In the name of God

High Resolution HLA typing Methods, Which one is more needed?

H.Farajifard, Ph.D.

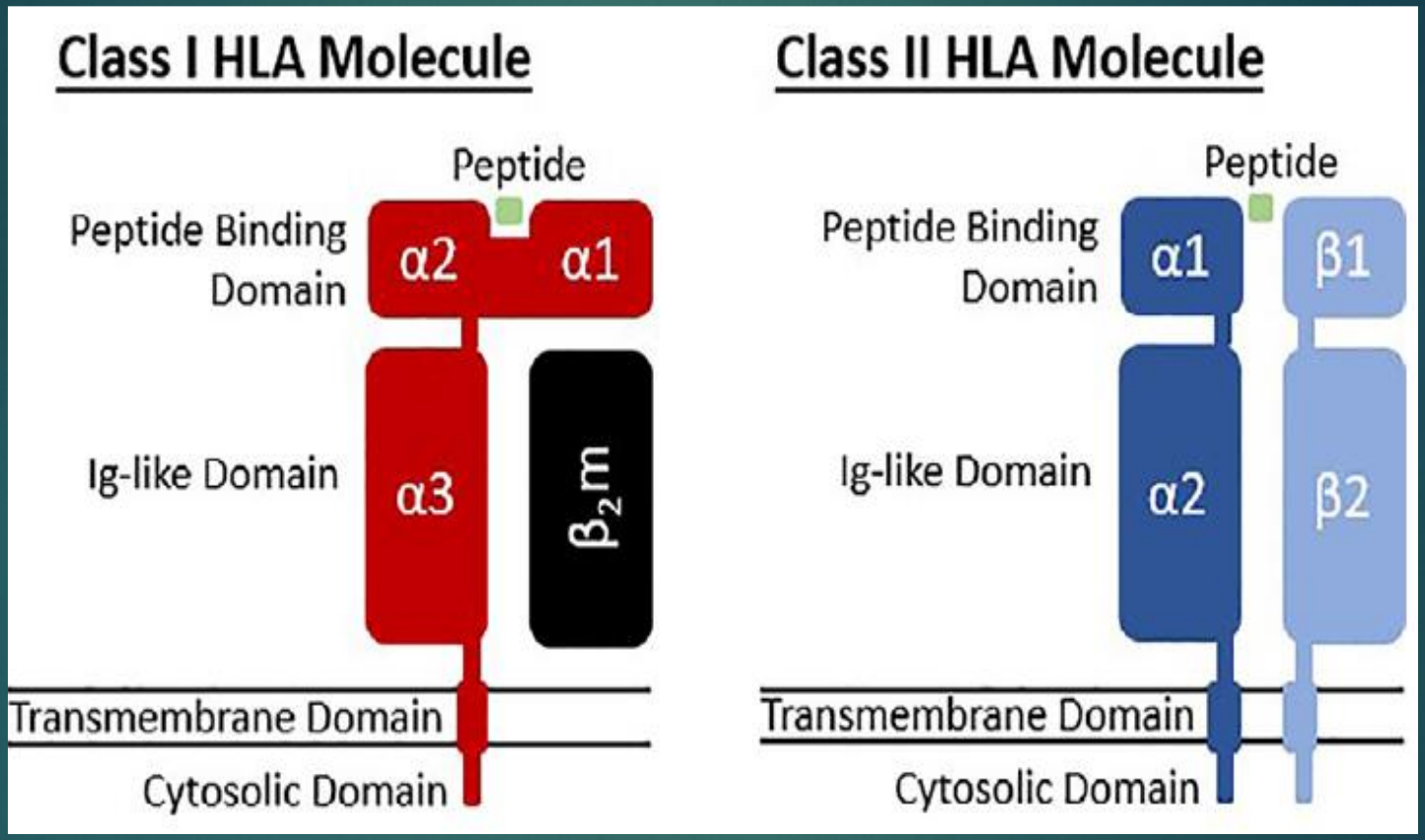
Assistant Professor of Medical Immunology,

Hematopoietic Stem Cell Transplantation Ward,

Pediatric Cell & Gene Therapy Research Center,

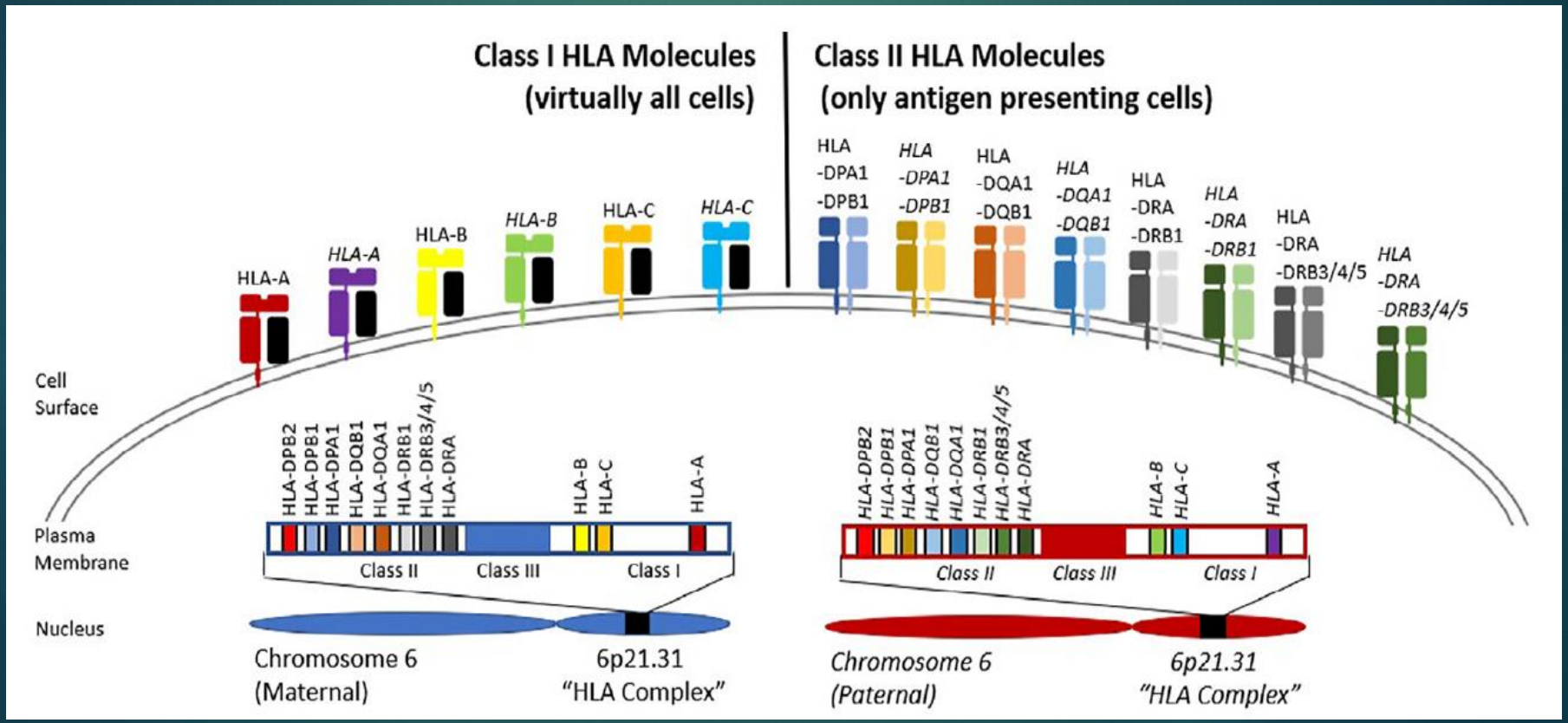
Tehran University of Medical Sciences (TUMS)

