International
Summer School 2013

Post-Transplant Monitoring in Solid Organ Transplant and HSC Transplant

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Post Transplant Monitoring

For Solid Organ Transplantation –

1. Donor Specific Antibody Identification to help rule in/rule out or predict antibody-mediated Rejection
2. Immune Function testing to adjust immunosuppressive medications or to assess donor specific tolerance
3. Chimerism identification – organ derived graft vs. host dis.

For Allogeneic (!) Hematopoietic Stem Cell Transplantation –

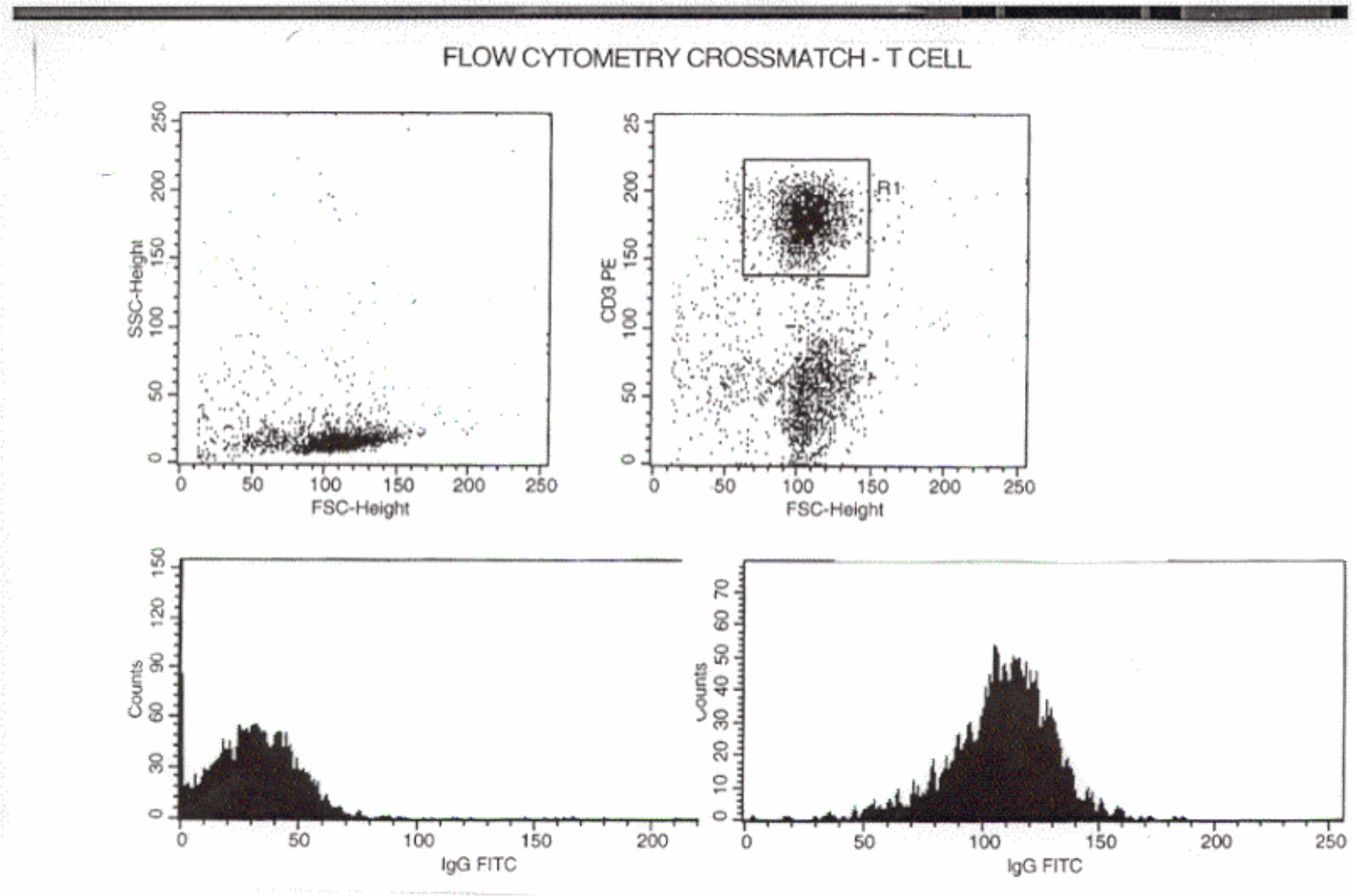
1. Donor Specific Antibody Identification for mismatches (to explain engraftment failure)
2. Chimerism identification to monitor engraftment
3. Identification of Non-HLA polymorphismss affecting GvHD

