Importance of Specimen Collection and Storage in Immunologic Biomarker Development

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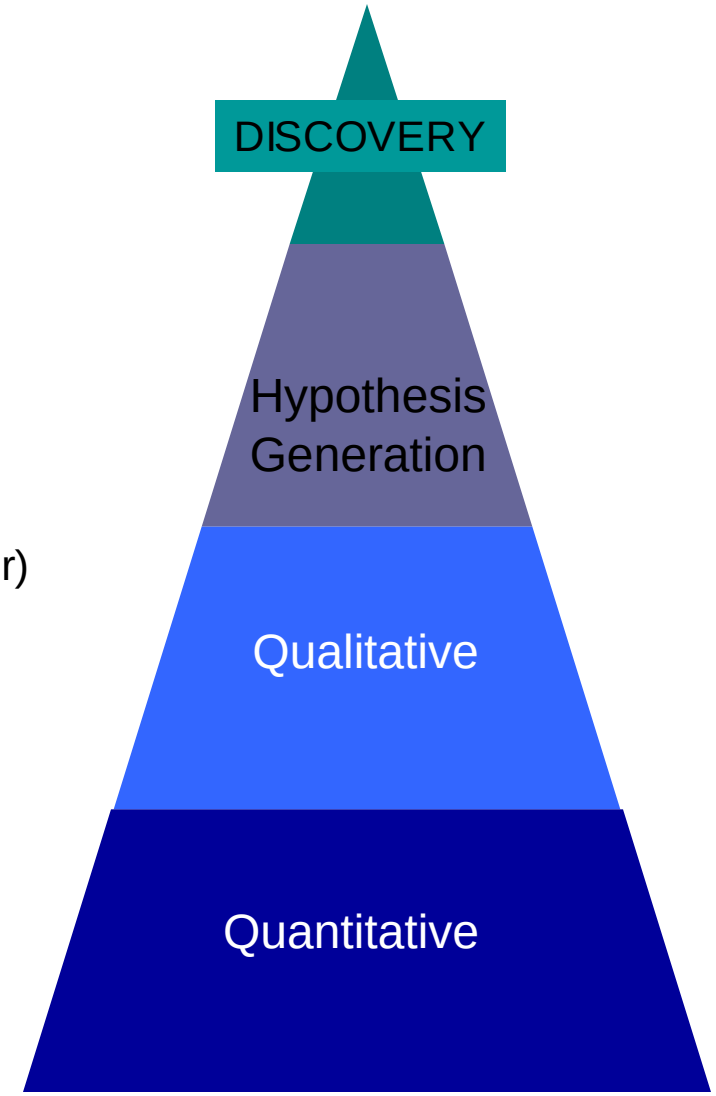
Need for High Quality Stored Specimens for Future Analysis

Effectors
- ELISPOT
- Tetramer
- Cytokine staining
- Humoral immunity
- NK, Th phenotype, etc.

Environment
- Cytokines
- Expression array (tumor)
- Epitope spreading
- GWAS

Host
- SNPs
- GWAS
- HLA



Biomarker Pyramid

Building a Pipeline for Immunologic Biomarker Validation

Sample Collection

Lymphocytes/PBMC
Peripheral Blood RNA
DNA
Serum
Tumor (DTH-Skin?)

Make sure there is enough material to analyze and bank

Quality Processing and Storage

SOP for each sample type which maintains sample integrity
QC Program for each sample type
Linked data
Prospectively defined clinical outcome assessment-long term F/U