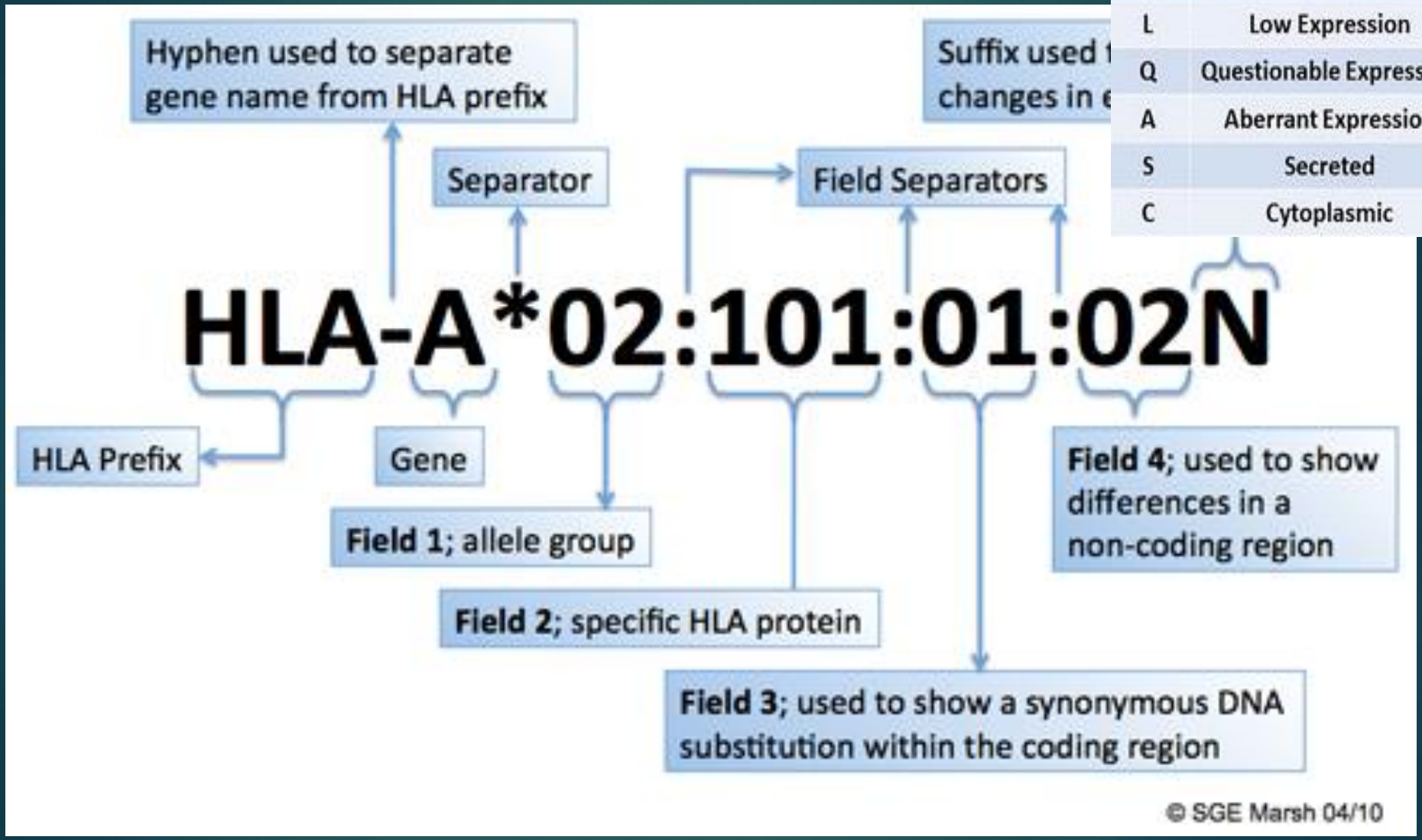
Major Histocompatibility Complex



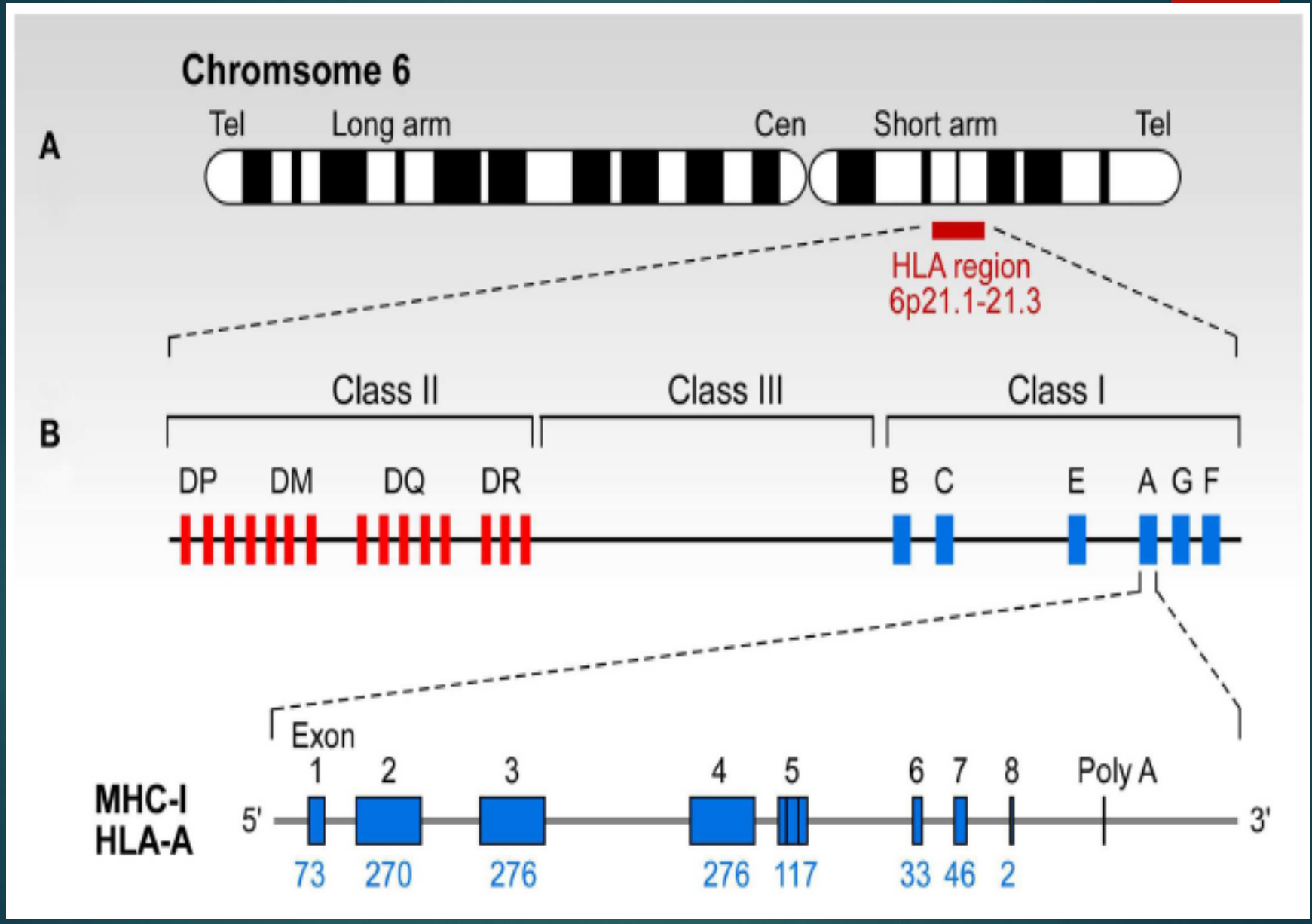
HLA or MHC

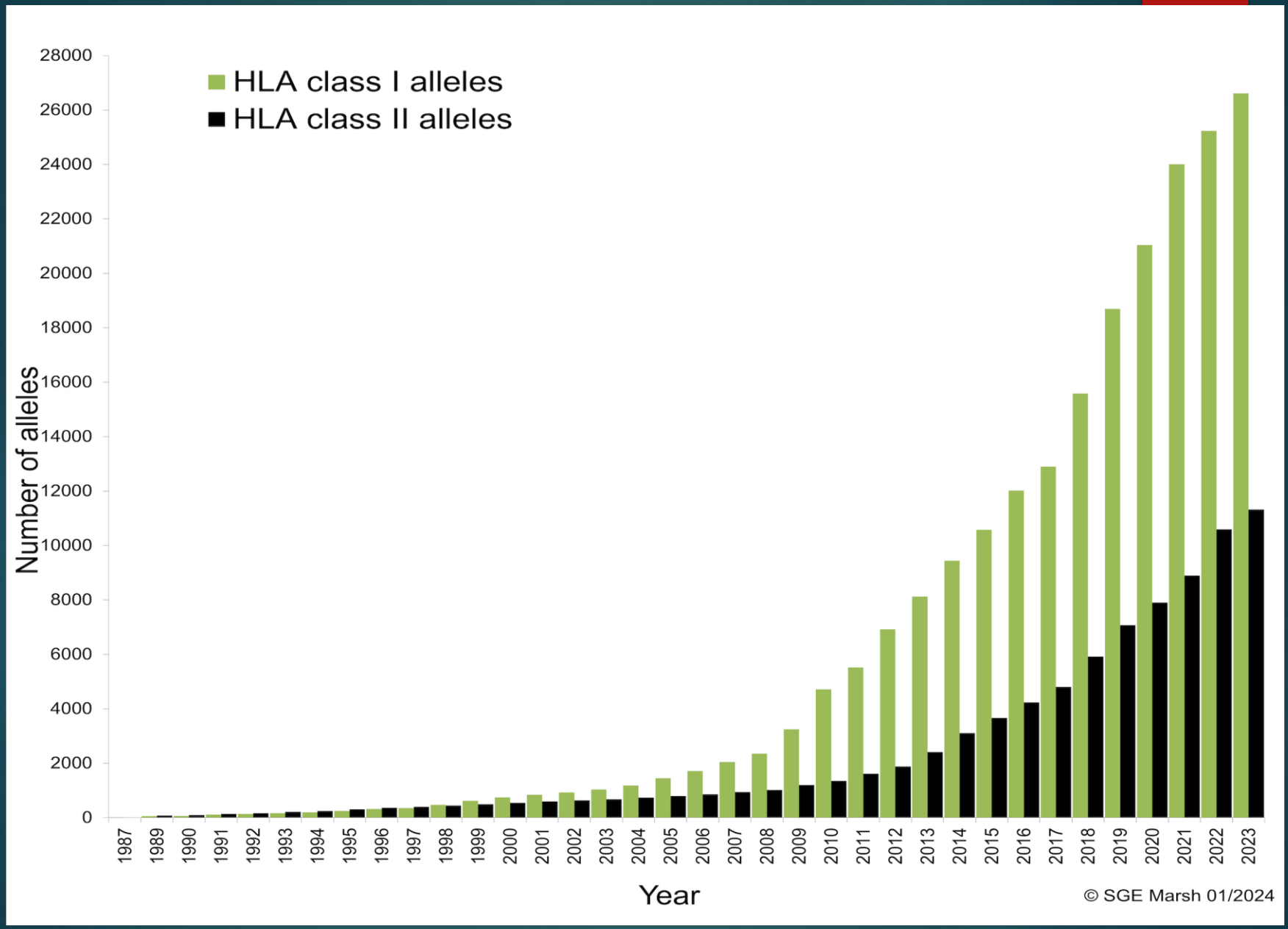
- CLASS I:
- A, B, C
- CLASS II:
- DR, DP, DQ





Suffix	Meaning
N	Null
L	Low Expression
Q	Questionable Expression
A	Aberrant Expression
S	Secreted
C	Cytoplasmic


Chromsome 6
A
Tel
Long arm
Cen
Short arm
Tel
HLA region
6p21.1-21.3
B
Class II
Class III
Class I
DP
DM
DQ
DR
B
C
E
A
G
F
Exon
1
2
3
4
5
6
7
8
Poly A
MHC-I
HLA-A
5'
3'
73
270
276
276
117
33
46
2


HLA class I alleles
HLA class II alleles
Number of alleles
28000
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
1987
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
2023
Year
© SGE Marsh 01/2024

Number of variant alleles at class I & II loci

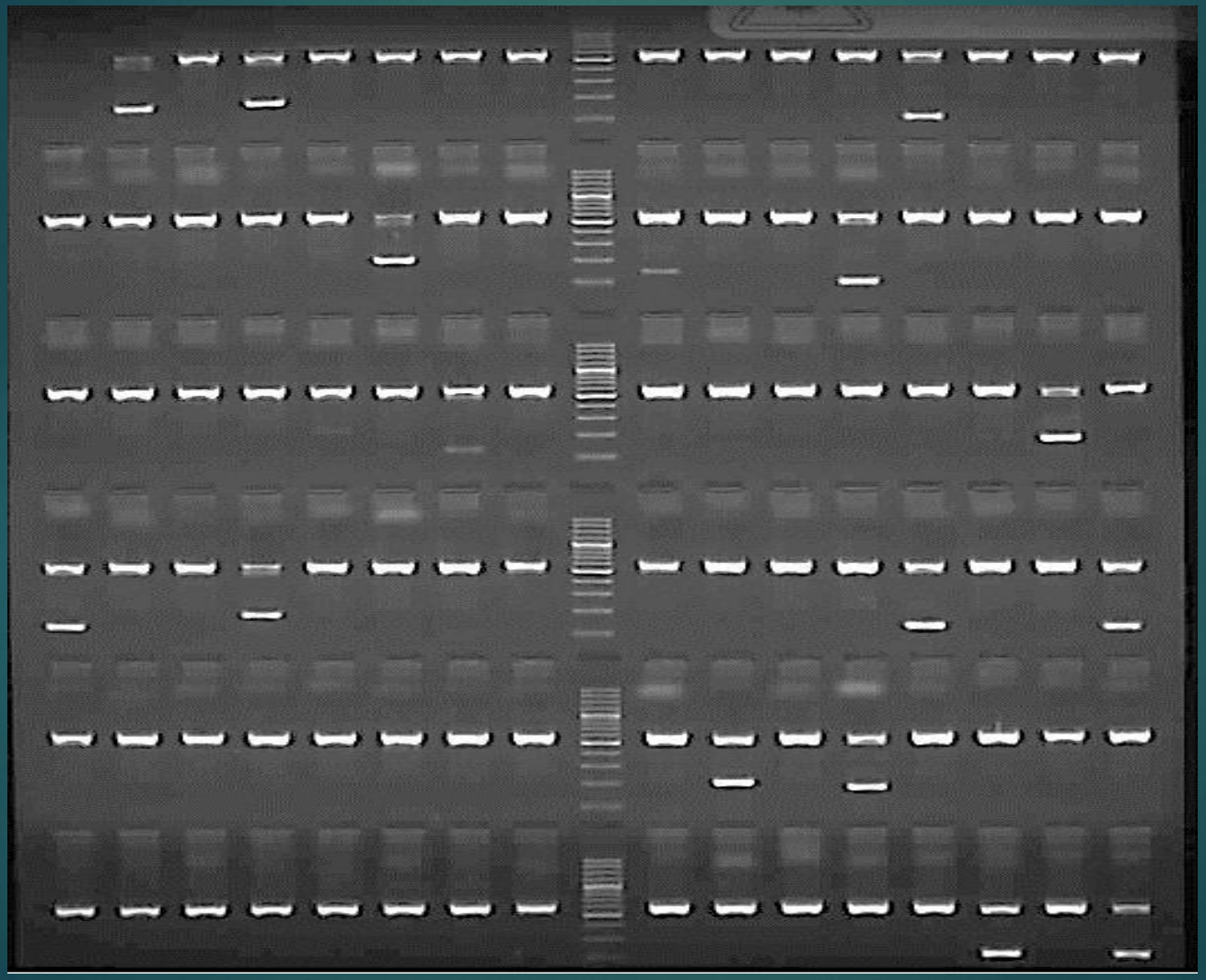
Numbers of HLA Alleles	
HLA class I alleles	26610
HLA class II alleles	11398
HLA alleles	38008
Other non-HLA alleles	901
Number of confidential alleles	0

High Resolution HLA Typing Methods

- Primer base HLA Typing:
- SSP & SSOP

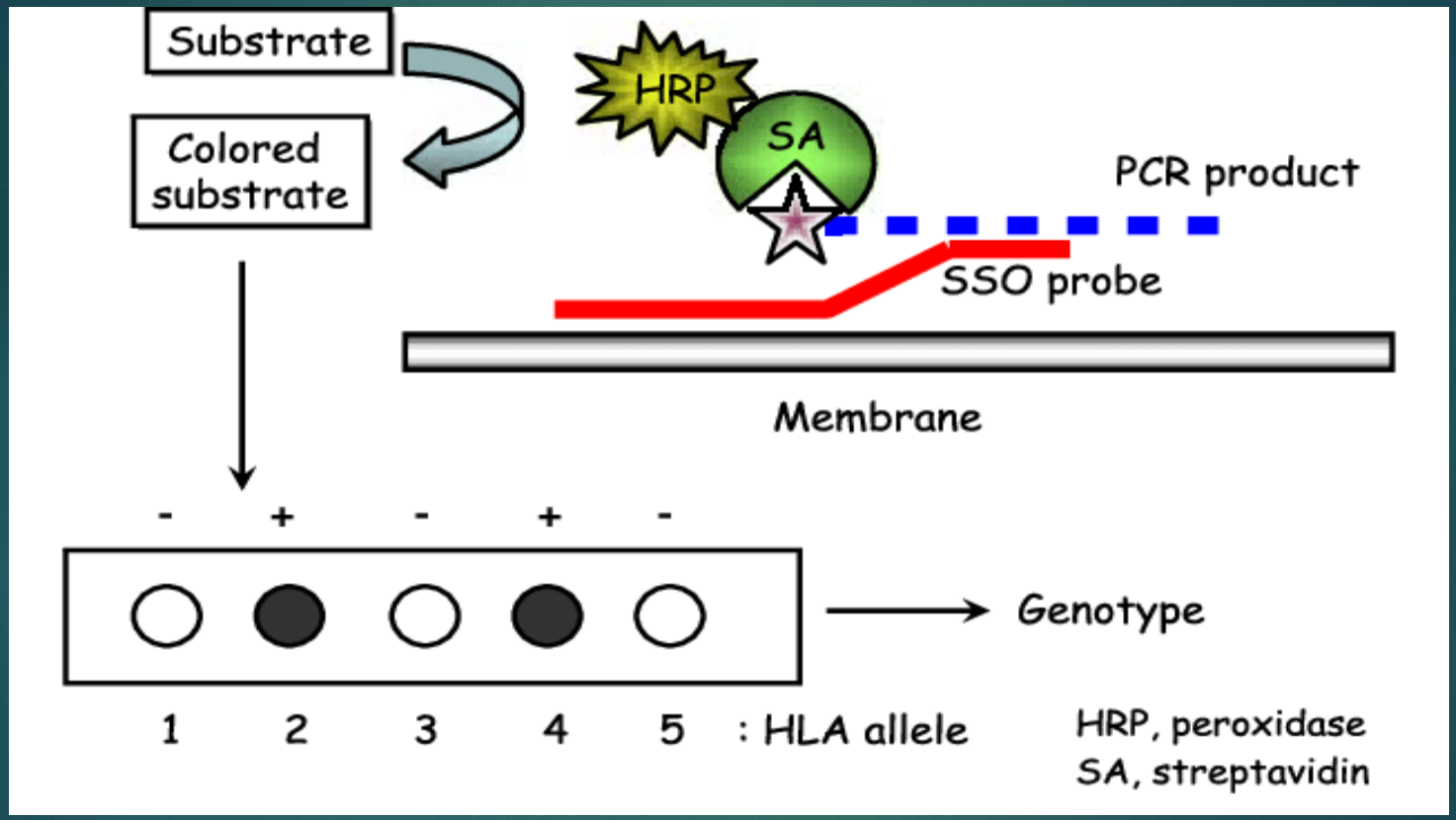
- Sequence base HLA Typing:
- SBT & NGS

SSP (sequence-specific primers)