NEW Type(s) of Organ Transplants – Vascularized Composite Tissue –

Will likely require Different Post – Transplant Monitoring Protocols

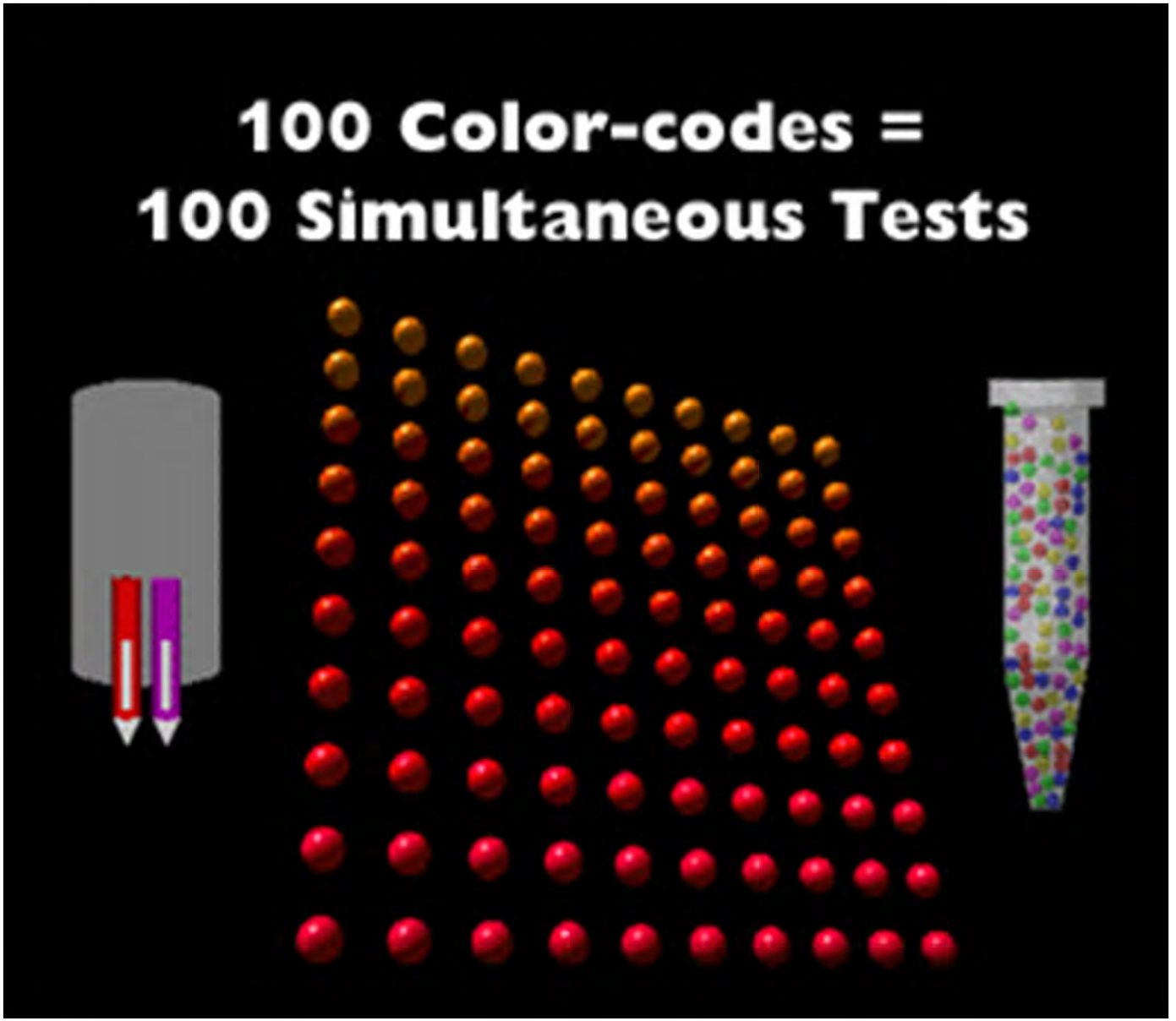
A few years ago, a team of military and civilian surgeons transplanted a new hand onto a retired Air Force master sergeant who lost hers nine years ago when a package bomb exploded at Lackland AFB, TX. Starting in 7/2014 such grafts will be regulated by UNOS/OPTN

Flow Cytometry Crossmatching – could be used to identify DSA post-transplant but would require surrogate cells*



*Currently, labs rarely store viable donor cells – high cost for liquid nitrogen

Instead, Microarray Assays with Donor Specific Beads or Panels are Used



Post-Organ Transplant Monitoring

Donor Specific Antibody (DSA) Identification to help rule in or rule out (or predict) Antibody-mediated Rejection (AMR) and to monitor the effectiveness of treatment for AMR

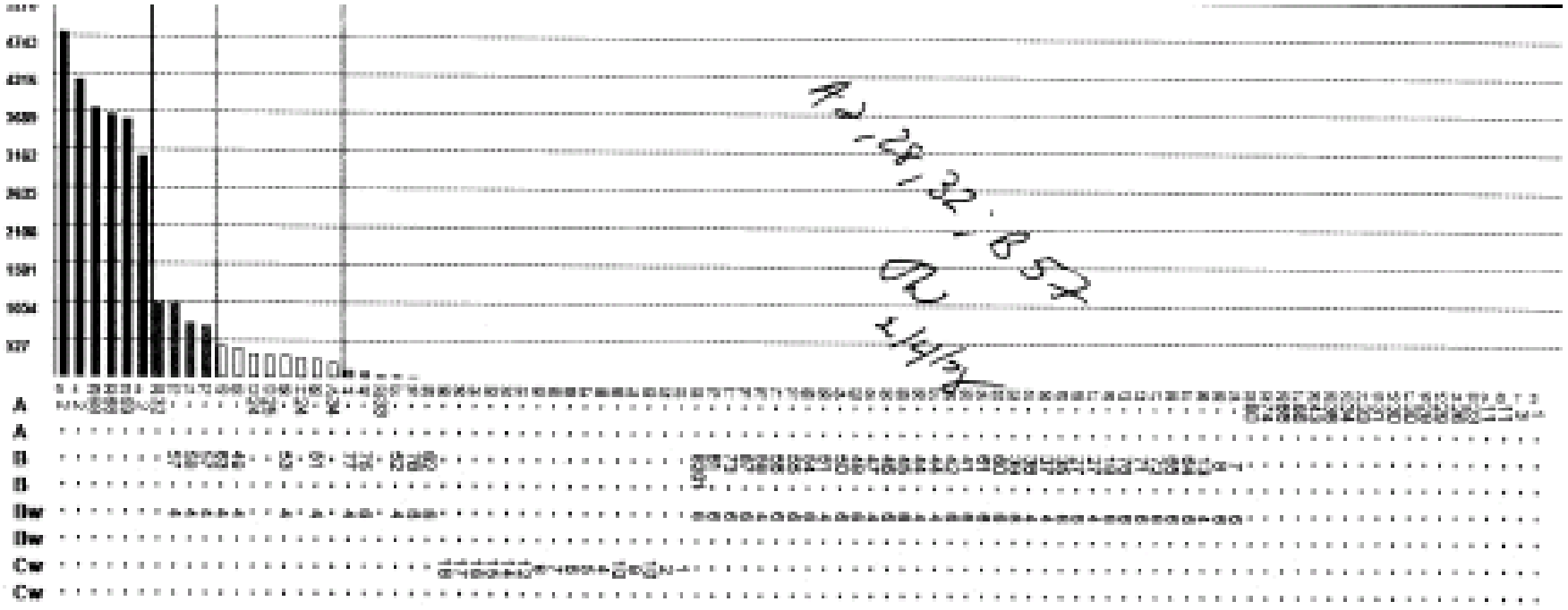
- Post-transplant Crossmatch Tests usually not possible (viable donor cells required – could use surrogate cells but difficult
- Identification of DSA is highly correlated with C4d Staining
- Correlations are with HLA-A,B and/or DR antibodies
- The role of HLA-C, DQ and/or DP antibodies is controversial
- The absence of DSA could actually be due to absorption in the graft

Immune Function Tests to consider adjustment of immunosuppressive meds or assess donor tolerance

- Blood drug levels don't accurately predict drug effects
- Lymphocyte counts don't predict lymphocyte function
- Some IS withdrawal protocols require DS tolerance

Chimerism Tests can Help Diagnosis Organ Derived GvHD

Example of Class I Microarray Antibody Identification Results



Immune Function and Other Traditional Cellular Assays in Transplantation and Immunogenetics

- **Mixed Leukocyte Culture (MLC)/ Primed Lymphocyte Typing-**
  - Assessment of HLA Class II compatibility measured as uptake of ^{3}H thymidine after 5 days (MLC) or 2 days (primed) co-incubation - CAN ASSESS THE DEVELOPMENT OF DONOR SPECIFIC TOLERANCE
- **Cytotoxic Lymphocytes (CTL)**

Assessment of immune reactivity as cytotoxic responses of stimulated responder cells against labeled target cells - measured as release of ^{52}Chromium.