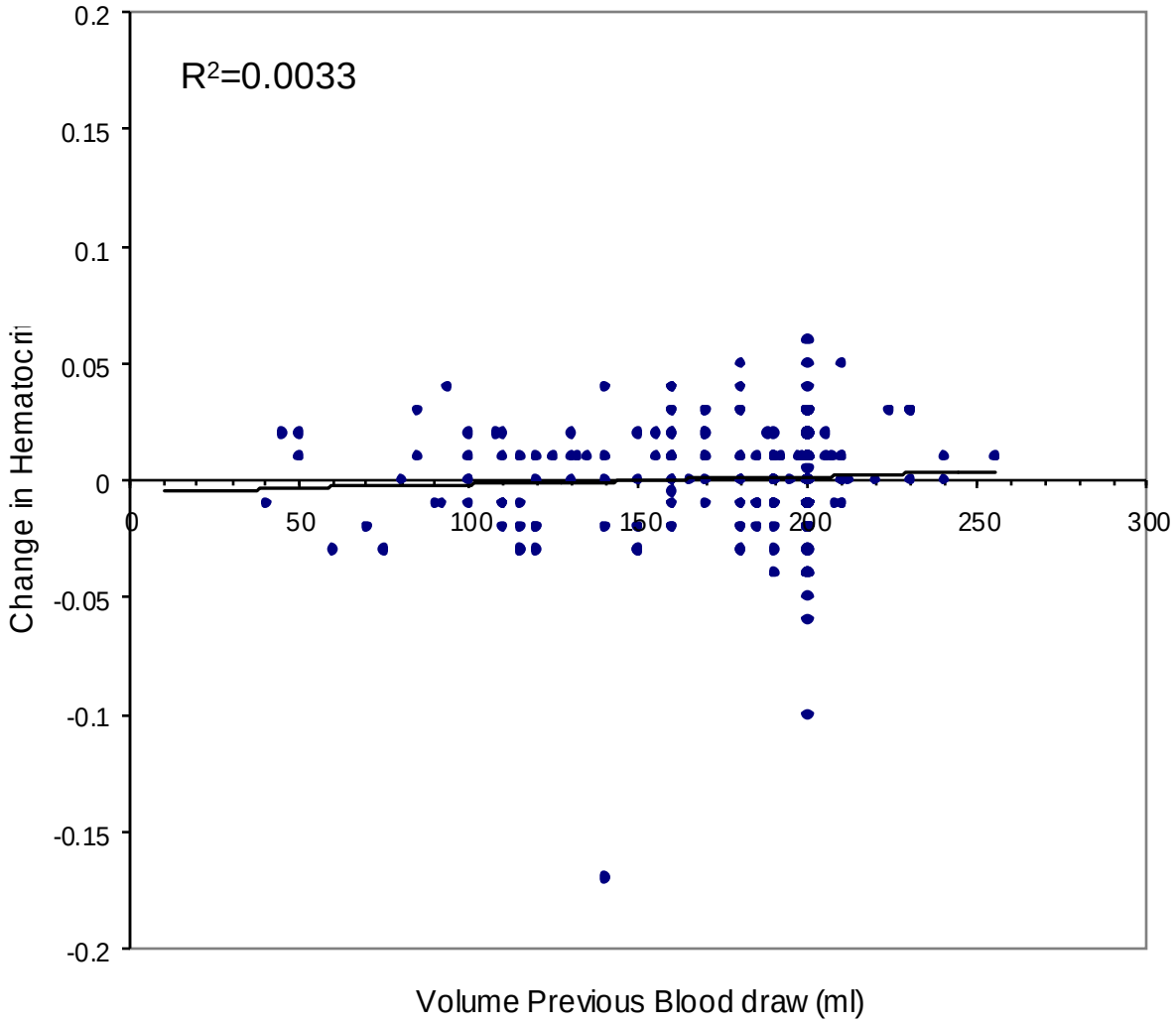
Ensure sample and data integrity via validated SOP

Banked Samples: Feasibility of Large Volume Blood Collection

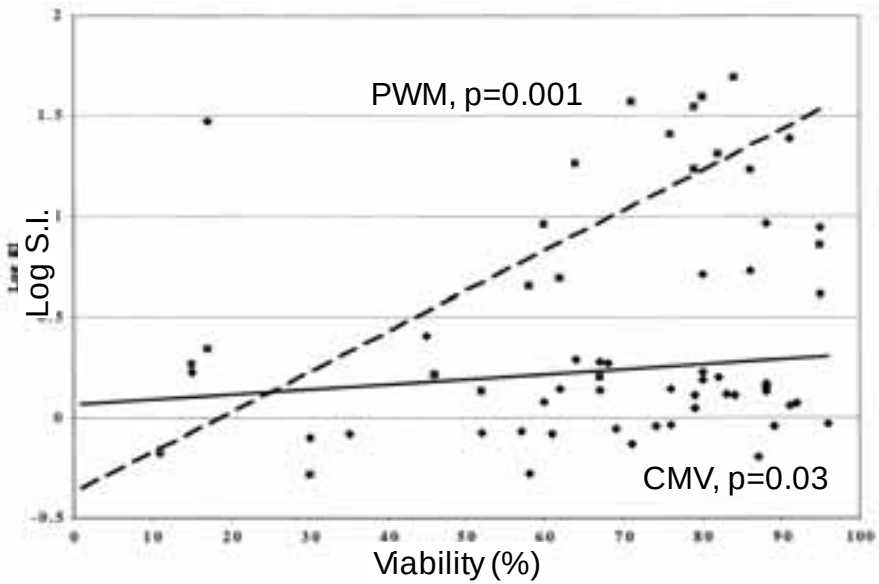
Parameter	Volume Blood Drawn (ml)	Cells/ml whole blood	Hct
Desired value	250	10^6/ml	>30
# of samples	**416**	**376**	**416**
Median	200	$0.8x10^6$	37
Mean	184	$0.9x10^6$	37
Minimum	10	$0.1x10^6$	29
Maximum	255	$3.2x10^6$	54