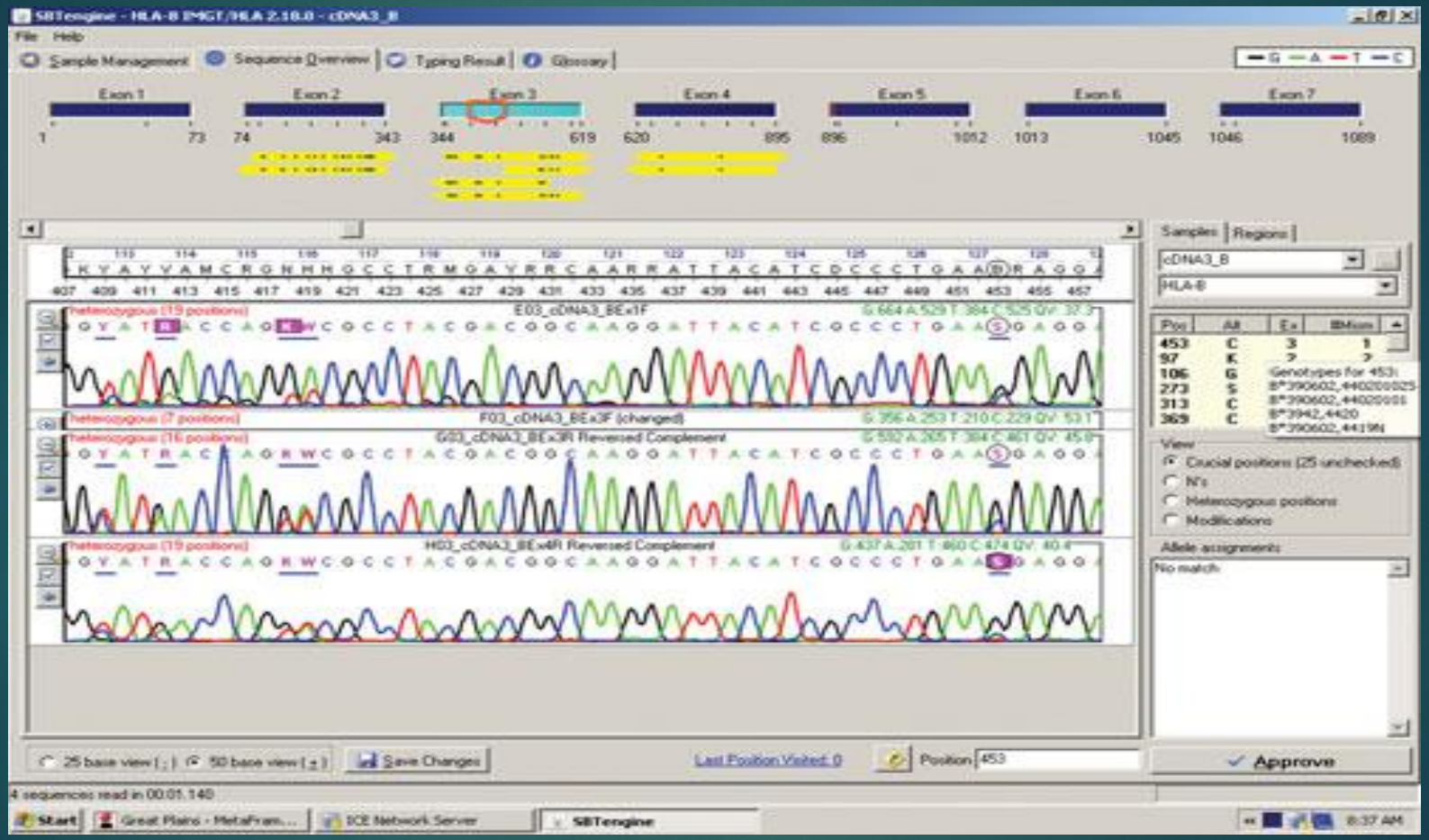


A	B	C	DRB1	DQB1	DPB1
1	7	1	1	2	1
2	8	2	3	3	2
3	13	3	4	4	3
11	14	4	7	5	4
23	15	5	8	6	5
24	18	6	9		6, 8
25	27	7	10		9
26	35	8	11		10
29	37	12	12		11
30	38	14	13		13
31	39	15	14		14
32	40	16	15		15
33	41	17	16		16
34	42	18			17
36	44				18
43	45				19
66	46				20
68	47				21, 22
69	48				23, 24, 25
74	49				26, 27
80	50				28
	51				29
	52				30
	53				31, 32, 33
	54				34
	55				35, 36, 37, 38
	56				39, 40, 41, 44
	57				45
	58				46, 47, 48 ,49
	59				50, 51, 52, 53
	67				54, 55, 56, 57
	73				58, 59, 60, 61
	78				62, 63
	81				...1454
	82, 83				

SSOP(Sequence-specific Oligonucleotide Probe)



SBT (Sequence Base Typing)



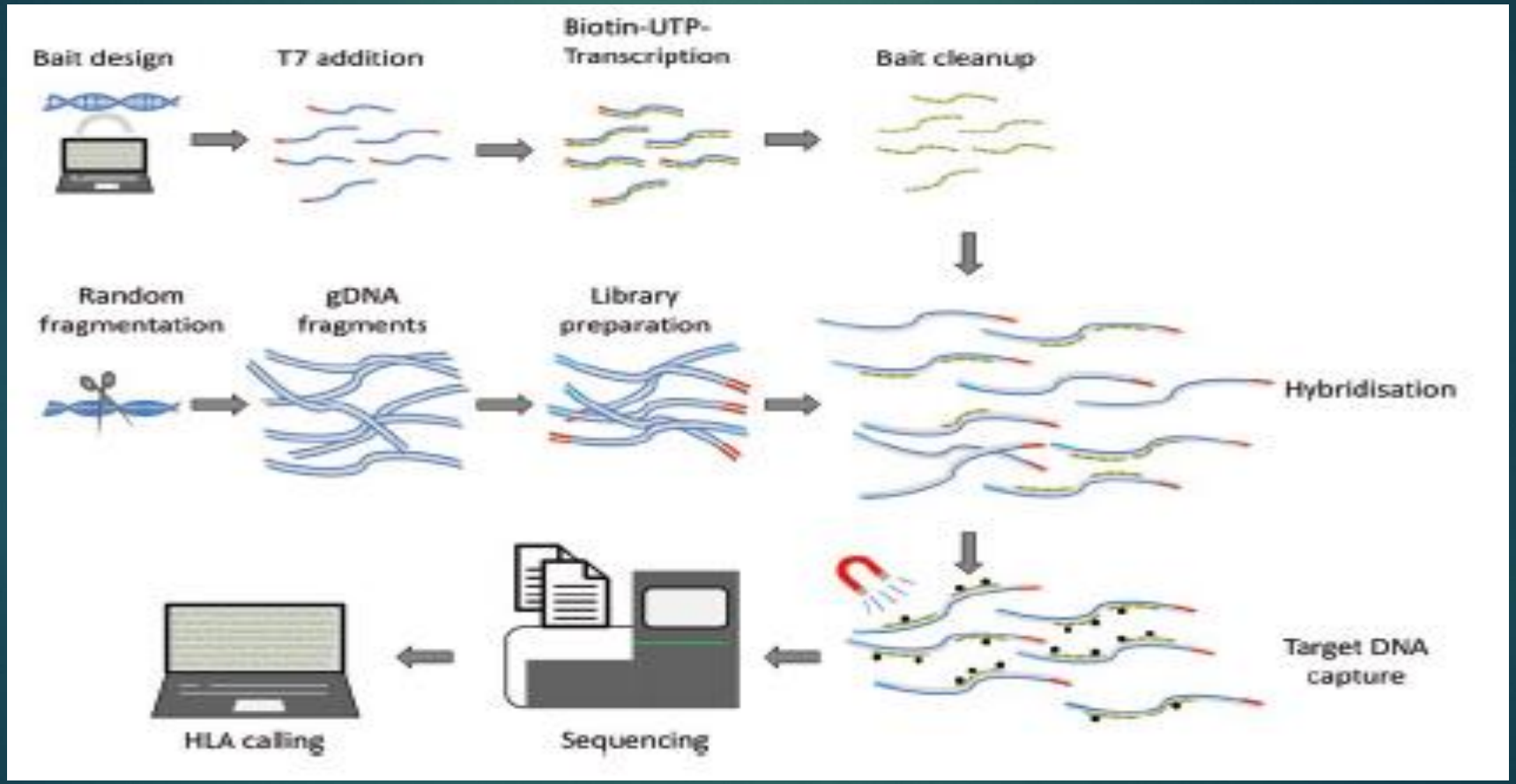
Class I : A / B / C

Class II: DRB1

Class II: DQB1, DRB345, DQA1

Class II: DPA1, DPB1

NGS (Next generation sequencing)



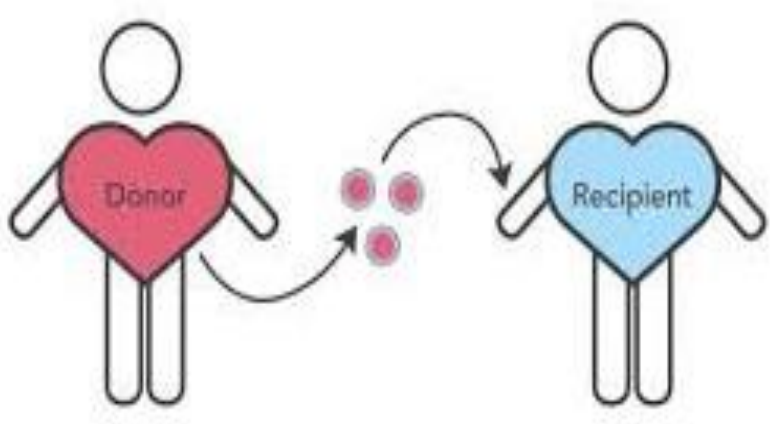
The Importance of Anti-HLA antibody after HSCT

Presented By: Dr Leila Jafari

2024 January 25

Main reason for pre-transplant immunological evaluation?

- Recipient/donor selection
- Risk stratification of transplant
- Better plane for post operative immunological complication
- Decreasing post operative complication
- **To decrease rejection risk**



HLA Ab in HSCT

- The HLA genes are the **most polymorphic** in the human genome, which have evolved to allow the immune system to sense foreign pathogens and provide wide-ranging protection

- Due to the high degree of polymorphism and the necessity of transplanting across **HLA antigen mismatches in some cases**, the presence of HLA antibodies directed against mismatched donor antigens has been recognized as a significant barrier to graft acceptance

Main reasons for Anti-HLA Ab forming?

- Pregnancy, Blood transfusion, History of prior HSCT, inflammatory events such as vaccination, infection, or trauma can result in the formation of HLA antibodies.
- In candidates to HSCT, the rate of HLA sensitization has been reported to range from 20% to 40% and the rate of DSA to at least one potential donor from 1.4% to 24%.

Main reason for pre-transplant immunological evaluation?

- Circulating DSAs can cause hyper-acute graft rejection that presents within minutes of revascularization of the transplanted organ.
- DSA developed post-transplant from pre-transplant antigen exposure is a major cause of chronic or recall graft rejection

Considerations

- The classical HLA genes are comprised of the class I and class II genes
- Over 30,000 class I and II alleles have been identified
- HLA,**B**,C
- HLA **DR**,DQ,DP
- **Non-malignant disorder patients** are more susceptible for HLA antibody formation

Importance of anti HLA antibody

MINI REVIEWS

The Role of Anti-HLA Antibodies in Hematopoietic Stem Cell Transplantation

Daniele Focosi, Alessandra Zucca, Fabrizio Scatena

Donor-specific antihuman leukocyte antigen antibodies (DSHA) have been clearly implicated in graft rejection in solid organ transplantation. Their role in allogeneic hematopoietic stem-cell transplantation (allo-HSCT) remains unclear. We summarize here evidence supporting a role for DSHA in graft failure in animal models of allo-HSCT and in clinical settings whenever no full HLA matching occurs.

Biol Blood Marrow Transplant 17: 1585-1588 (2011)

KEY WORDS: Anti-HLA antibodies, Hematopoietic stem cell transplantation, Mismatch, Luminex

- There was significant impact of anti-HLA antibodies on engraftment, incidence of relapse, and incidence of acute graft-vs-host disease.
- The presence of anti-HLA antibodies was associated with significantly worse overall survival.

Importance of anti HLA antibody

frontiers in Immunology

MINI REVIEW
published: 12 August 2016
doi: 10.3389/fimmu.2016.00307

Donor-Specific Anti-HLA Antibodies in Allogeneic Hematopoietic Stem Cell Transplantation

Sarah Morin-Zorman, Pascale Loiseau, Jean-Luc Taupin and Sophie Caillat-Zucman*

Laboratoire d'Immunologie et Histocompatibilité, Hôpital Saint-Louis, Assistance Publique Hôpitaux de Paris (APHP), Université Paris Diderot, Paris, France

Reference	Patients (*n*)	Stem cell source	Conditioning	Anti-HLA%	DSA%	Graft failure with/without DSA
Spellman et al. (34)	115	Mismatched unrelated	RIC	ND	9	24 versus 1%
Ciurea et al. (36)	592	10/10 and 9/10 unrelated	MACorRIC	19.6	1.4	37.5 versus 2.7%
Yoshihara et al. (39)	79	Haplo-identical	RIC	20.2	14	27 versus 3%
Ciurea et al. (36)	24	Haplo-identical	RIC	ND	21	60 versus 5%
Chang et al. (40)	345	Haplo-identical	MAC	25.2	11.3	61% (MFI›10,000) versus 3.2%
Ciurea et al. (36)	122	Haplo-identical	Non-specified	ND	18	32 versus 4%
Takanashi et al. (41)	386	Single CBU	MAC	23.1	5	83 versus 32%
Cutler et al. (42)	73	Double CBU	MACorRIC	ND	24	57 versus 5.5%
Ruggeri et al. (43)	294	Single and double CBU	RIC	23	5	81 versus 44%
Yamamoto et al. (44)	175	Single CBU	MACorRIC	39.4	ND	50% if anti-HLA-C, DP, DQ, DRB1/2/3 versus 16%

Case Reports

Case 1	Case 2
Female,42 y	Female, 55 y
T-NHL	AML
HID (Sister)	HID
DSA (A2,MFI2,263+)	DSA (DR7)
FCXM -	FCXM -
Desensitization Treatment	Desensitization Treatment
PLT engraftment (+15)	PLT engraftment (+18)
Neu engraftment (+64)	Neu engraftment (+27)
Alive after 4 y	Relapse after 12 months Anti HLA I antibody +

How we detect of Anti HLA antibodies?