- **Donor Specific Precursor Frequency**

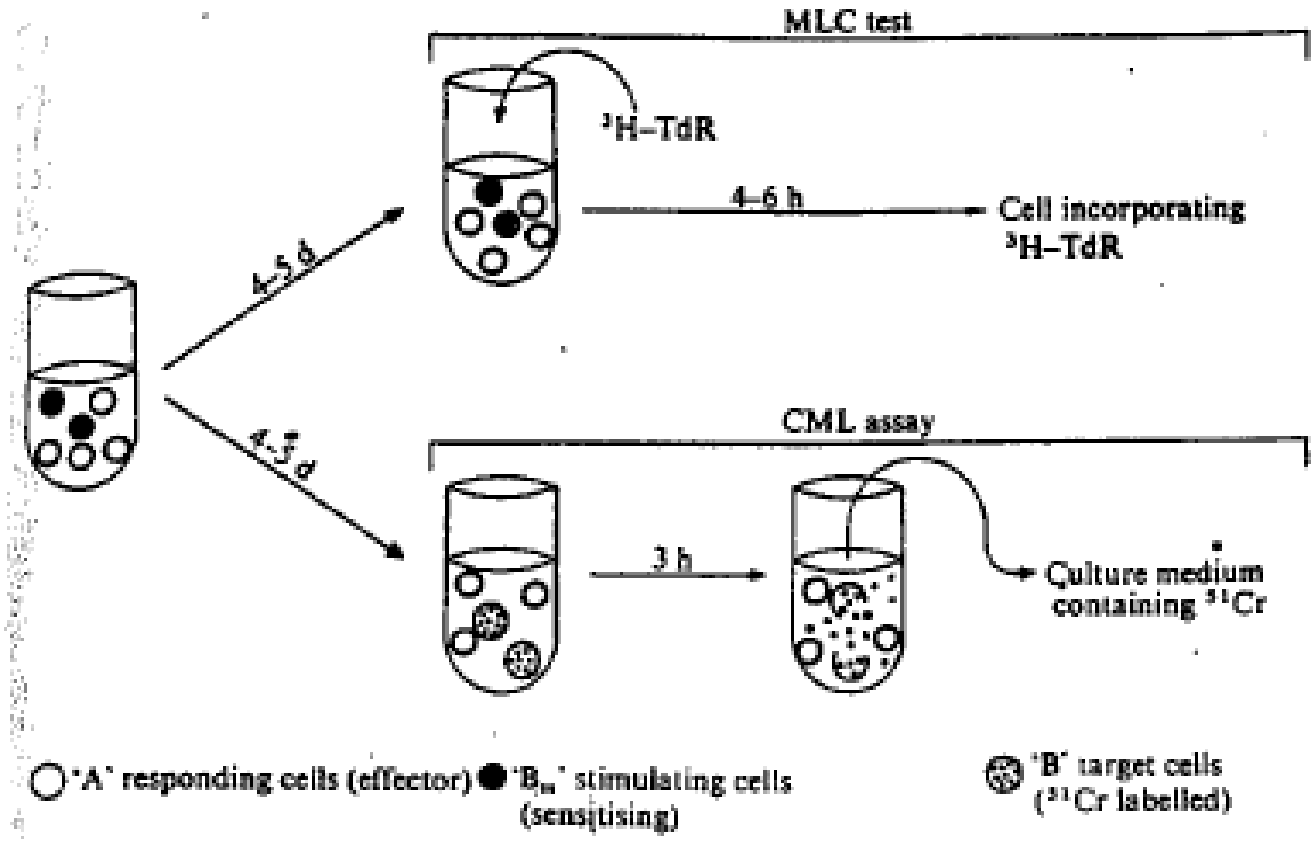
Limiting dilution assays for MLC, PLT or CT

- **General Immune Function - Conventional**

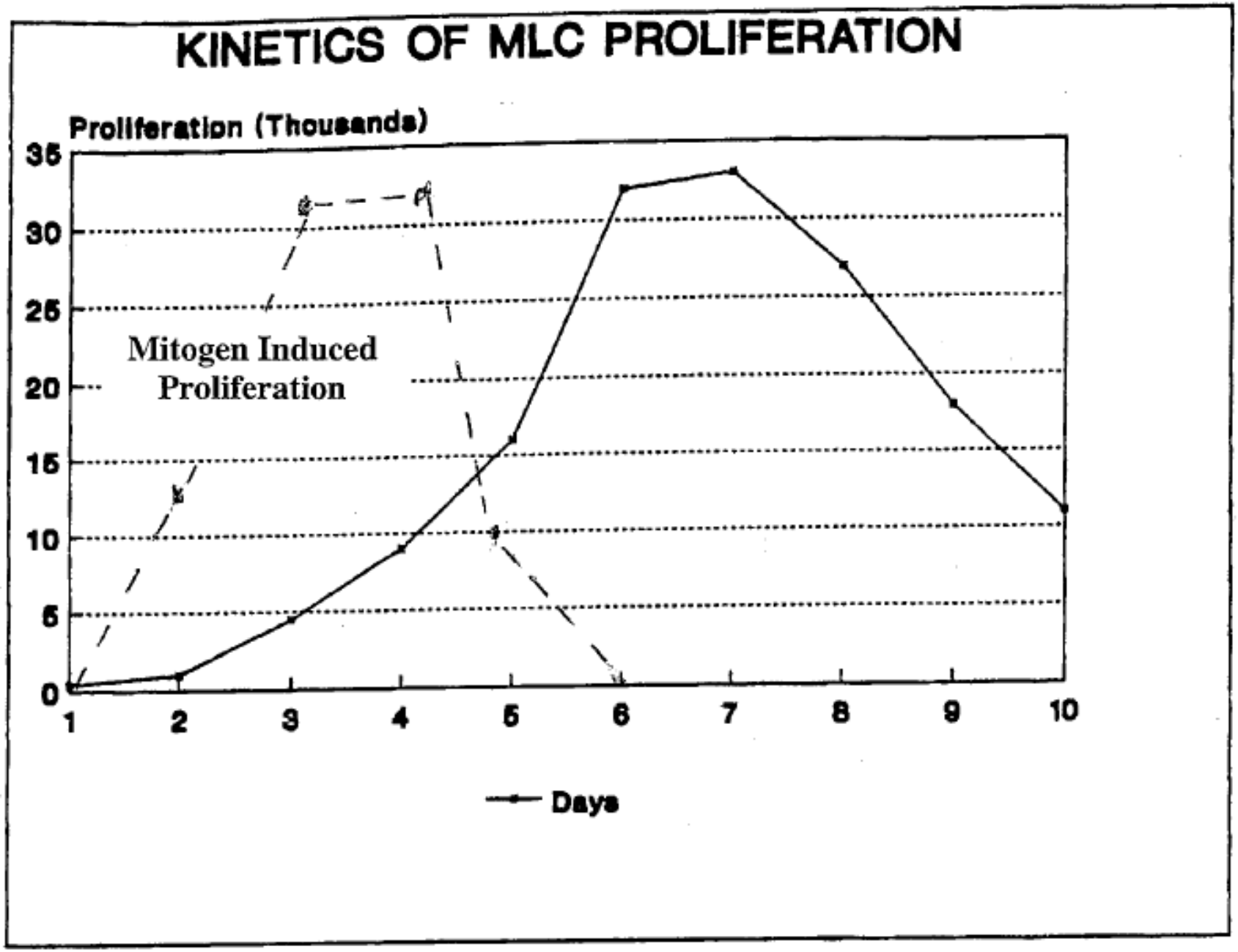
The capacity of subject lymphocytes to proliferate in response to individual mitogens, antigens, and/or pooled allogeneic cells (^{3}H thymidine–3 days)