- Stage III/IV breast cancer patients across 3 trials (115 patients)
- Minimum 30 days from chemotherapy
- Large volume blood (200-250 cc) every 3 months for 1 year
- Majority of patients collected via venipuncture

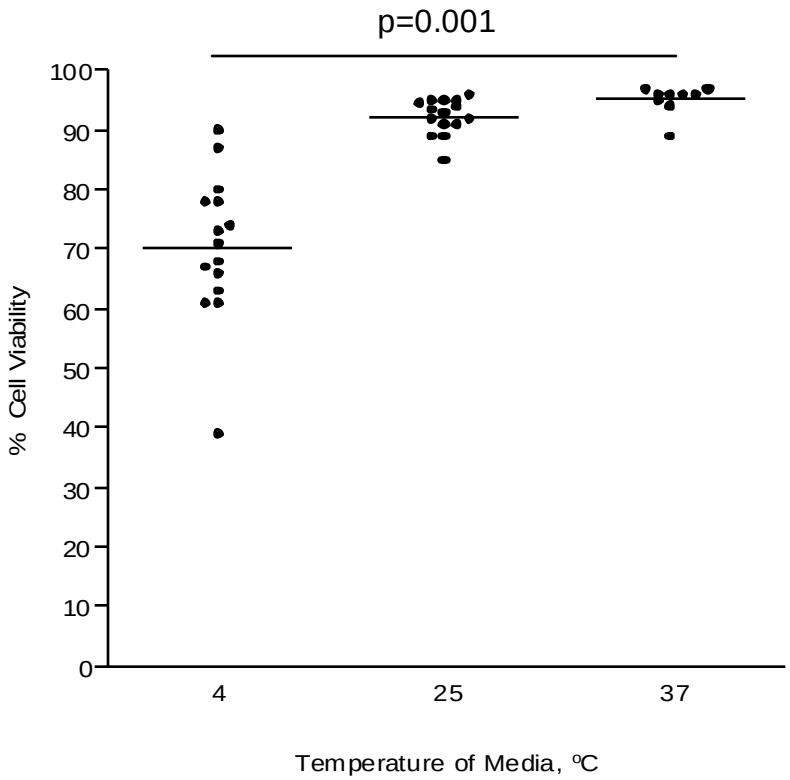
Change in Hematocrit vs. Volume of Previous Blood Draw