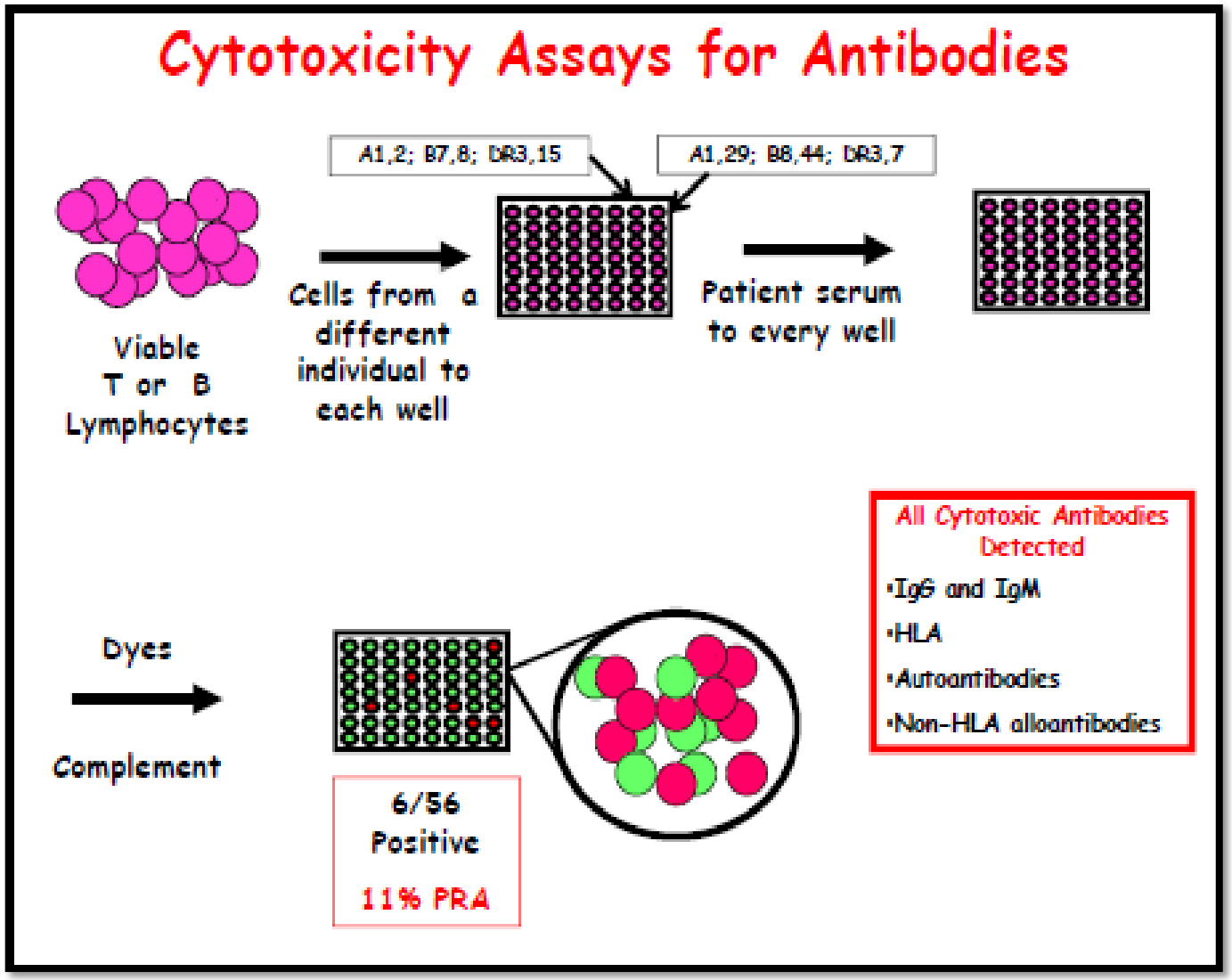
- **Live cell:** Cytotoxicity Assay (CDC)
- **Solid phase assay**: screening or single antigen assay

✓Elisa assay

✓Flow cytometery

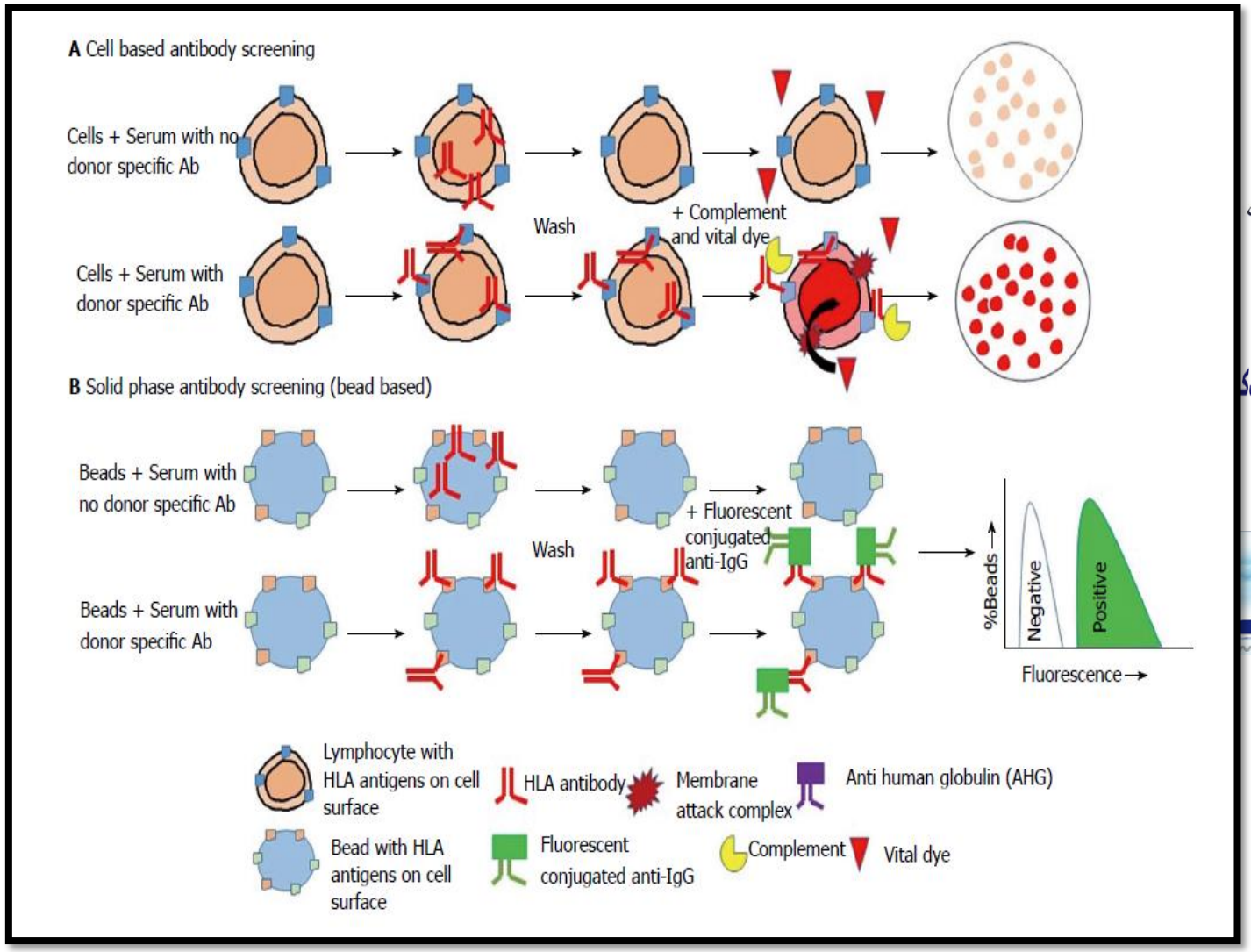
✓Luminex

CDC Assay



Mostly Class I HLA

Cell based vs Solid phase



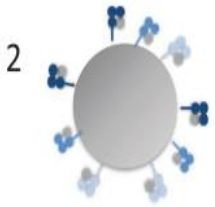
Human leukocyte antigen typing and crossmatch: A comprehensive review, *World J Transplant* 2017 December 24; 7(6): 276-363

Bead characteristics

Bead characteristics : 3 different formats

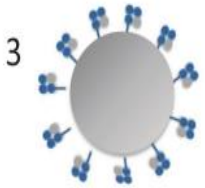
Pooled antigen bead: Class I or II Phenotype of several individuals

Screen
Inexpensive
Yes / No
Increase/ Decrease



Phenotype bead: Class I or II Phenotype of one individual

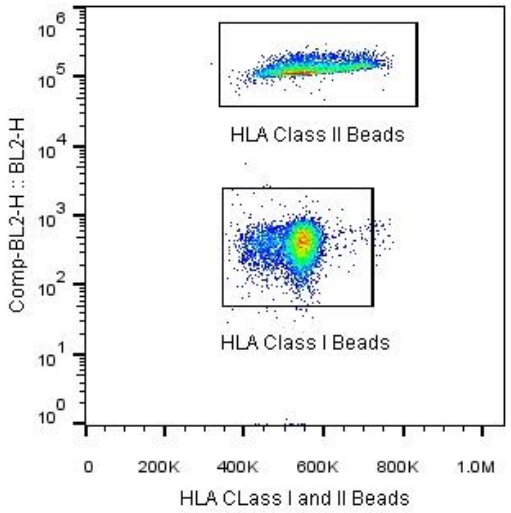
HLA expression density similar to cells
Better correlation to crossmatch

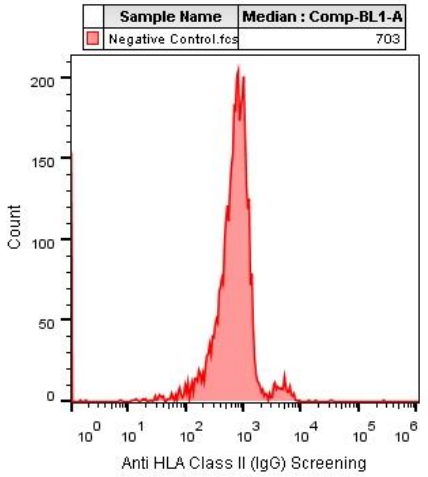


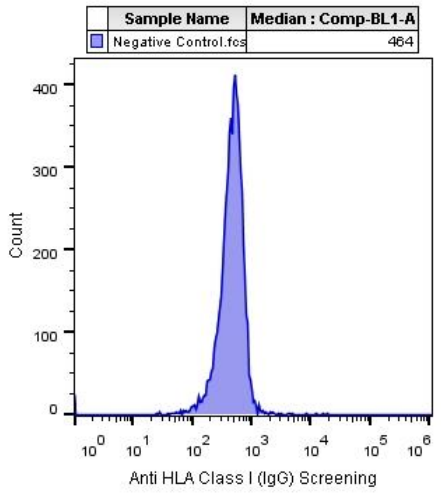
Single antigen bead (SAB): Recombinant product of one allele

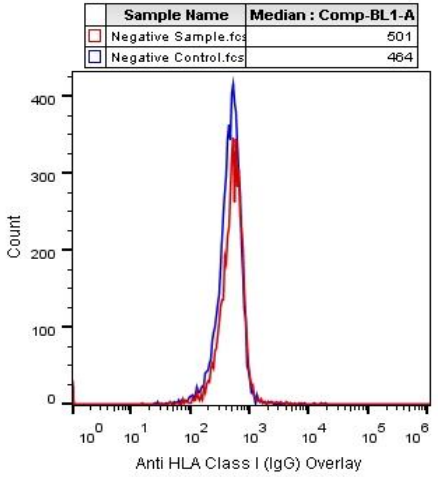
Highest specificity and sensitivity
Difficult to assess antibody strength

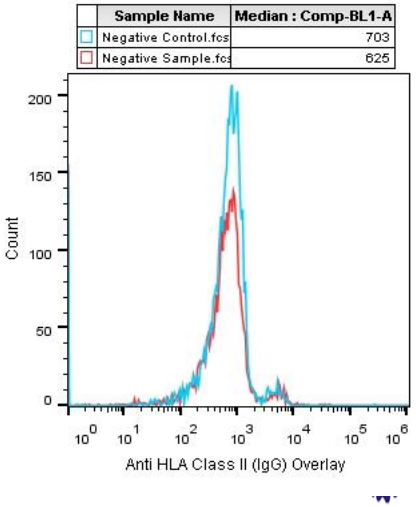
Flow PRA

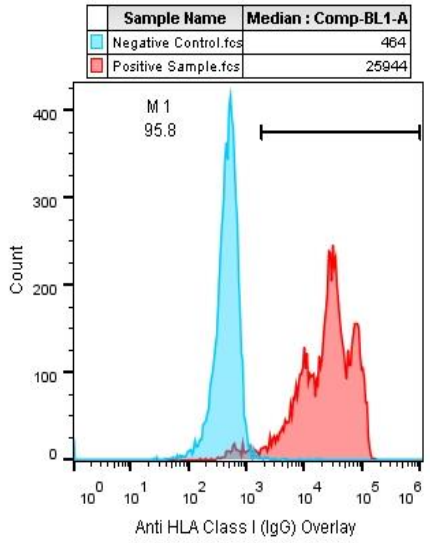

Comp-BL2-H :: BL2-H
HLA Class II Beads
HLA Class I Beads
0
200K
400K
600K
800K
1.0M
HLA CLass I and II Beads

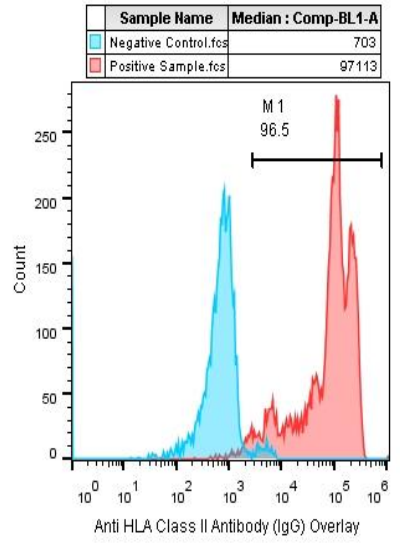

Sample Name
Median : Comp-BL1-A
Negative Control.fcs
703
Count
Anti HLA Class II (IgG) Screening