- **General Immune Function "Cylex®"**
  - Response to PHA measured in 16 hours
  - Measured as ATP formation (early response measure).

Cell Culture for MLC (or CML) Tests –



F. H. Bach, M. L. Bach, and P. M. Sondel



Time course kinetics of a primary MLC response. Incorporation of tritiated thymidine by proliferating cells usually reaches a maximum at day 6-7.

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IMMUNE RESPONSE TEST REPORT*

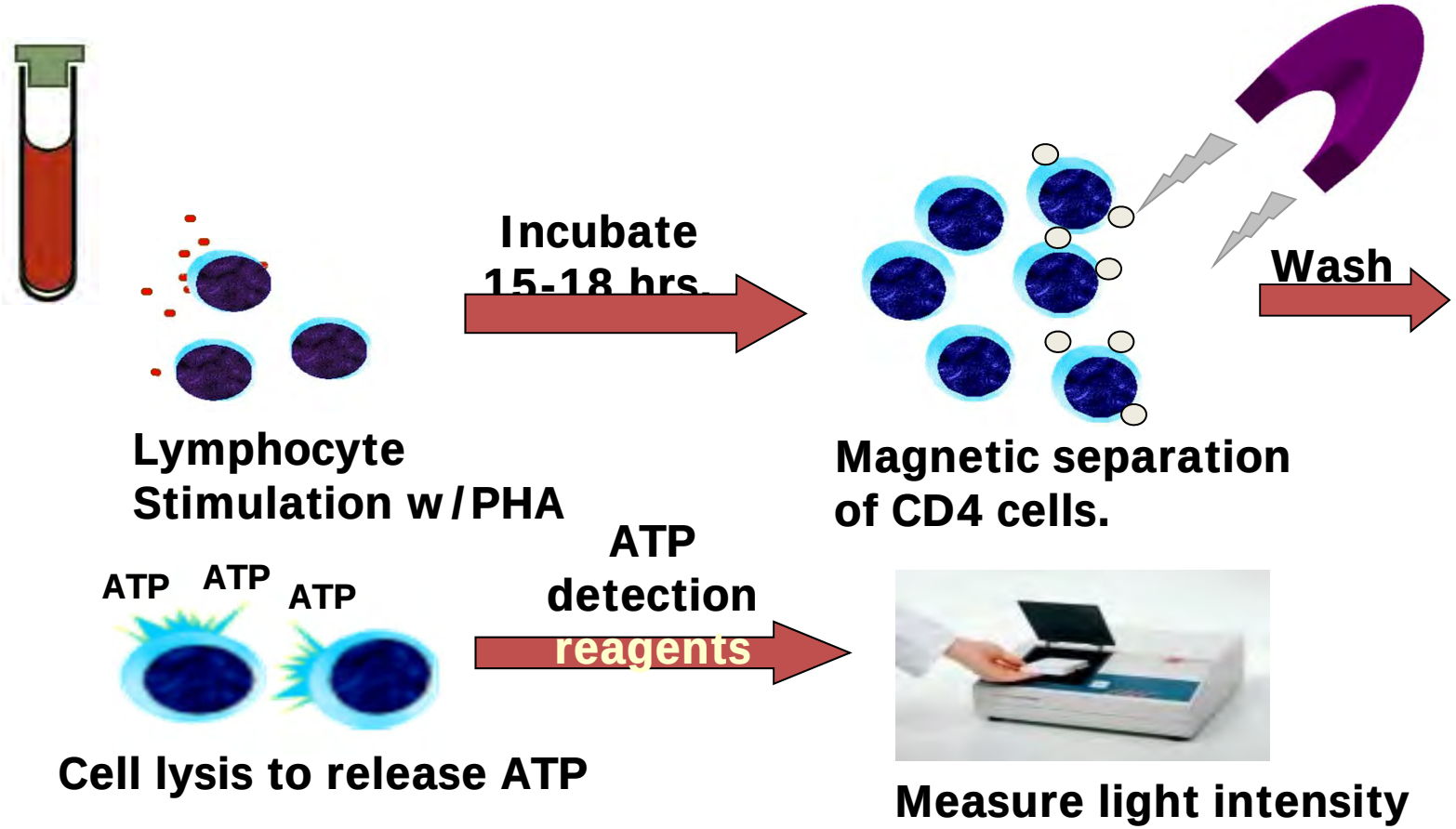
Test Date(s): 1/10/07-1/16/07
Technologist (s): KMR/BCH/LLS
Sample Date: 1/9/07
Specimen#: W00827708