Banked Samples: Ensuring Functional Capacity of T Cells



Weinberg et al, Clin Diag Lab Immunol, 2000



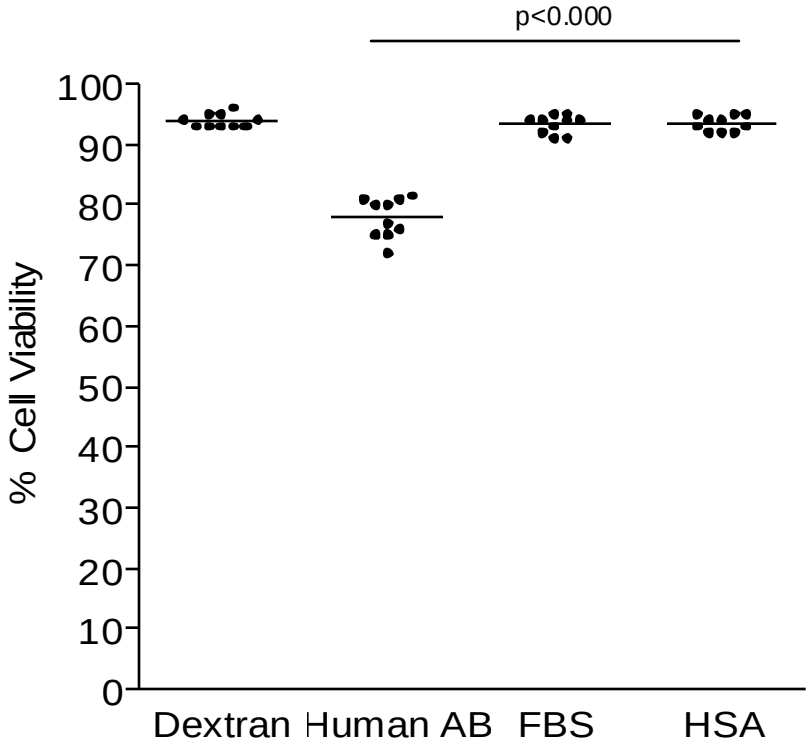
Impact of thawing method

Factors That Did Not Matter

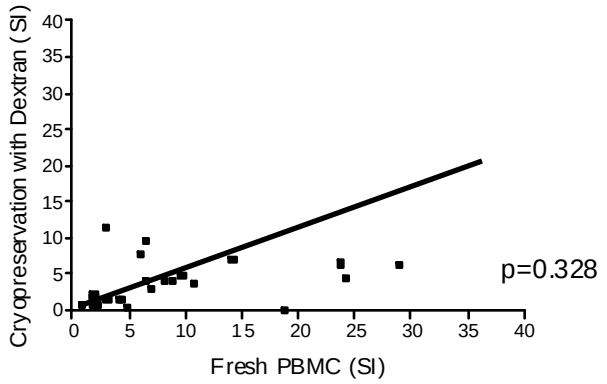
- Shipping on dry ice, 24, 48, 72 hours (p>0.05)
- Large (100cc) vs. small volume (25cc) thaw (p>0.05)
- Speed of spin at wash (p>0.05)
- # cells/vial (p>0.05)