Sample Name
Median : Comp-BL1-A
Negative Control.fcs
464
Count
Anti HLA Class I (IgG) Screening

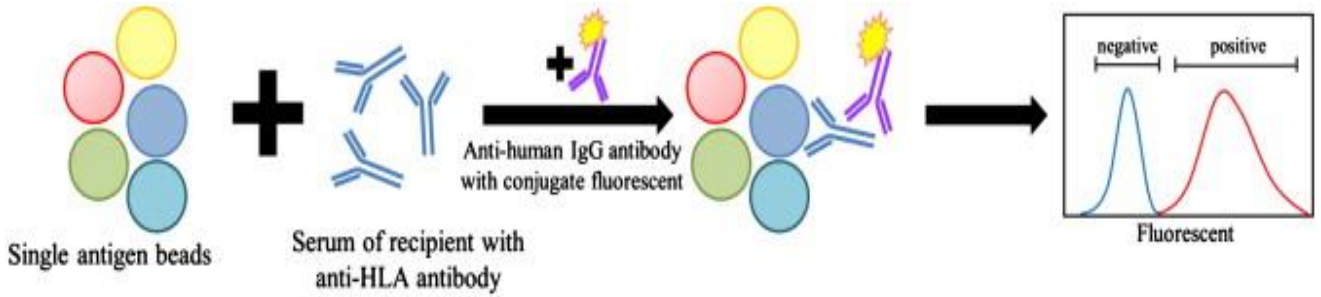

Sample Name
Median : Comp-BL1-A
Negative Sample.fcs
501
Negative Control.fcs
464
Count
Anti HLA Class I (IgG) Overlay


Sample Name
Median : Comp-BL1-A
Negative Control.fcs
703
Negative Sample.fcs
625
Count
Anti HLA Class II (IgG) Overlay


Sample Name
Median : Comp-BL1-A
Negative Control.fcs
464
Positive Sample.fcs
25944
M 1
95.8
Count
Anti HLA Class I (IgG) Overlay

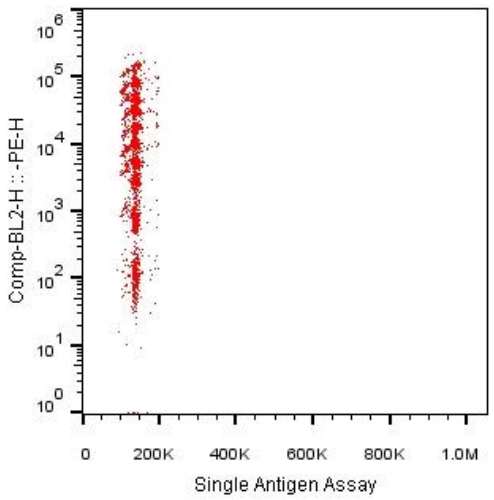

Sample Name
Median : Comp-BL1-A
Negative Control.fcs
703
Positive Sample.fcs
97113
M 1
96.5
Count
Anti HLA Class II Antibody (IgG) Overlay

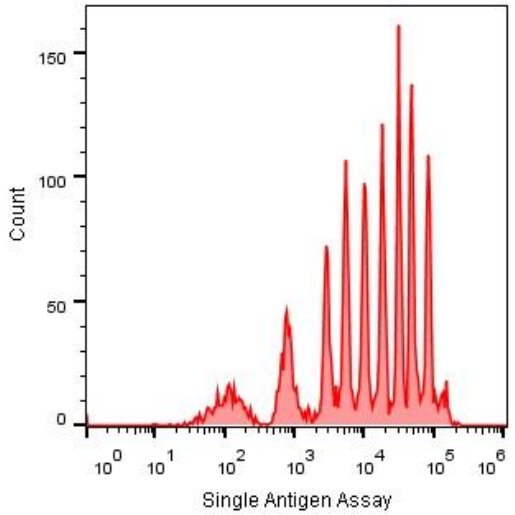
Flow Cytometric



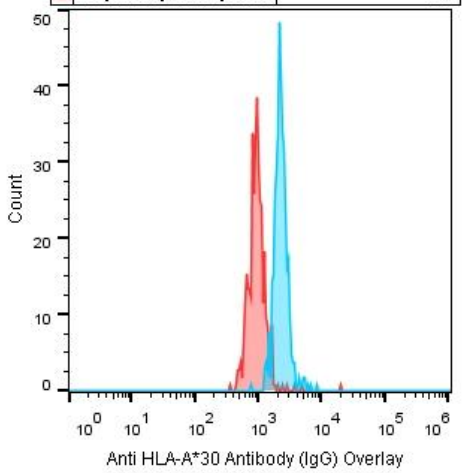
HLA class I	HLA class II
A*01-A*02-A*03-A*11-A*23-A*24-A*25-A*26-A*29-A*30-A*31-A*32-A*33-A*34-A*36-A*66-A*68-A*69-A*74-A*80 B*07- B*08- B*13-B*18- B*27-B*35-B*37-B*38-B*39-B*41-B*42-B*44-B*45-B*46-B*47-B*48-B*49- B*50-B*51-B*52-B*53 -B*54-B*55-B*56-B*57-B*58-B*59-B*60-B*61-B*62- B*63-B*64-B*65-B*67-B*71-B*72-B*73-B*75- B*78-B*81-BW4-BW6 Cw1-Cw2-Cw4-Cw5-Cw6-Cw7-Cw8-Cw9-Cw10-Cw12-Cw14-Cw15-Cw16-Cw17-Cw18	DRB1*01-DRB1*01:03-DRB1*03:01-DRB1*03:02-DRB1*04-DRB1*07-DRB1*08-DRB1*09-DRB1*10-DRB1*11-DRB1*12-DRB1*13-DRB1*14- DRB1*15-DRB1*16-DRB3-DRB4-DRB5 DQB1*02-DQB1*04-DQB1*05-DQB1*06-DQB1*03:01-DQB1*03:02-DQB1*03:03 DPB1*01-DPB1*02-DPB1*03-DPB1*04-DPB1*05-DPB1*08-DPB1*09-DPB1*10-DPB1*11-DPB1*13-DPB1*14-DPB1*17-DPB1*18-DPB1*19-DPB1*105-DPB1*20-DPB1*21-DPB1*40

Flow Cytometric Single Antigen Assay

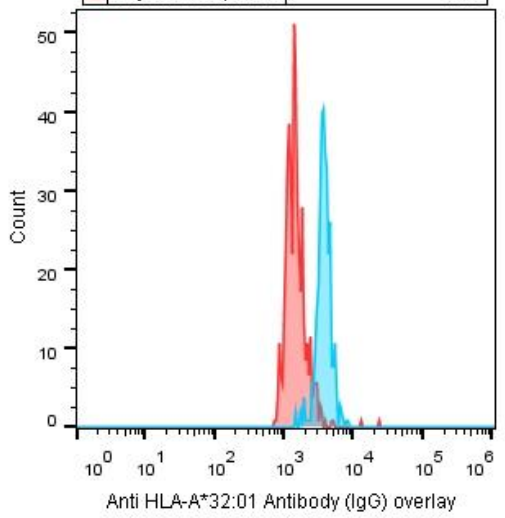




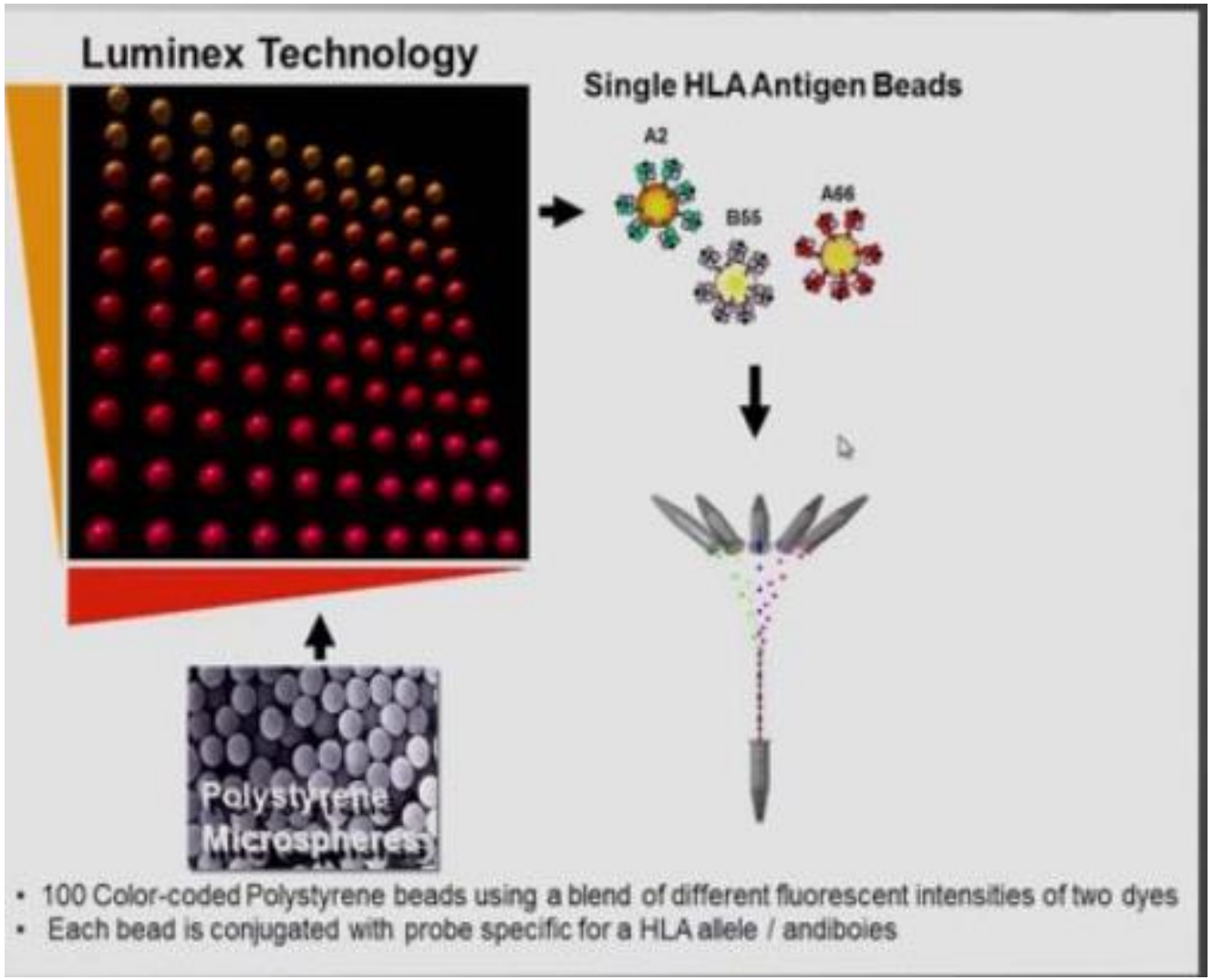
Sample Name	Median : Comp-BL1-A
Single Antigene Positive.fcs	2142
Single Antigene Negative.fc	903



Sample Name	Median : Comp-BL1-A
Positive Sample 2.fcs	3594
Negative Sample 2.fcs	1365



Luminex single antigen bead assay

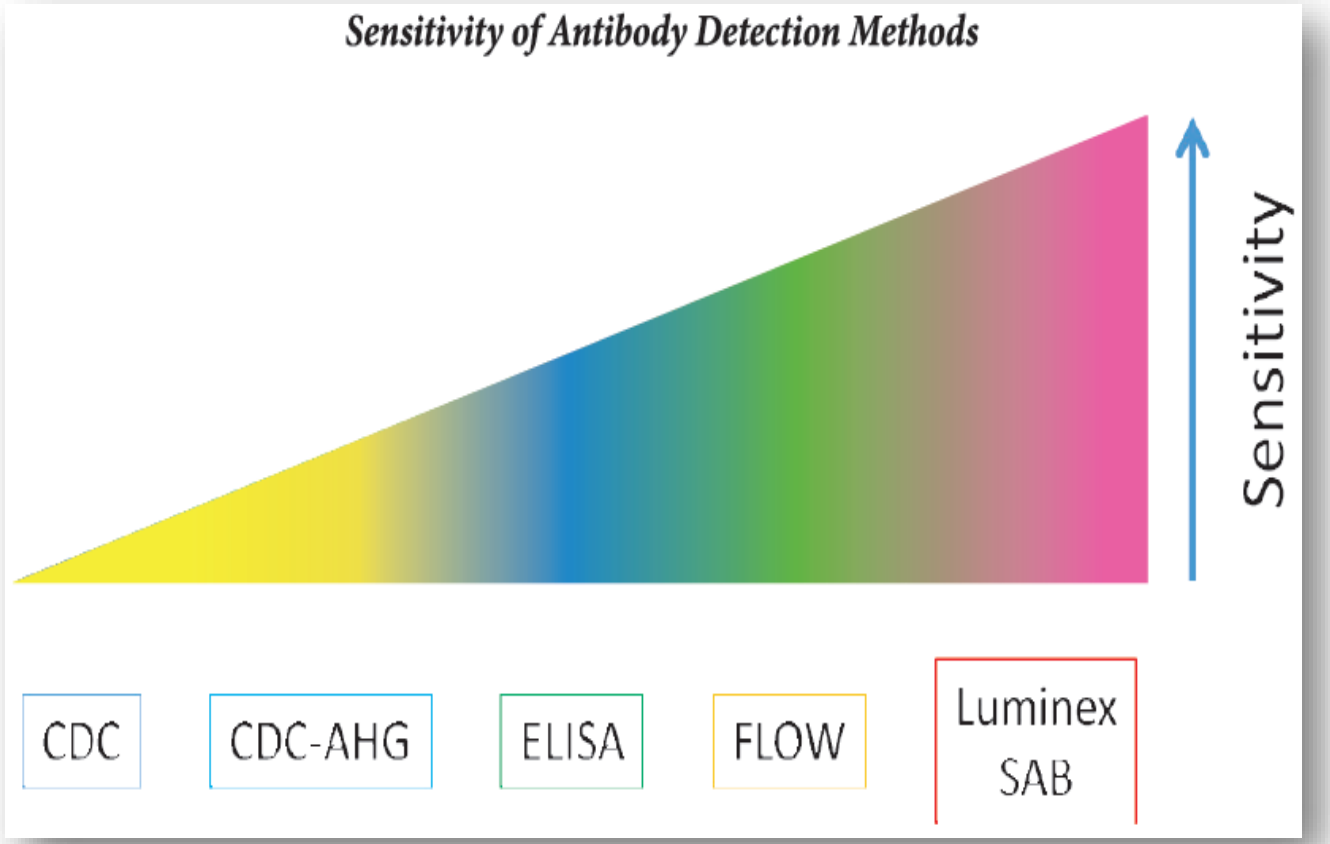


What we can do with Single antigen data?