Patient: MC
Report Date: 1/17/07
Physician: Dr. Atkins
Hospital: SWTMH

Net CPM**

	Patient	Control #1	Control #2	Control #3	Mean of Controls	RPI***
PHA						
20 ug/ml	245,246	217,170	247,665	186,283	217,039	**1.13**
10 ug/ml	220,431	210,875	230,728	153,740	198,447	**1.11**
5 ug/ml	161,047	177,240	206,555	149,203	177,666	**0.91**
2.5 ug/ml	56,856	120,584	112,211	101,972	111,589	**0.51**
CON A						
50 ug/ml	125,153	93,687	152,108	158,631	134,809	**0.93**
10 ug/ml	67,839	60,249	110,365	135,234	101,949	**0.67**
5 ug/ml	53,882	50,826	102,900	130,393	94,706	**0.57**
2.5 ug/ml	32,588	30,845	77,929	110,125	72,966	**0.45**
PWM						
1:100	45,221	33,889	36,431	105,003	58,441	**0.77**
1:500	73,146	37,831	59,405	129,294	75,510	**0.97**
1:1500	79,185	48,742	74,714	131,792	85,083	**0.93**
1:4500	98,152	57,097	88,228	154,618	99,981	**0.98**
Pooled Allogeneic Cells Test 1	134,295	94,234	138,916	163,398	136,223	**0.99**
Pooled Allogeneic Cells Test 2	122,185	116,004	151,037	165,657	93,887	**1.30**

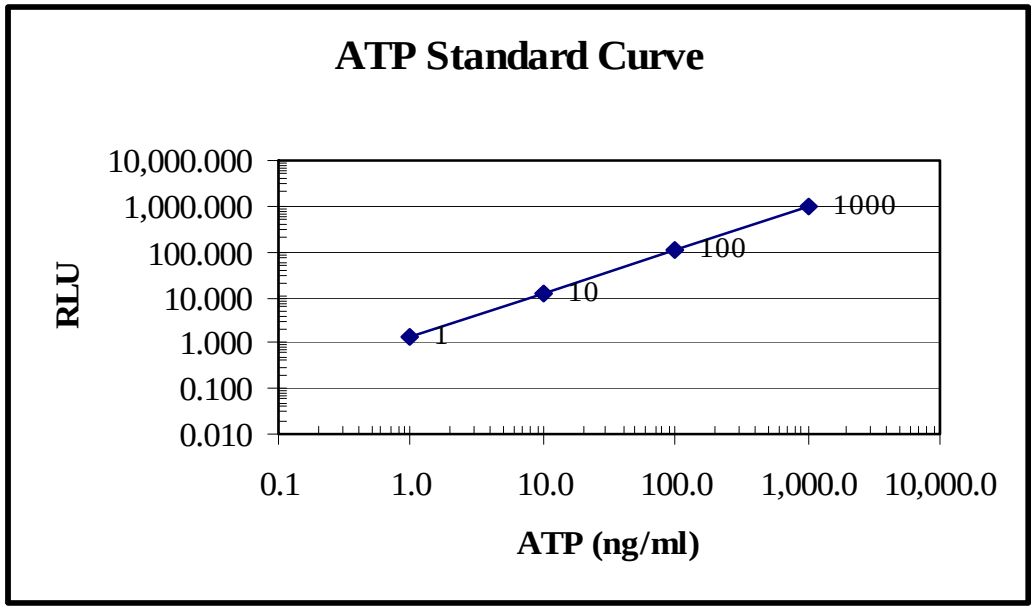
Cylex® Test for “General” Immune Function