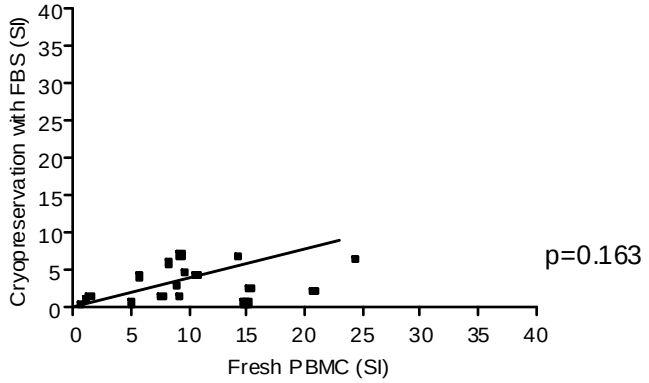
Disis et al, J Immunol Meth, 2005

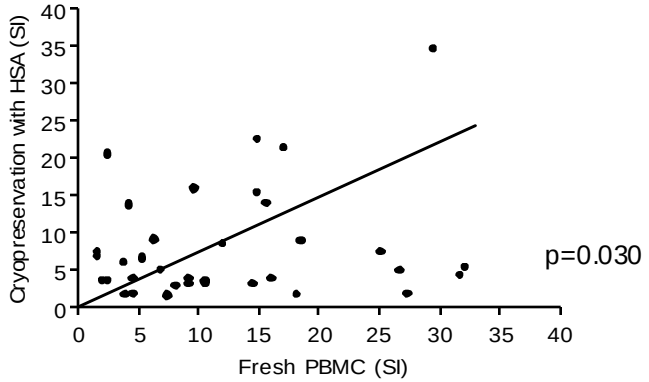
Validation of SOP



Impact of media additives



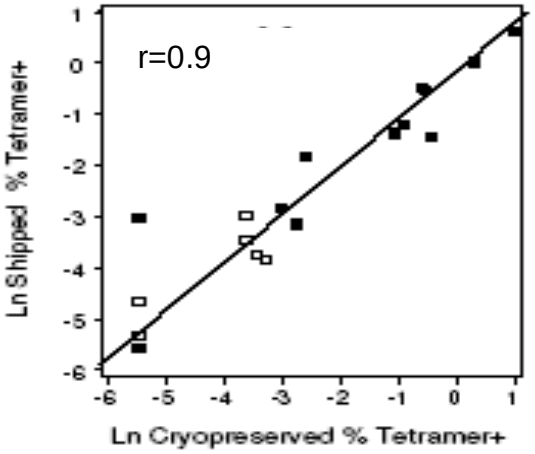




Disis et al, J Immunol Meth, 2005

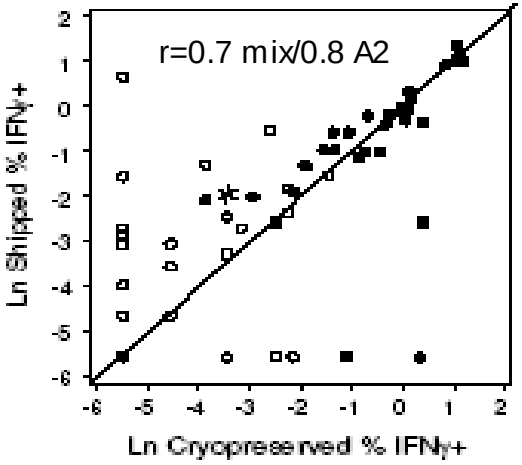
Validation Across Multiple Assays of Same Class