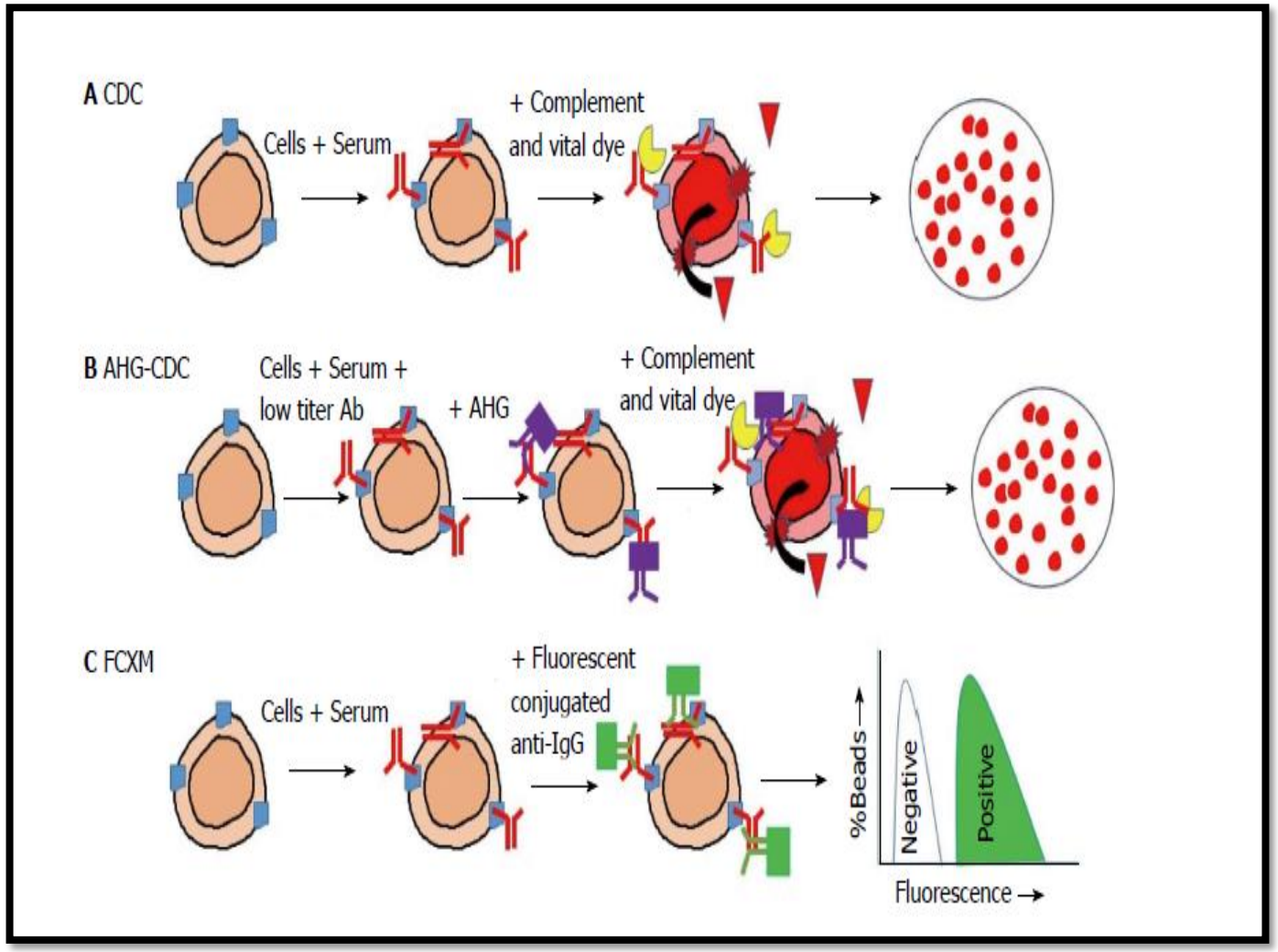
Reactive Antigens	
High Risk Antigens (MFI >1000)	A*11:01- A*23:01- A*24:02- A*32:01- B*08:01- B*14:02- B*27:05- B*35:01- B*38:01- B*52:01- B*57:01-
Moderate Risk Antigens (MFI 500-1000)	A*31:01- A*33:01- A*68:01- B*07:02- B*18:01- B*51:01

Reactive Antigens	
High Risk Antigens (MFI >1000)	DRB1*01:01- DRB1*01:02- DRB1*01:03- DRB1*03:01- DRB1*03:02- DRB1*04:01- DRB1*04:04- DRB1*04:05- DRB1*08:01- DRB1*09:01- DRB1*10:01- DRB1*11:01- DRB1*12:01- DRB1*12:02- DRB1*13:01- DRB1*13:03- DRB1*14:01- DRB1*15:01- DRB1*15:02- DRB1*16:01- DRB3*02:02- DRB5*01:01-
Moderate Risk Antigens (MFI 500-1000)	DQB1*02:01

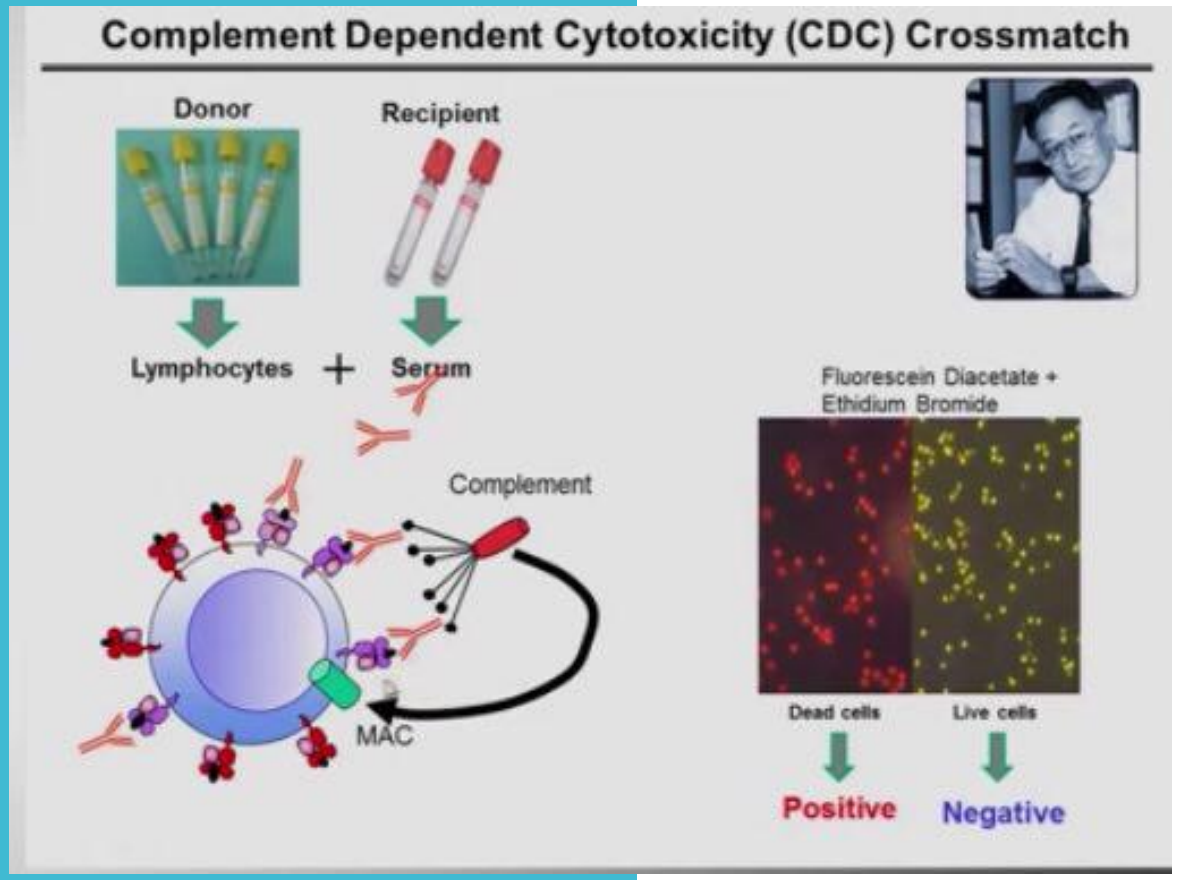
Difference between Anti-HLA antibody detection methods

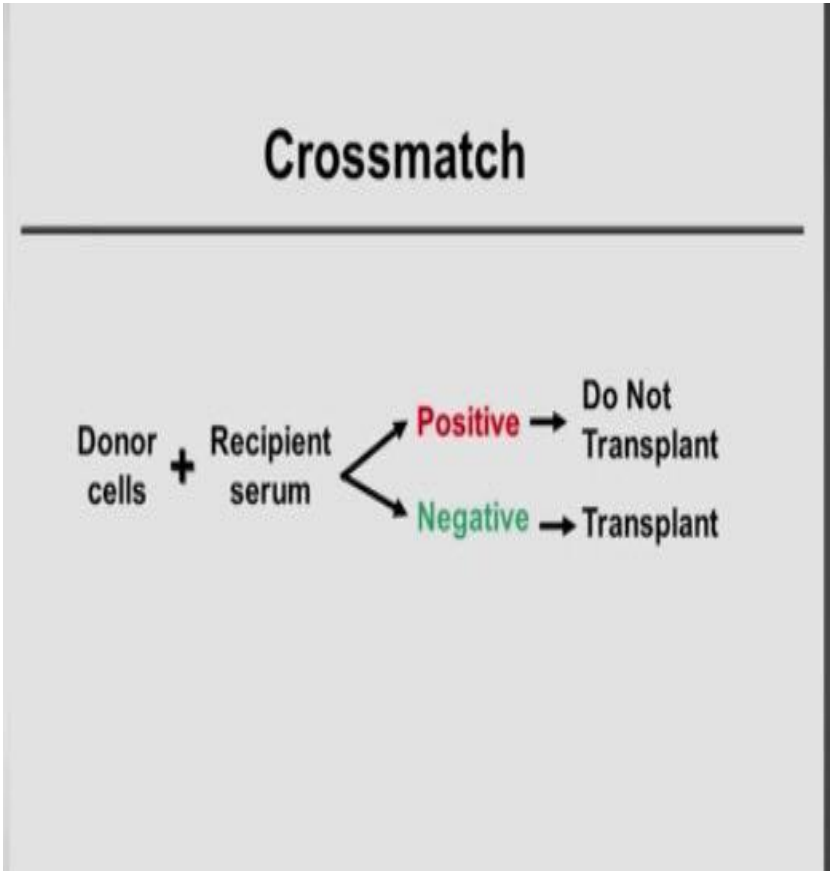


Cross match



Human leukocyte antigen typing and crossmatch: A comprehensive review, *World J Transplant* 2017 December 24; 7(6): 276-363