J. Britz et. al., “In Vitro CMI: Rapid Assay for Measuring Cell-Mediated Immunity”, CRC Press, 2002

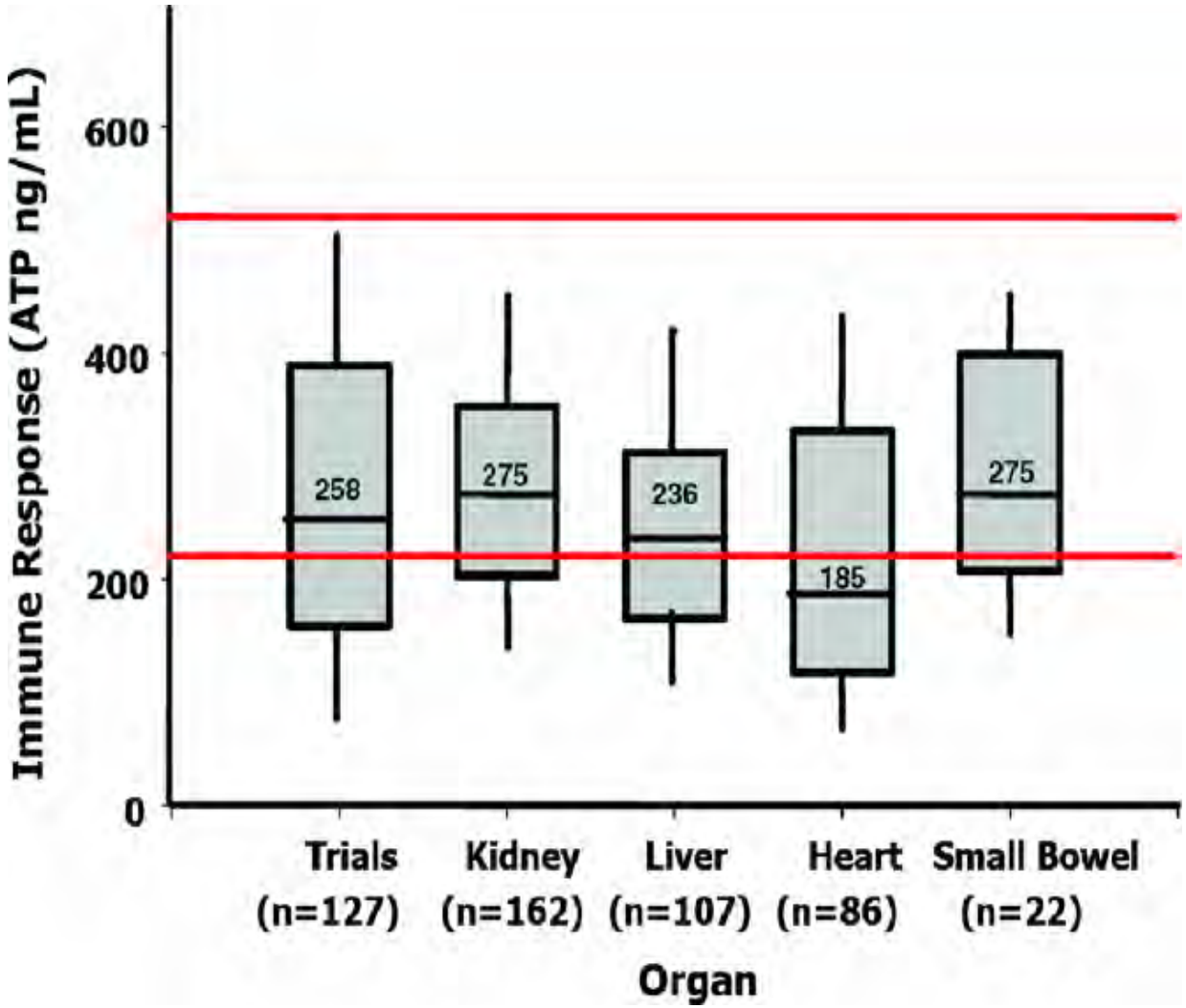
ImmuKnow™

An ATP Standard Curve is Used to Calculate Results for Patient Tests



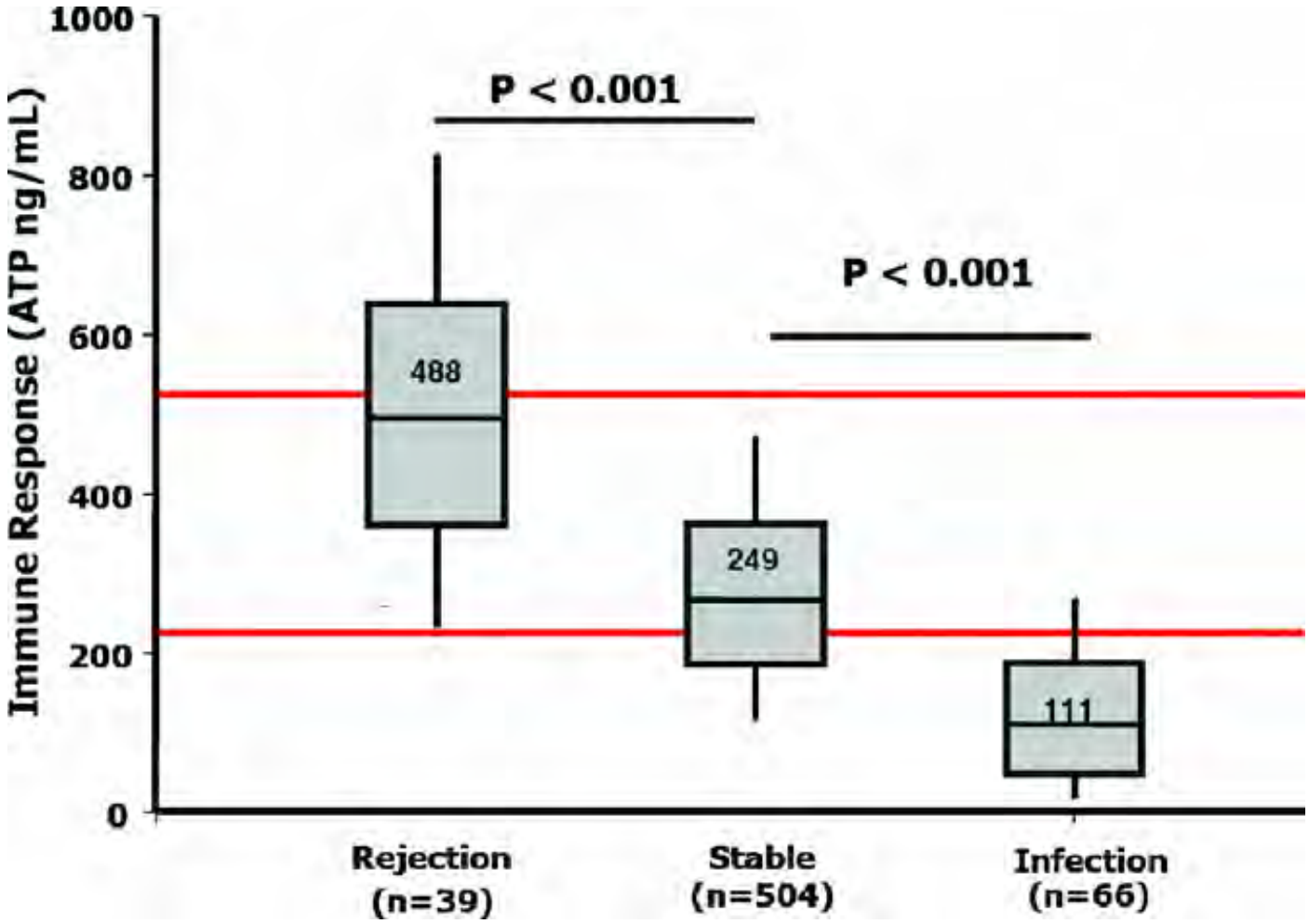
The intensity of the light emitted is directly proportional to the amount of ATP in the sample. Comparison to ATP standards allows quantifiable measurement of ATP produced by activated cells.

Cylex Responses in “Stable” Transplant Recipients



From Kowalski et al, Transplantation, 2006

Cylex Responses of Solid Organ Transplant Recipients
During Periods of Confirmed Rejection or Documented Infection