Tetramer



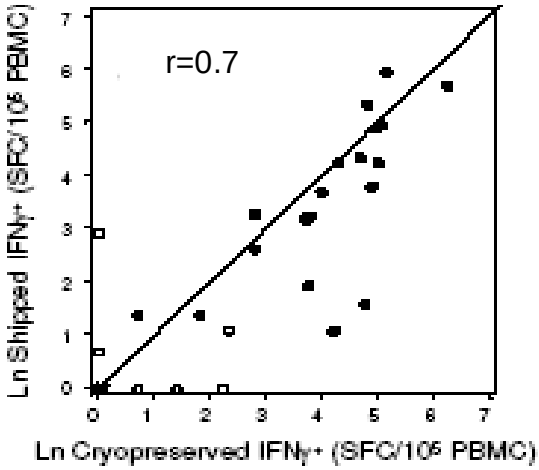
HLA-A2 Peptide

Intracellular Cytokine Staining



HLA-A2 Peptide
Peptide Pool-pp65

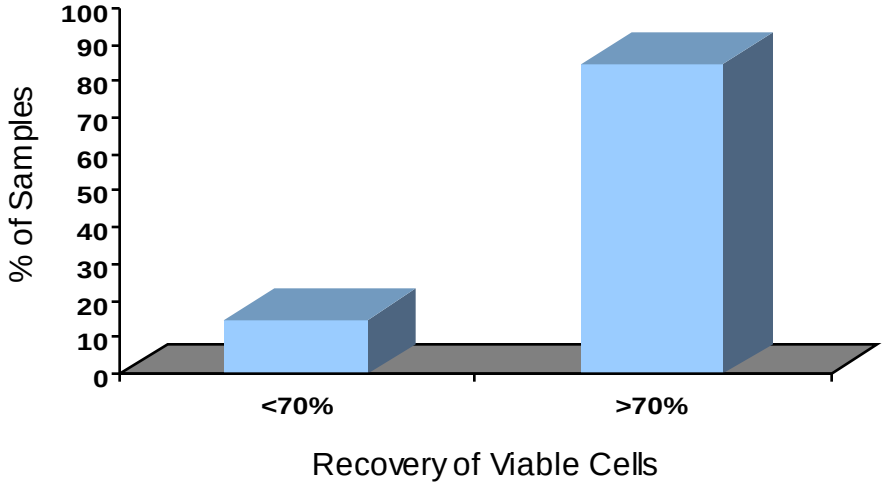
ELISPOT



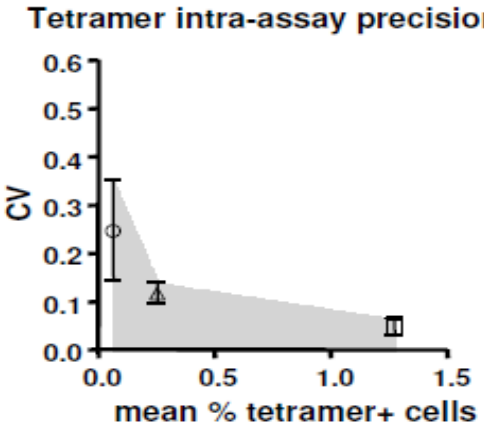
Peptide Pool-pp65

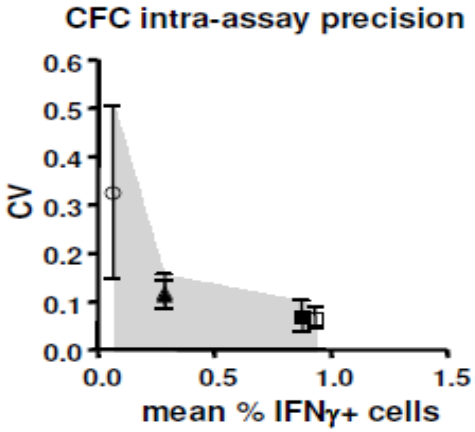
Maecker et al, BMC Immunol, 20005

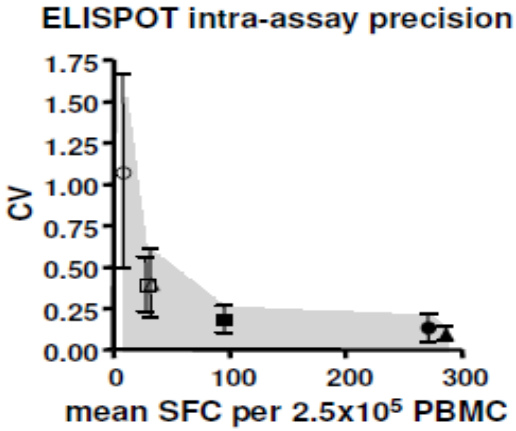
Validation in Stored Samples: Ongoing Performance



Analysis of 80 samples
Age range: 30-2500 days, av. 600
Median recovery: 70%
Mean recovery: 70%
Range: 22-130%







Maecker et al, BMC Immunol, 2008

Open Access Protocols

Protocol for Isolation, Cryopreservation, and Thawing of PBMCs

Description

Cryopreserved PBMCs are a common specimen source for studies of immunological responses to vaccines, immunotherapies, etc. The health and viability of cells recovered post-cryopreservation are of course critical to the success and accuracy of immunological assays performed on them. We have developed this protocol to help standardize PBMC isolation and cryopreservation techniques, specifically for the assessment of thawed cells by cytokine flow cytometry.

Cryopreservation of PBMCs

The following protocol for freezing PBMCs uses a final concentration of 10% dimethylsulfoxide (DMSO) and 11.25% protein (human serum albumin) in cRPMI. Cryoprotectants, such as DMSO, reduce the amount of ice present during freezing and reduce solute concentration, thus reducing ionic stress. However, these compounds can themselves cause osmotic injury since they are hypertonic and can cause damage during their addition or removal.

1. Resuspend PBMCs (from **Isolation** section of **Processing of Fresh PBMCs**, above) at 1 x 10^7 viable lymphocytes/ml in 4°C 12.5% HSA in RPMI medium, in a 50 ml conical polypropylene tube.
2. While *gently* swirling the tube, add dropwise enough 4 C 2X freezing medium to double the volume of the cell suspension.
3. Immediately place the tube on ice. Avoid any further mixing or agitation of the cells. Slowly remove the cell suspension into a pipet and dispense 1 ml per cryovial on ice.
4. Place the cryovials in a pre-cooled Mr. Frosty-style freezing container that has been filled with 70% isopropanol according to the manufacturer's instructions. Place the freezing container at –80°C.

Thawing of PBMCs

If PBMCs are not thawed properly, viability and cell recovery can be compromised; and cells may not perform optimally in functional assays. In general, cells should be thawed quickly but diluted slowly to remove DMSO. Cells with DMSO intercalated into their membranes are very fragile, and must be pelleted and handled gently.

1. Warm cRPMI to 22°-37°C in a 37°C water bath before beginning thawing procedure.
2. Transfer the cryovial from liquid nitrogen to a 37°C water bath. If liquid nitrogen has seeped into the cryovial, loosen the cap slightly to allow the nitrogen to escape during thawing.

Disis et al, J Immunol Meth, 2005
Maecker et al, BMC Immunol, 2005