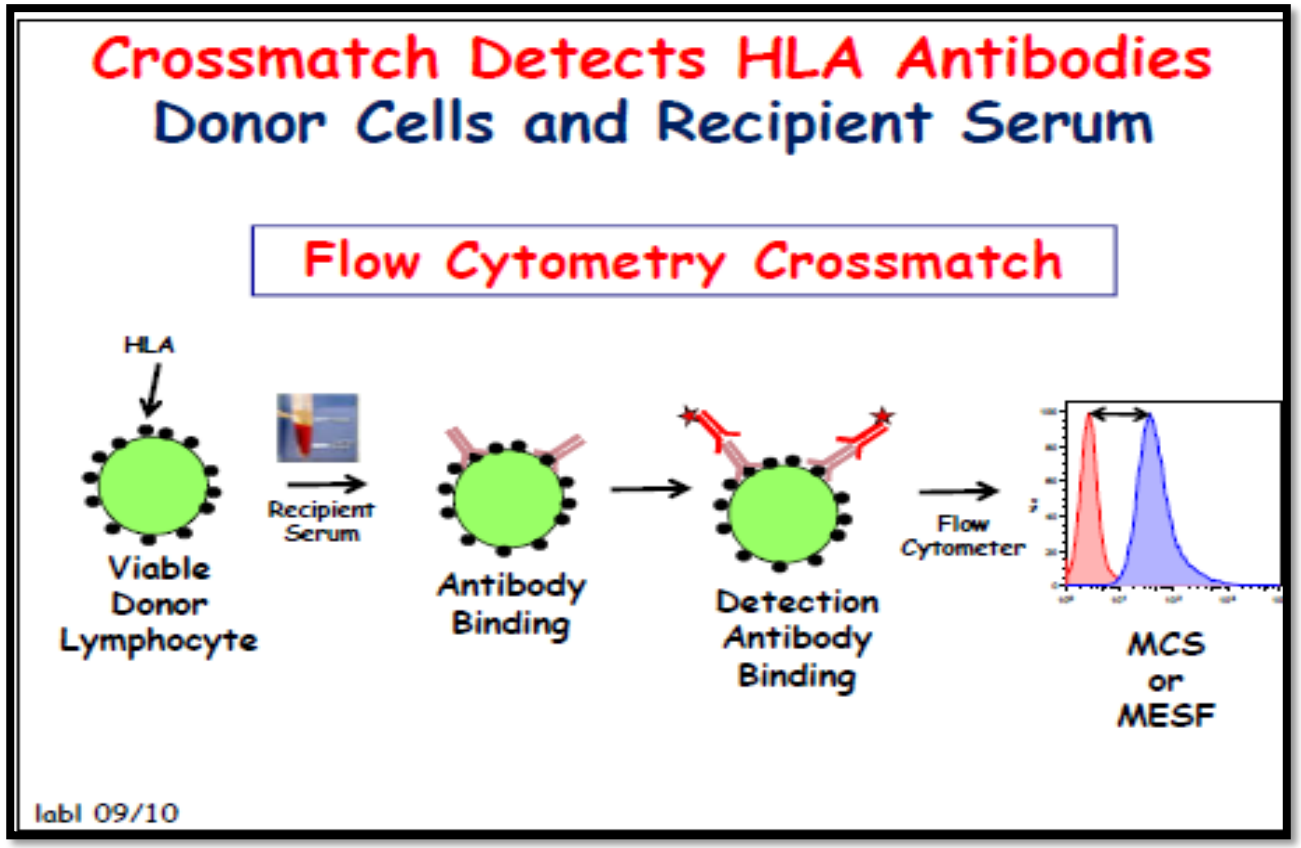


دانشگاه علوم پزشکی
و خدمات بهداشتی درمانی تهران

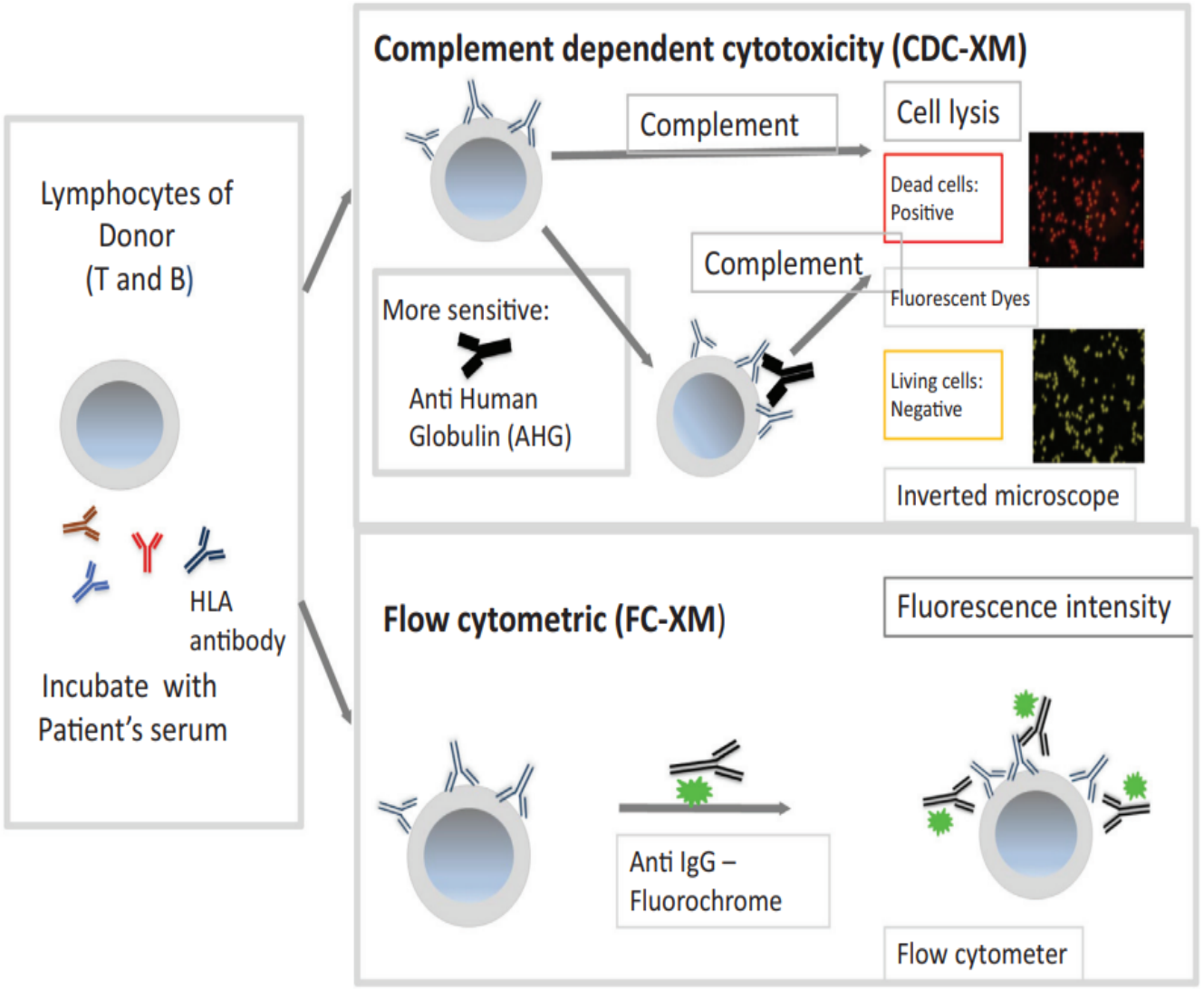
مرکز تحقیقات سلول و ژن درمانی کودکان

www.epezeshk.com

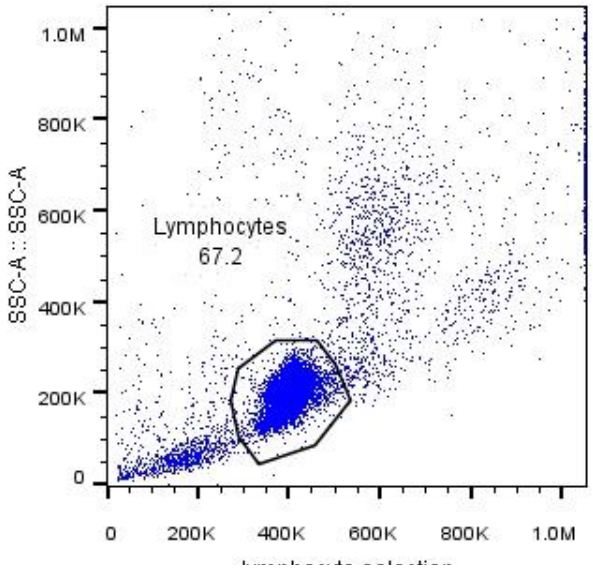
Flowcytometry cross match

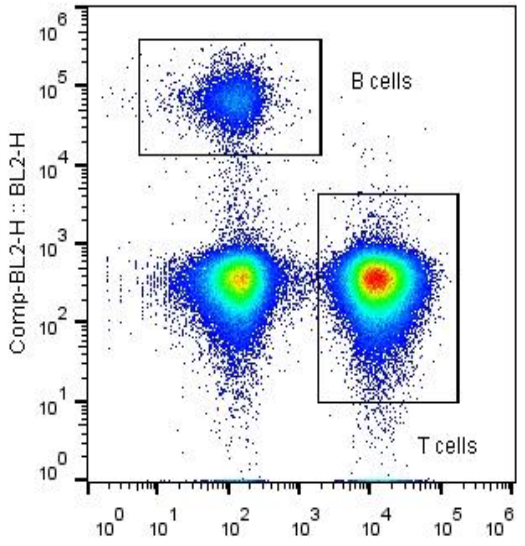


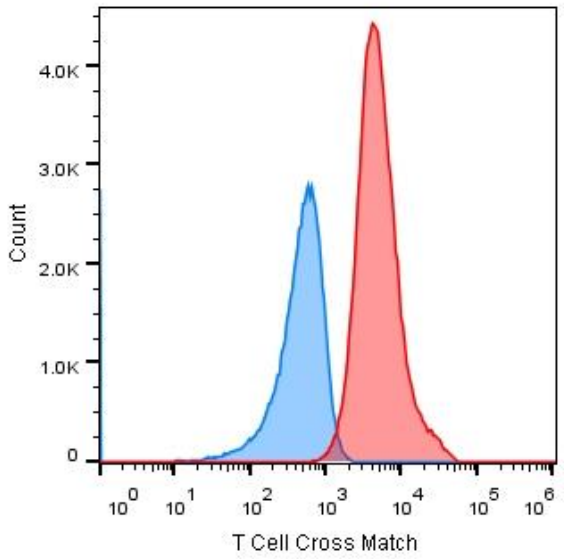
Cross match

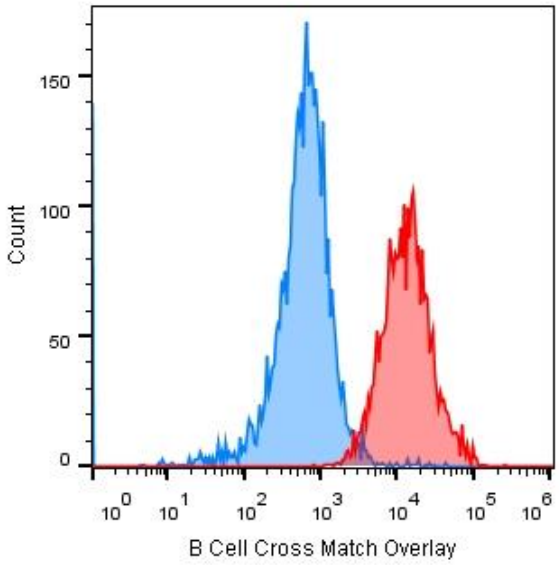

Lymphocytes of Donor (T and B)
HLA antibody
Incubate with Patient's serum
Complement dependent cytotoxicity (CDC-XM)
Complement
Cell lysis
Dead cells: Positive
More sensitive:
Anti Human Globulin (AHG)
Complement
Fluorescent Dyes
Living cells: Negative
Inverted microscope
Flow cytometric (FC-XM)
Fluorescence intensity
Anti IgG – Fluorochrome
Flow cytometer

Flow cytometric cross match









Desensitization of HLA antibody in patients

- Desensitization of HLA antibodies in HSCT patients
- Rituximab
- Proteasome inhibitor bortezomib
- Therapeutic plasma exchange
- Intravenous immunoglobulin

Single center Study

frontiers | Frontiers in Immunology

TYPE Original Research
PUBLISHED 26 May 2023
DOI 10.3389/fimmu.2023.1165759

Check for updates

OPEN ACCESS

EDITED BY
Eric Spierings,
Utrecht University, Netherlands

REVIEWED BY
Shigeo Fuji,
Osaka International Cancer Institute, Japan
Luca Castagna,
Azienda Ospedaliera Ospedali Riuniti Villa Sofia Cervello, Italy

*CORRESPONDENCE
Mi Kwon
mi.kwon@salud.madrid.org
Rebeca Bailén
rebeca.bailen@salud.madrid.org

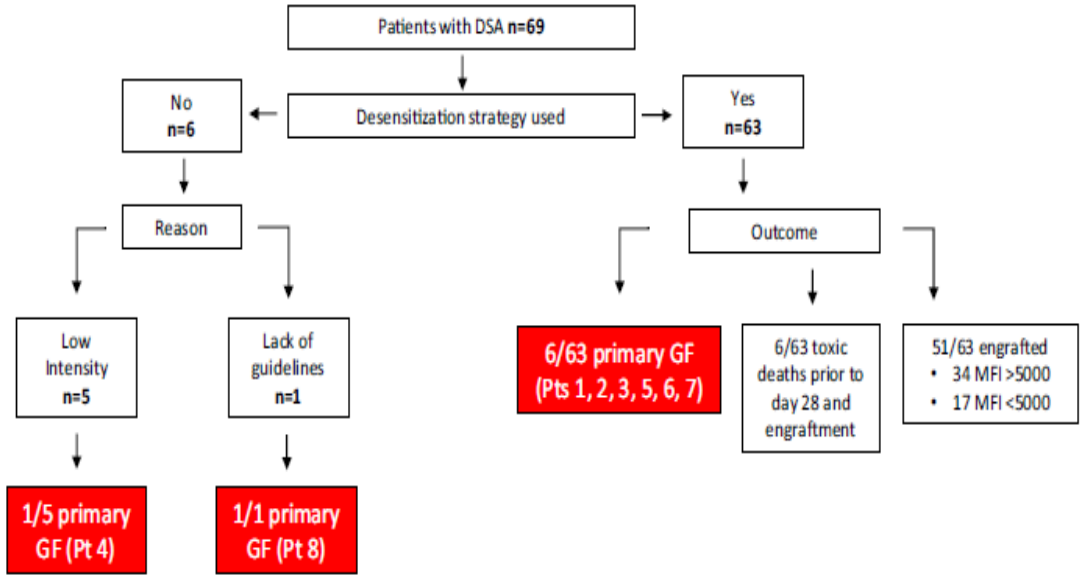
RECEIVED 14 February 2023
ACCEPTED 16 May 2023
PUBLISHED 26 May 2023

CITATION
Bailén R, Alenda R, Herruzo-Delgado B, Acosta-Fleitas C, Vallés A, Esquirol A, Fonseca M, Solán L, Sánchez-Vadillo I,

Results of haploidentical transplant in patients with donor-specific antibodies: a survey on behalf of the Spanish Group of Hematopoietic Transplant and Cell Therapy