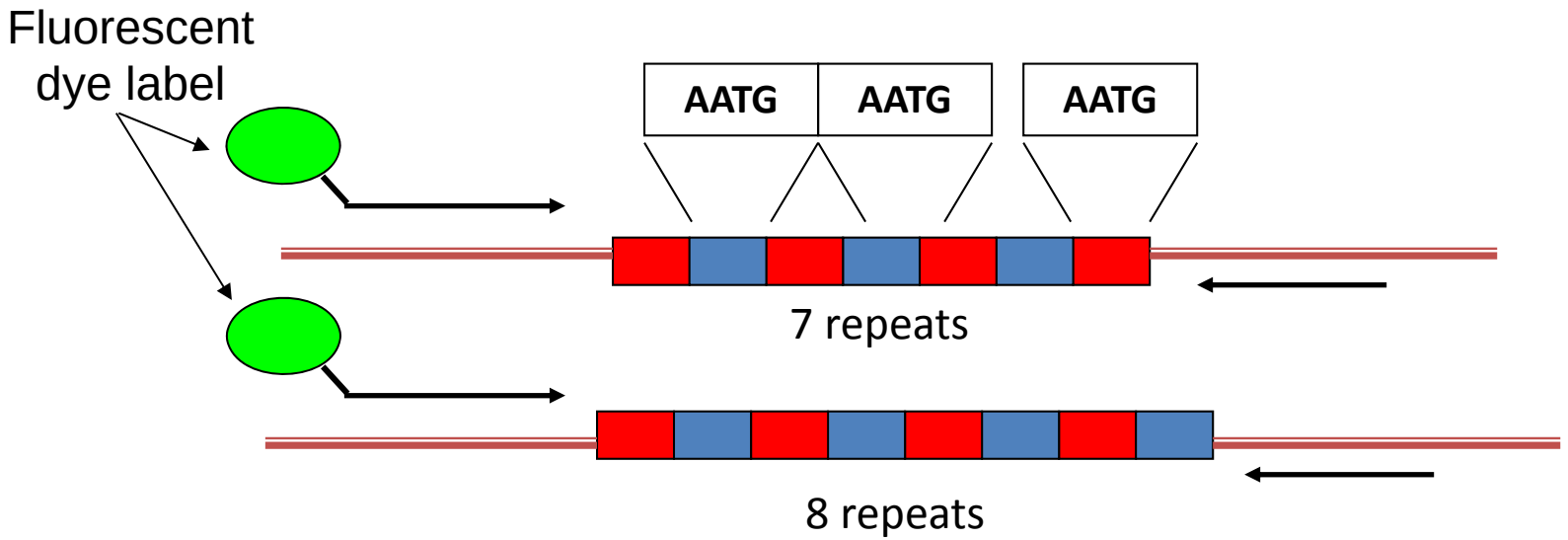


From Kowalski et al, Transplantation, 2006

THE USE OF POLYMORPHIC PCR-STR (and -VNTR) SYSTEMS TO TEST FOR CHIMERISM AFTER ORGAN TRANSPLANTATION

- Some Tolerance Induction Protocols infuse organ donor bone marrow or lympycytes to try to create tolerance/donor-specific suppressor cells
- For those protocols, systems are needed to monitor chimerism
- Organ Donor-Derived Lymphocytes can cause Graft vs. Host Disease, especially after Liver or Small Bowel Transplantation
  - Grafts contain large numbers of donor lymphocytes
  - Immunosuppression can prevent rejection of donor lyphocytes

PCR Reaction for (STR) Short Tandem Repeats



the repeat region is variable between samples while the flanking regions where PCR primers bind are constant

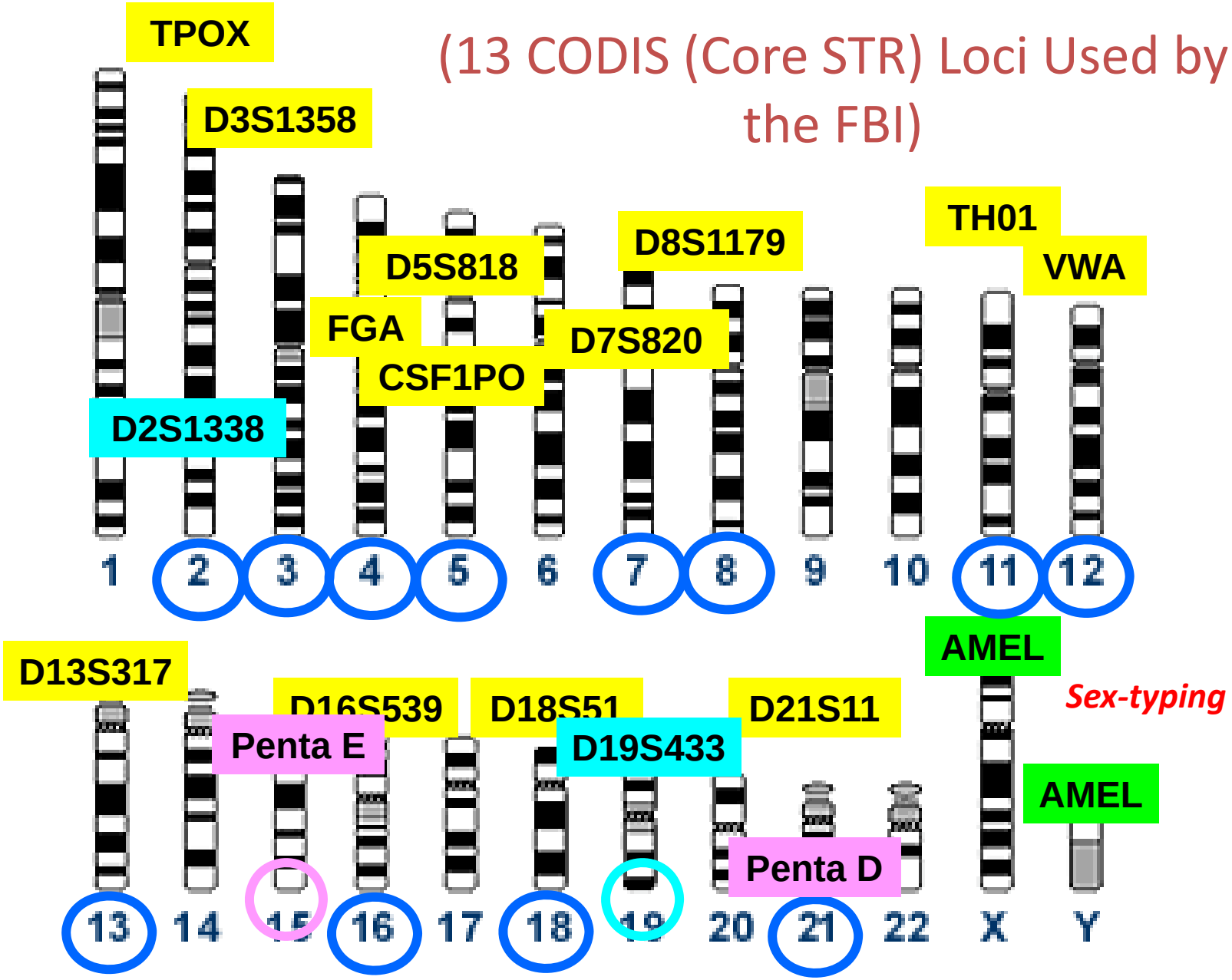
Homozygote = both alleles are the same length

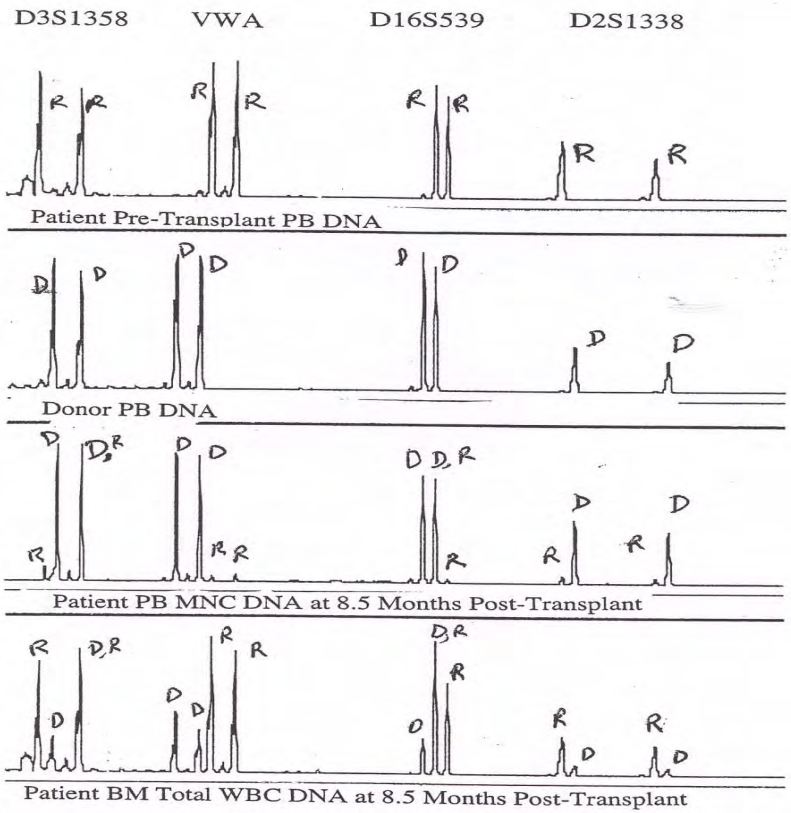
Heterozygote = alleles differ and can be resolved from one another

In multiplex systems, different primers can use different dyes, but the same dyes can be used when products are different sizes.

