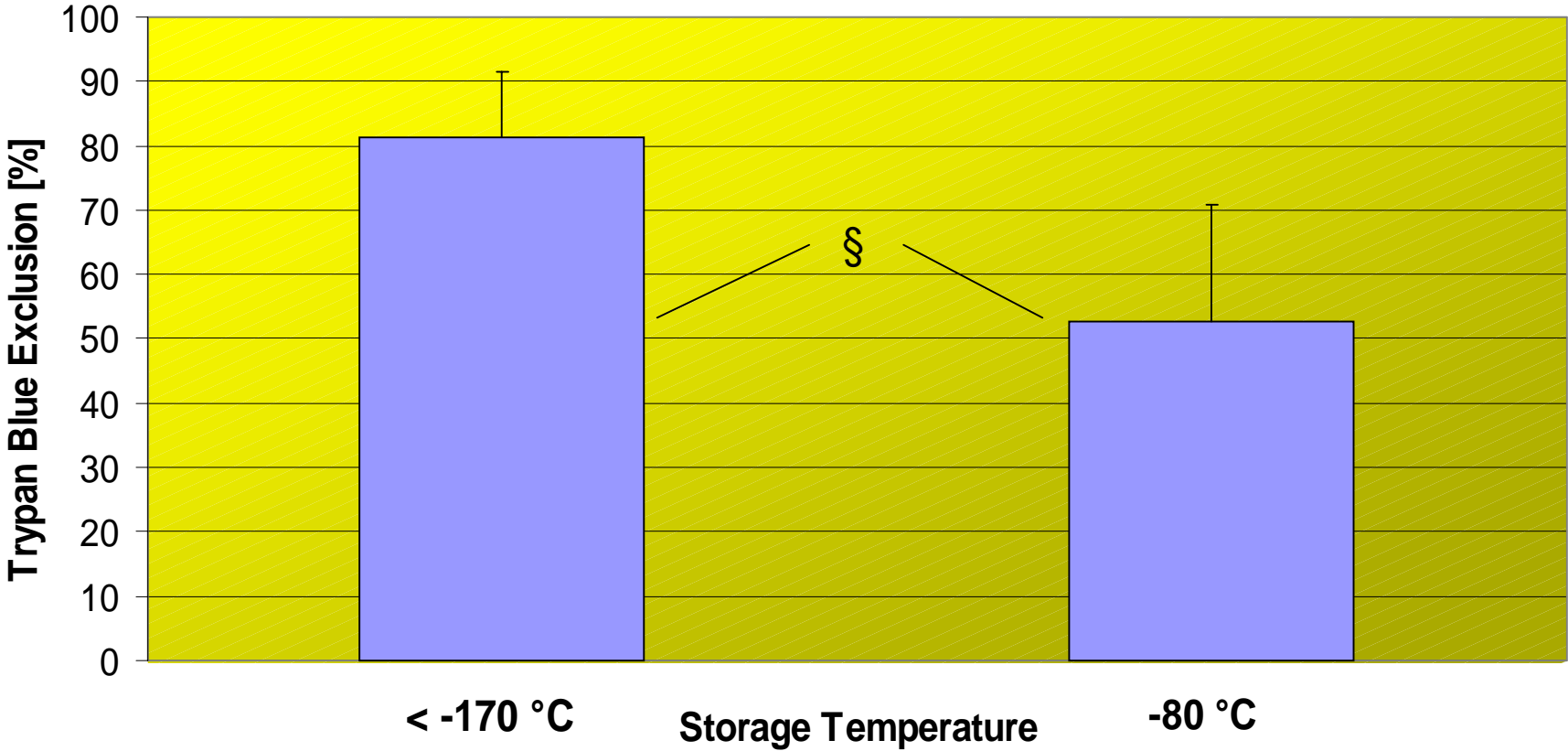


§ = not significantly different

# Membrane Integrity (after Thawing and Washing) [%]

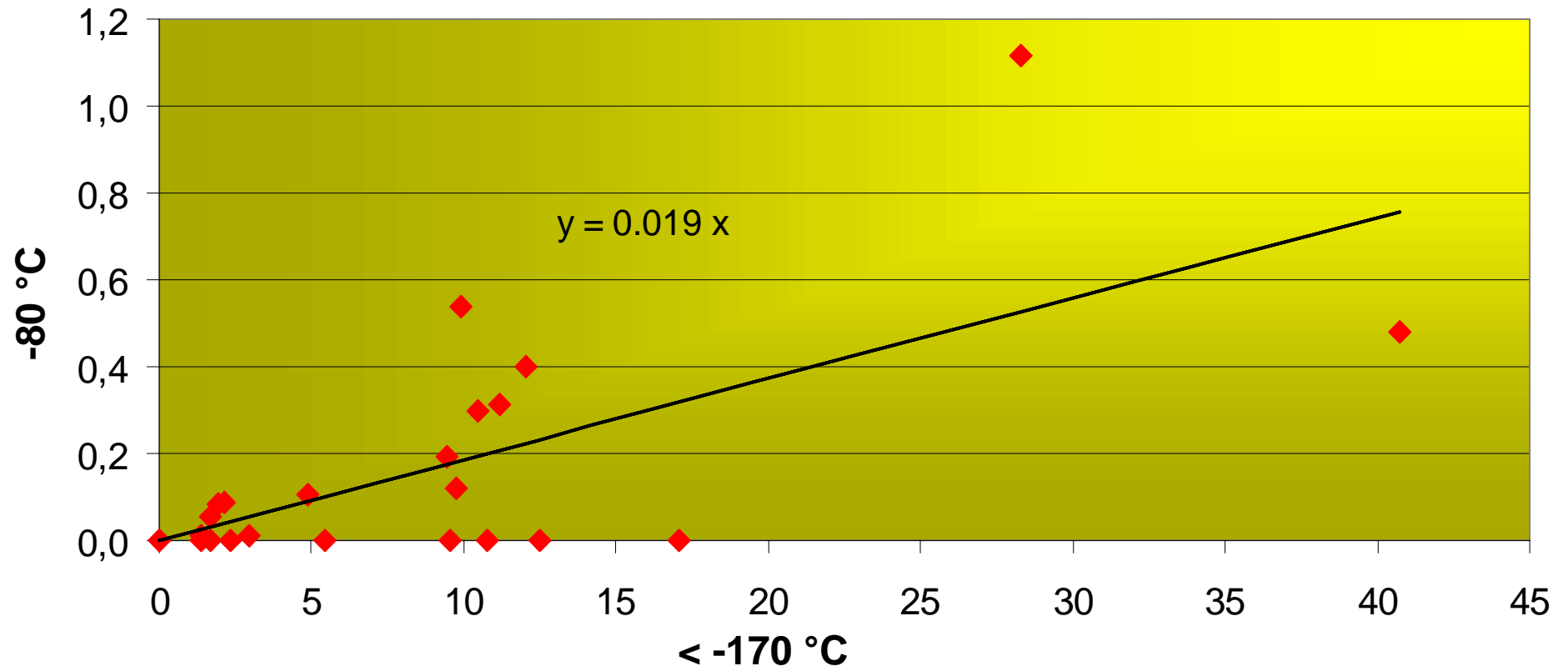


# Membrane Integrity (after Thawing and Washing)

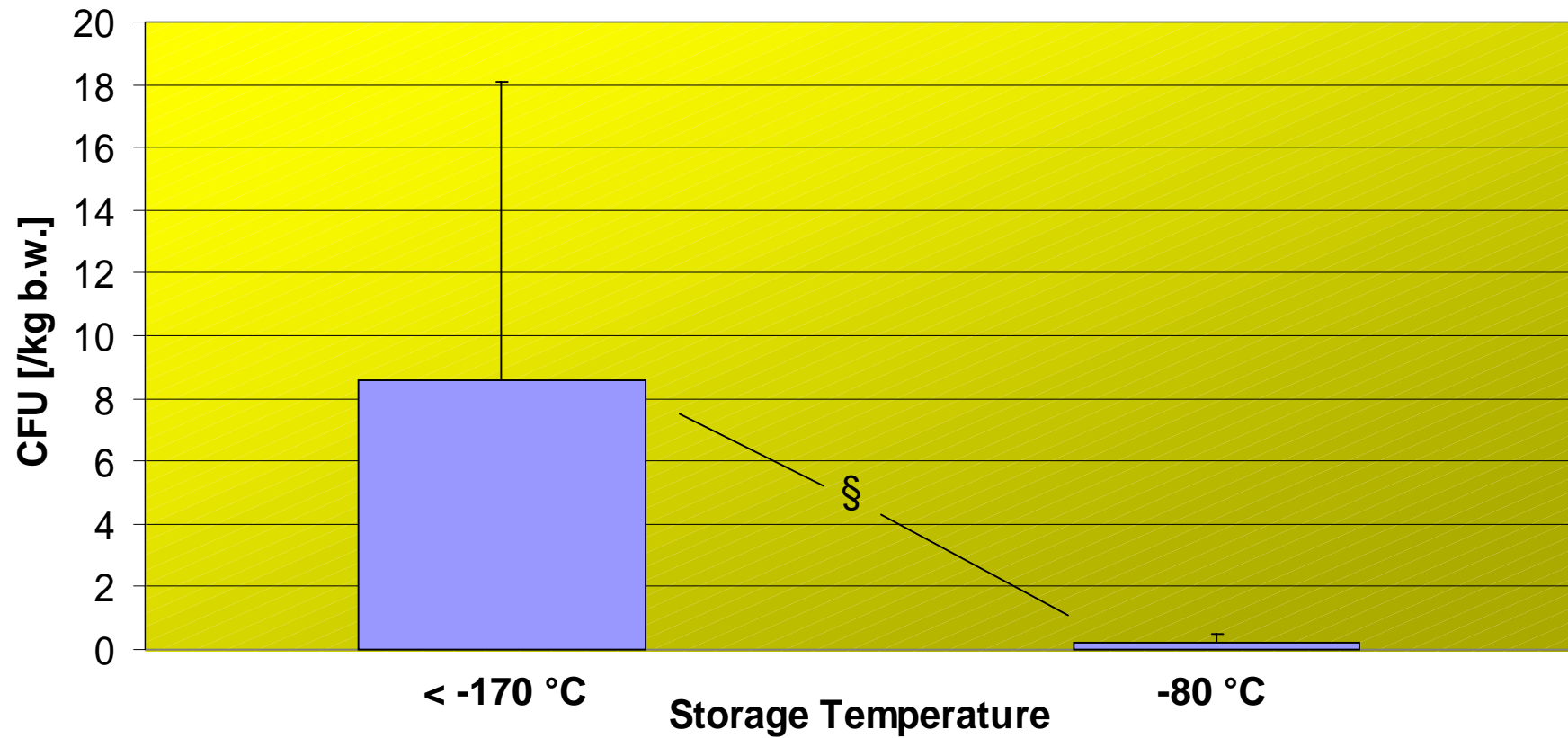


§ = significantly different, p < 0.05

# Clonogenicity (after Thawing and Washing) [CFU /kg b.w.]



## Clonogenicity (after Thawing and Washing)



§ = significantly different,  $p < 0.05$

# Summary

- **WBC Recovery**

Numerical WBC recovery showed no significant difference if storage was continued at  $-80\text{ }^{\circ}\text{C}$  or below  $-170\text{ }^{\circ}\text{C}$ .

- **Membrane Integrity**

Membrane integrity (in terms of trypan blue exclusion) was significantly lower after 13 - 29 months of storage at  $-80\text{ }^{\circ}\text{C}$ .

- **Clonogenicity**

The colony forming potential was significantly lower as well; there was nearly no growth of cultures after long-term storage at  $-80\text{ }^{\circ}\text{C}$  following storage below  $-170\text{ }^{\circ}\text{C}$ .

# Conclusions

- Long-term storage of PBPC in mechanical refrigerators at -80 °C after long-term storage below -170 °C cannot be recommended.
- Looking for numerical WBC recovery is insufficient for the validation of storage conditions.
- Membrane integrity is a more sensitive parameter; however, unsuitable storage conditions are most easily detected by semi-solid cell culture assays.
- **Hypothesis A**: The relatively “high” storage temperature of -80 °C leads to the damage.
- **Hypothesis B**: The relatively “high” storage temperature of -80 °C leads to the damage *after* preceding storage at < -170 °C.