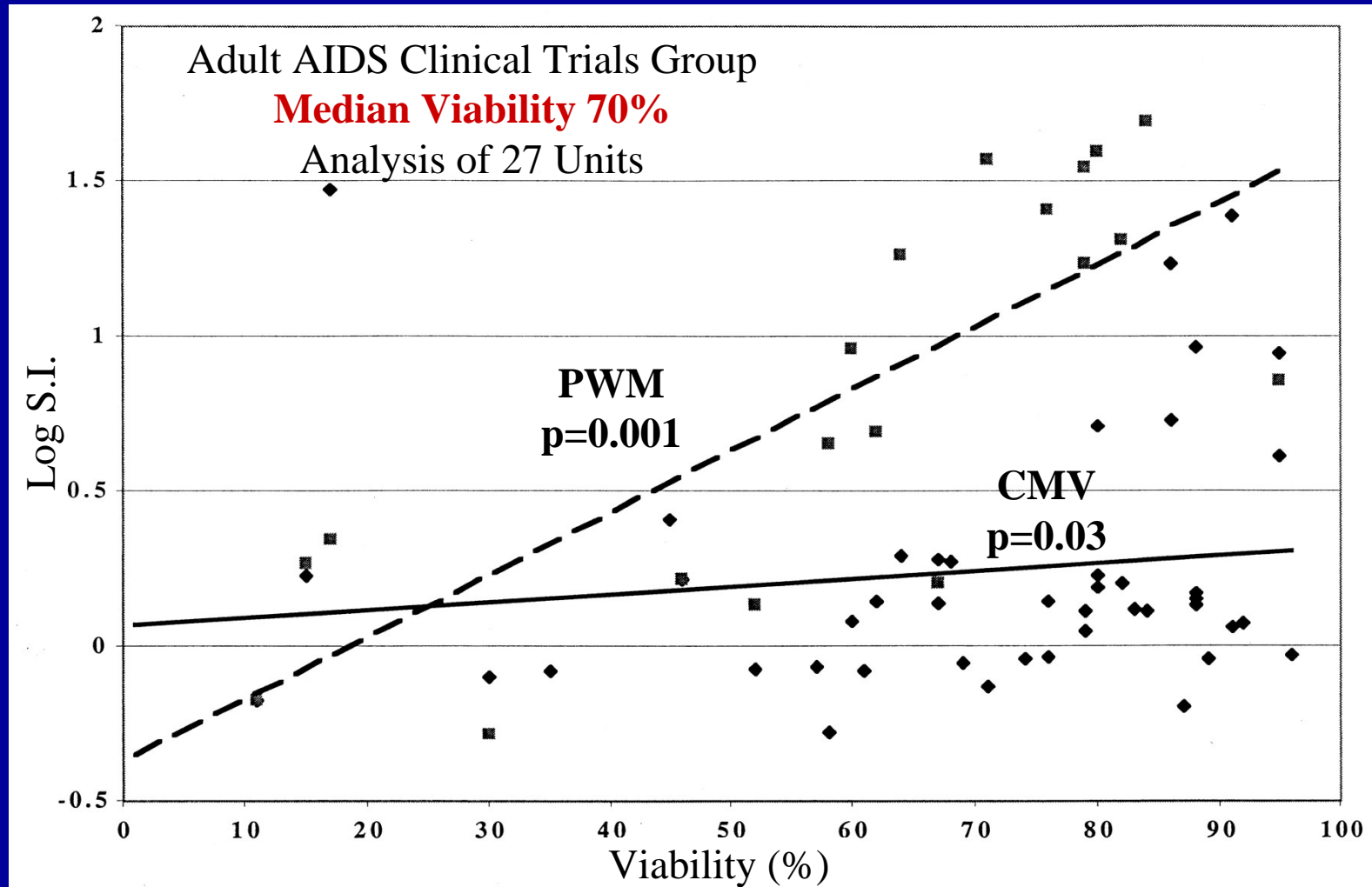


# Maximizing the Retention of Antigen Specific Lymphocyte Function After Cryopreservation

# Clinical Importance of Cryopreservation

- Transfusion for therapeutic purposes
- Evaluate all samples at the same time
- Retrospectively analyze specimens
- Improve assay precision and accuracy