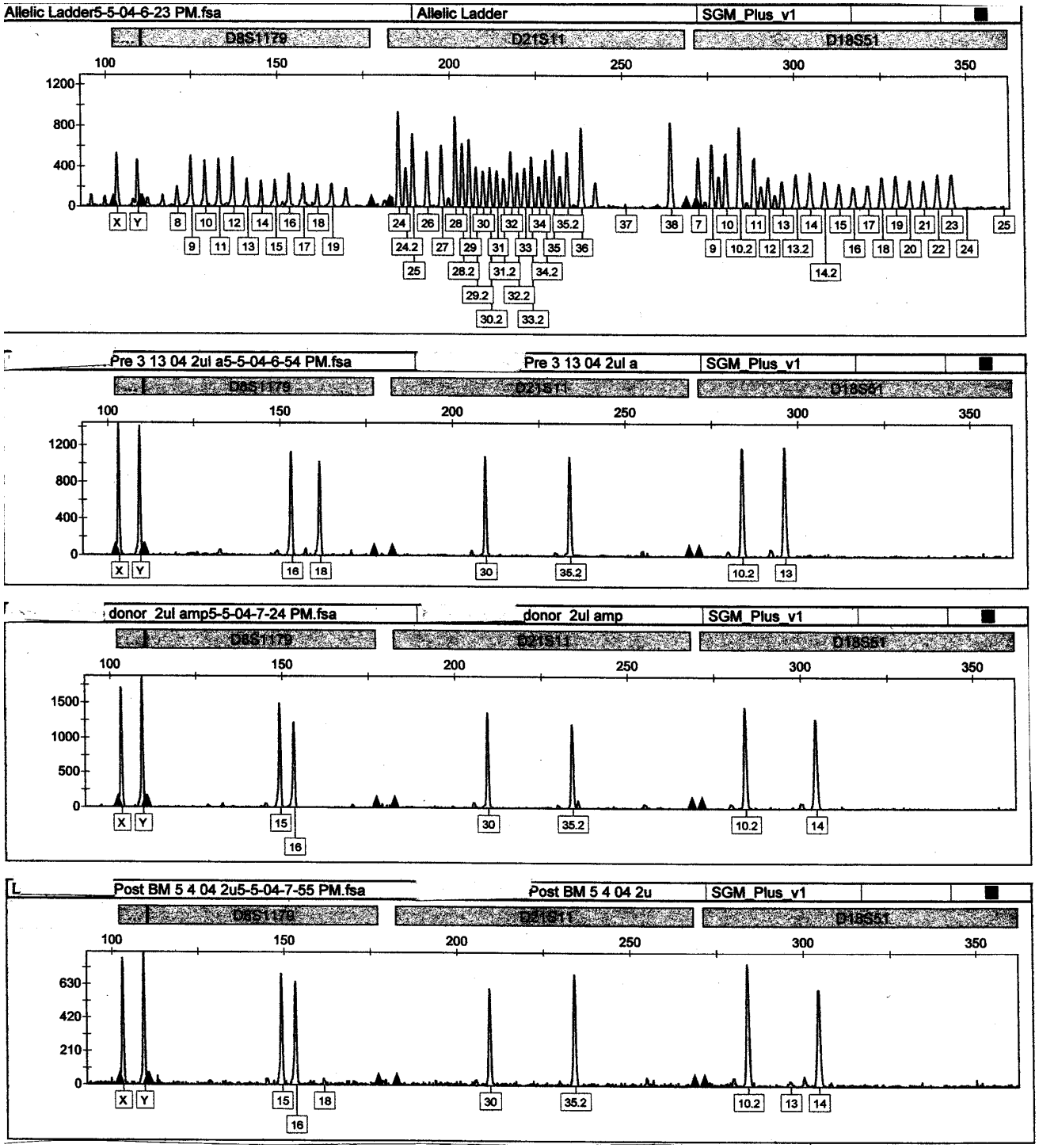
Commonly used STR Marker Locations

(13 CODIS (Core STR) Loci Used by the FBI)





Pollack MS, et al., Severe, Late-onset Graft-Versus-Host Disease in a Liver Transplant Recipient Documented by Chimerism Analysis. Human Immunology. 2005



Example of typical STR multiplex analysis results: Note somewhat smaller areas of larger bands in most systems

- Row 1 = xy + known alleles for 3 different STR systems
- Row 2/3= recipient/ donor pre-transplant
- Row 4 = post-transplant sample – 2 informative systems without interfering stutter bands. (The last system would not have been usable if the 2nd recipient band had been 1 allele larger - interfering stutter band)

HLA haplotypes for a case report family: Monitoring Antibody mediated rejection to evaluate cause and treatment for HPC transplant*

Patient	a A*32, B*35, Cw*04, DRB1*11, DRB3
	d/c A*29/, B*07, Cw*07, DRB1*01
Father:	a A*32, B*35, Cw*04, DRB1*11, DRB3
	b A*24, B*38, Cw*NT, DRB1*14, DRB3
Mother:	c A*68, B*07, Cw*07, DRB1*01
	d A*29, B*45, Cw*NT, DRB1*12, DRB3
Brother#1: (donor)	a A*32, B*35, Cw*04, DRB1*11, DRB3
	c A***68**, B*07, Cw*07, DRB1*01
Brother#2:	b A*24, B*38, Cw*NT, DRB1*14, DRB3
	c A*68, B*07, Cw*07, DRB1*01
Sister:	a A*32, B*35, Cw*04, DRB1*11, DRB3
	d A*29, B*45, Cw*NT, DRB1*12, DRB3

***Pollack, MS and Ririe D. Clinical significance of recipient antibodies to stem cell donor mismatched class I HLA antigens. Human Immunology 2004**