# Viability Predicts Function after Cryopreservation



## MOCK TRIAL RUNS WITH STANDARD LAB ASSAYS

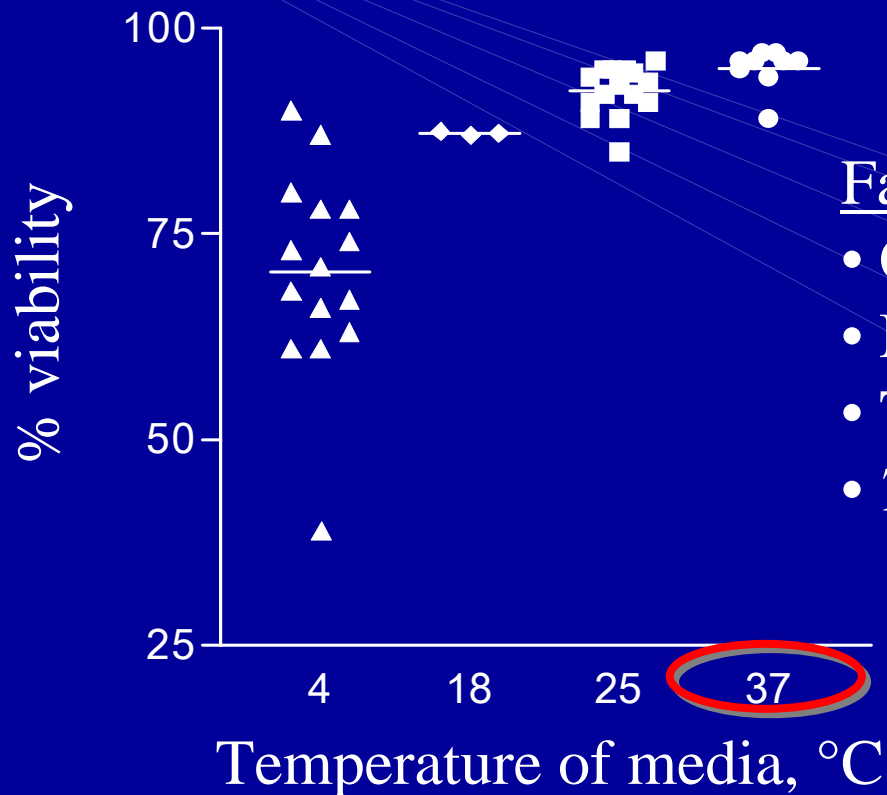


- # PROBLEM

- Cryopreservation methods

# Temperature During Thawing Effects Viability

## Cell Viability



## Factors that Effect Viability

- Cell clumping
- Media protein base
- Technical handling during processing
- *Temperature during thawing*

# Freezing Media

- 90% Fetal Bovine Serum/10% DMSO
- 90% Human AB serum/10% DMSO
- 89% Autologous plasma/1% Dextran-40/10% DMSO
- 11.5% Human serum albumin/10% DMSO

# Cost Analysis for Cryopreservation

Autologous plasma	Free
Fetal bovine serum	\$0.20
Human AB serum	\$0.95
Human serum albumin	\$3.75

**Tetanus toxoid**

Stimulation Index

Fresh PBMC FBS Dextran HSA

$p < 0.0001$

$p = 0.0040$

$p = 0.692$

Stimulation Index

Fresh PBMC FBS Dextran HSA

**p=0.692**



# Important Issues for Cryopreservation

- Standardization of additives
- Sera/dextran=cell clumping
- Controlled rate freezer not limiting
- Thawing as important as freezing
- Temperature at thawing
- Variability in counting effects recovery
- Volume and spin time tested for thawing-not critical

# Consensus SOP for Cryopreservation

- Cryopreservation
  - 11.5% human serum albumin in RPMI
  - 10% DMSO
  - 10 million PBMC/vial
- Thawing of PBMC
  - Thaw media at 37°C
  - Wash PBMC in 50 cc conical tube
  - 1200 rpm for 10 minutes

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