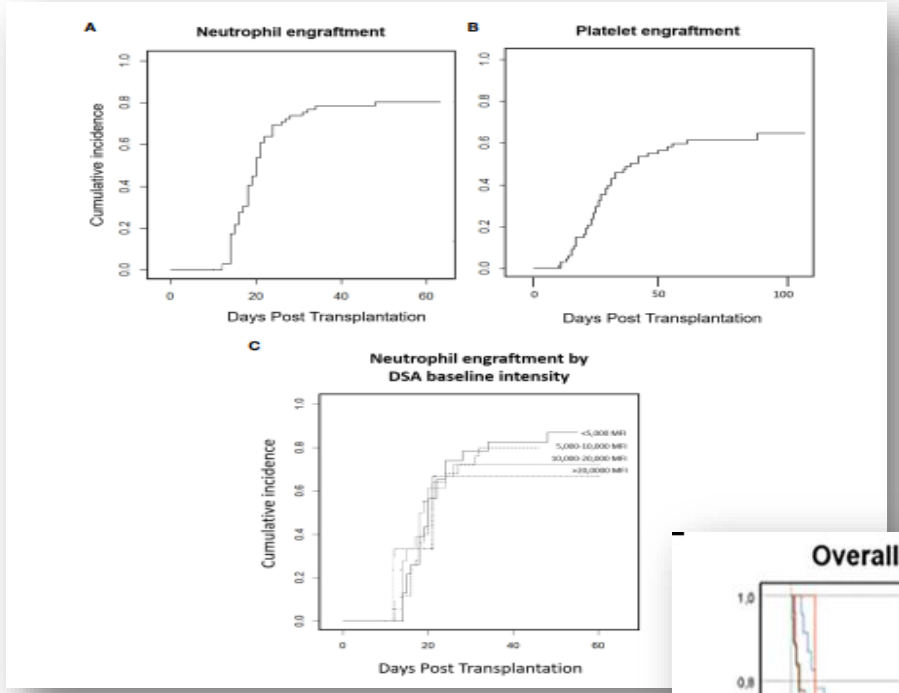
Rebeca Bailén[1,2*], Raquel Alenda[3], Beatriz Herruzo-Delgado[4], Cynthia Acosta-Fleitas[5], Ana Vallés[6], Albert Esquirol[7], Marta Fonseca[8], Laura Solán[9], Irene Sánchez-Vadillo[10], Guiomar Bautista[11], Leyre Bento[12], Oriana López-Godino[13], Ariadna Pérez-Martínez[14], Anna Torrent[15], Joud Zanabili[16], María Calbacho[17], Miguel Ángel Moreno[3], María Jesús Pascual-Cascón[4], Luisa Guerra-Domínguez[5],

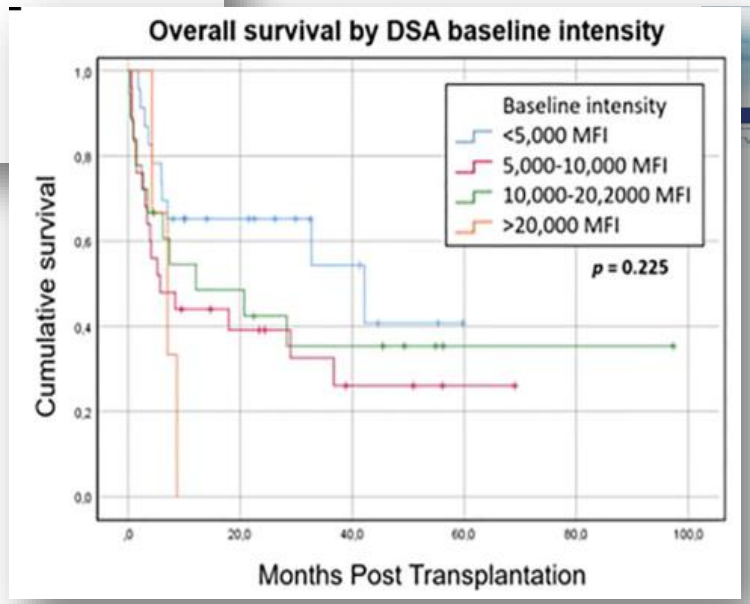
A



	Patients (n=69)
Baseline DSA characteristics (*n*, %)	
• DSA anti-MHC class I only	33 (48)
• *Intensity >5,000 MFI*	*24*
• DSA anti-MHC class II only	19 (27)
• *Intensity >5,000 MFI*	*7*
• DSA anti-MHC class I and II	17 (25)
• *Intensity >5,000 MFI*	*15*
Baseline DSA intensity (*n*, %)	
• >5,000 MFI	46 (67)
• >10,000 MFI	21 (30)
• >20,000 MFI	3 (4)
Complement fixation techniques available (*n*, %)	20 (29)
• *Positive C1q/C3d fixation*	*14*
Patients receiving desensitization (*n*, %)	63 (91)
• Rituximab	53 (84)
• IVIG	42 (67)
• TPE	33 (52)
• Incompatible platelet transfusion	26 (41)
• MMF	26 (41)
• Tacrolimus	13 (21)
• Buffy coat	12 (19)
• Bortezomib	2 (3)
• Steroids	1 (2)

Single center Study





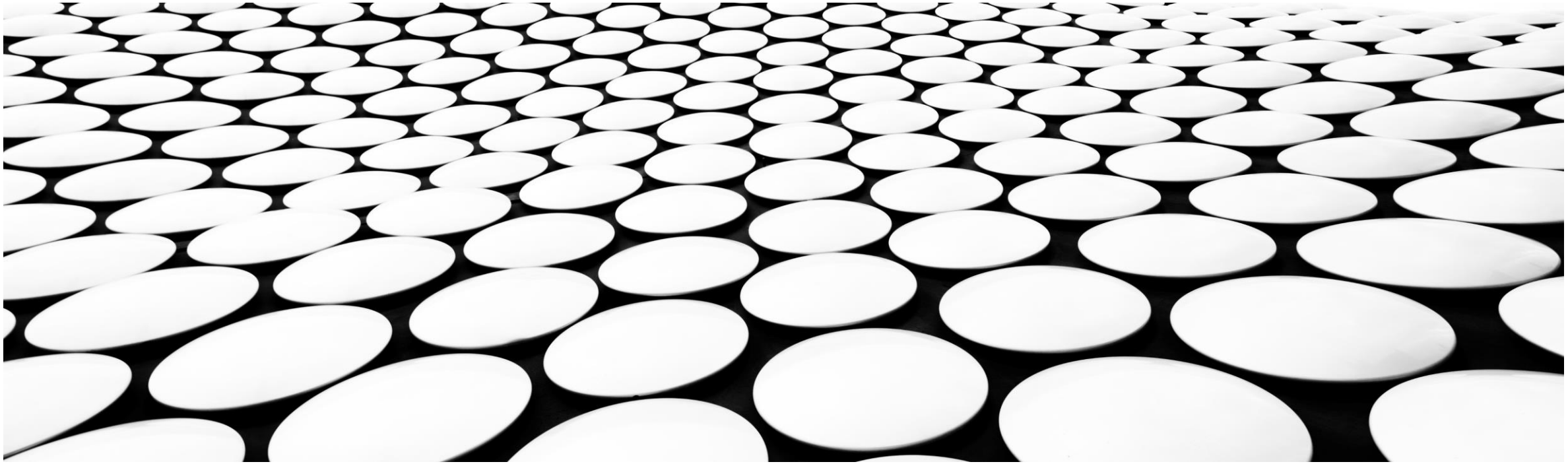
Conclusion

- Nowadays, almost every HSCT candidate has a donor.
- Circulating HLA antibodies specific for the donor's mismatched antigens may have delayed or failed engraftment.
- Desensitization treatments to lower donor specific antibody to levels compatible with engraftment
- The role of the HLA laboratory in support of HSCT has expanded to include HLA antibody testing and monitoring and assessment of the effectiveness of antibody reduction treatments.

QUALITY CONTROL PROCEDURES FOR STEM CELL THERAPY PRODUCTS

RASHIN MOHSENI

PEDIATRIC CELL & GENE THERAPY RESEARCH CENTER, TEHRAN UNIVERSITY OF MEDICAL SCIENCES



Quality Control

1. Viability

2. Karyology

3. Identity Testing
3.1. Confirmation of Species of Origin
3.2. DNA Profiling for Cell-Specific Identification
3.3. Antibody Markers
3.4. Pluripotency

4. Sterility
4.1. Mycoplasma Testing
4.2. Endotoxin Detection

5. Quality Control of Culture Conditions, Reagents and Media

6. Conclusions

Welcome to the presentation on Quality Control Procedures for Stem Cell Therapy products. This session will cover the essential aspects of ensuring excellence in stem cell therapy through rigorous quality control measures.

Stem cell therapy offers promising regenerative potential for various medical conditions. It is crucial to maintain the highest quality standards to ensure safety and efficacy.

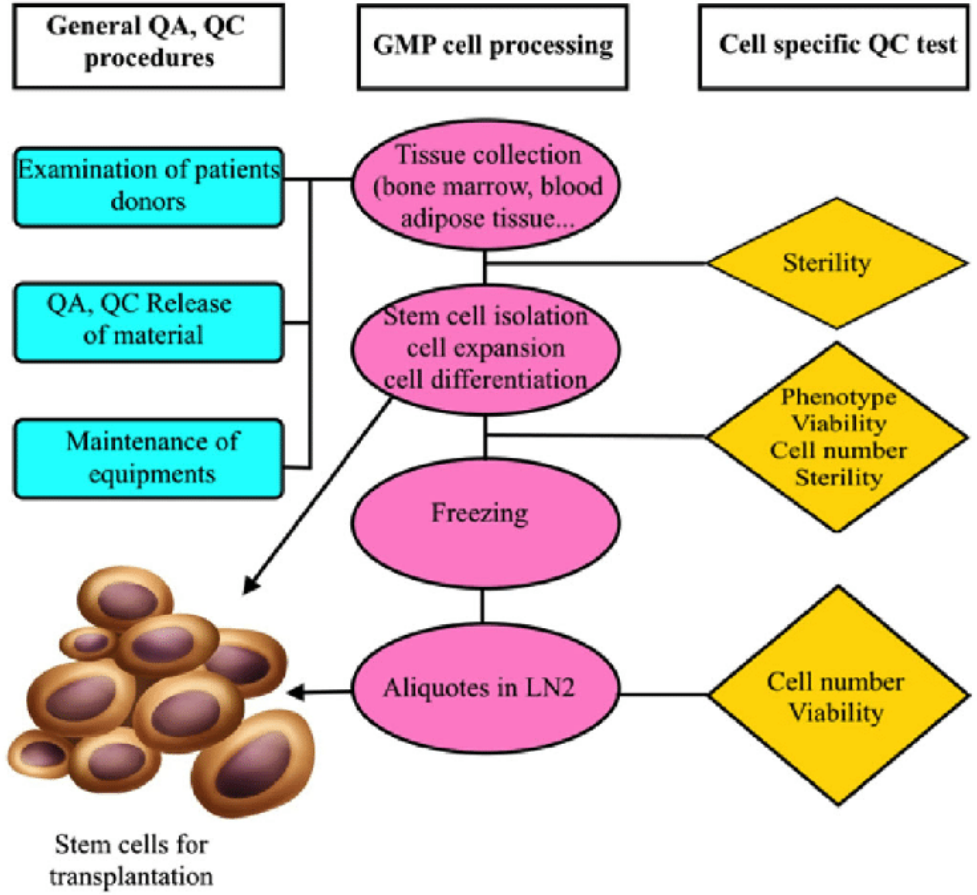
Adherence to regulatory guidelines is paramount in the development and manufacturing of stem cell therapy products. Stringent compliance ensures patient safety and product reliability.

Quality Control Measures

Robust quality control measures encompass rigorous testing, validation, and monitoring at every stage of stem cell product development. This ensures consistency and purity of the final product.

Good Manufacturing Practices (GMP)

Adhering to GMP standards is essential to maintain the highest level of quality and safety in stem cell therapy production. GMP guidelines cover all aspects of production and quality control.



1. Viability Testing for Cell Cultures

1.1. Dye exclusion (e.g., Trypan Blue, Naphthalene Black)

Dyes that penetrate cells are excluded by the action of the cell membrane in viable cells; thus cells containing no dye have functional membranes and are probably viable.

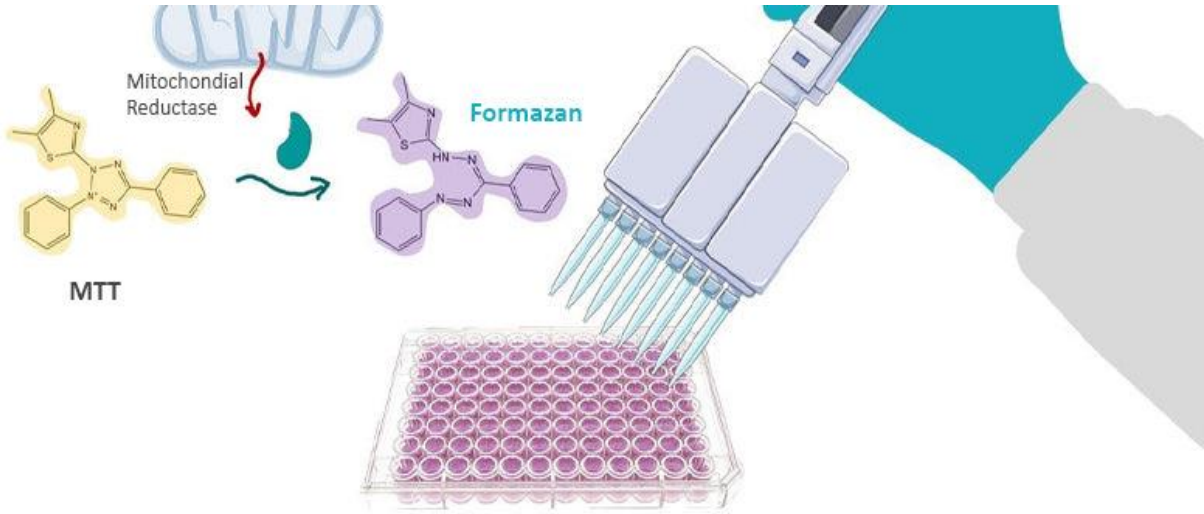
Advantages: Rapid and usually easy to interpret
Disadvantage: May overestimate viability since
apoptotic cells continue to have active membranes and may appear viable.

1.2. Fluorescein diacetate assay

Fluorescein diacetate enters the cell and is degraded by intracellular esterases, releasing fluorescein that cannot escape from cells with intact membranes, and thus the cells fluoresce when observed under UV light.
Advantages: Rapid setup
Disadvantages: Requirement for UV microscope or flow cytometer

1. Viability Testing for Cell Cultures

1.3. 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium (MTT) assay

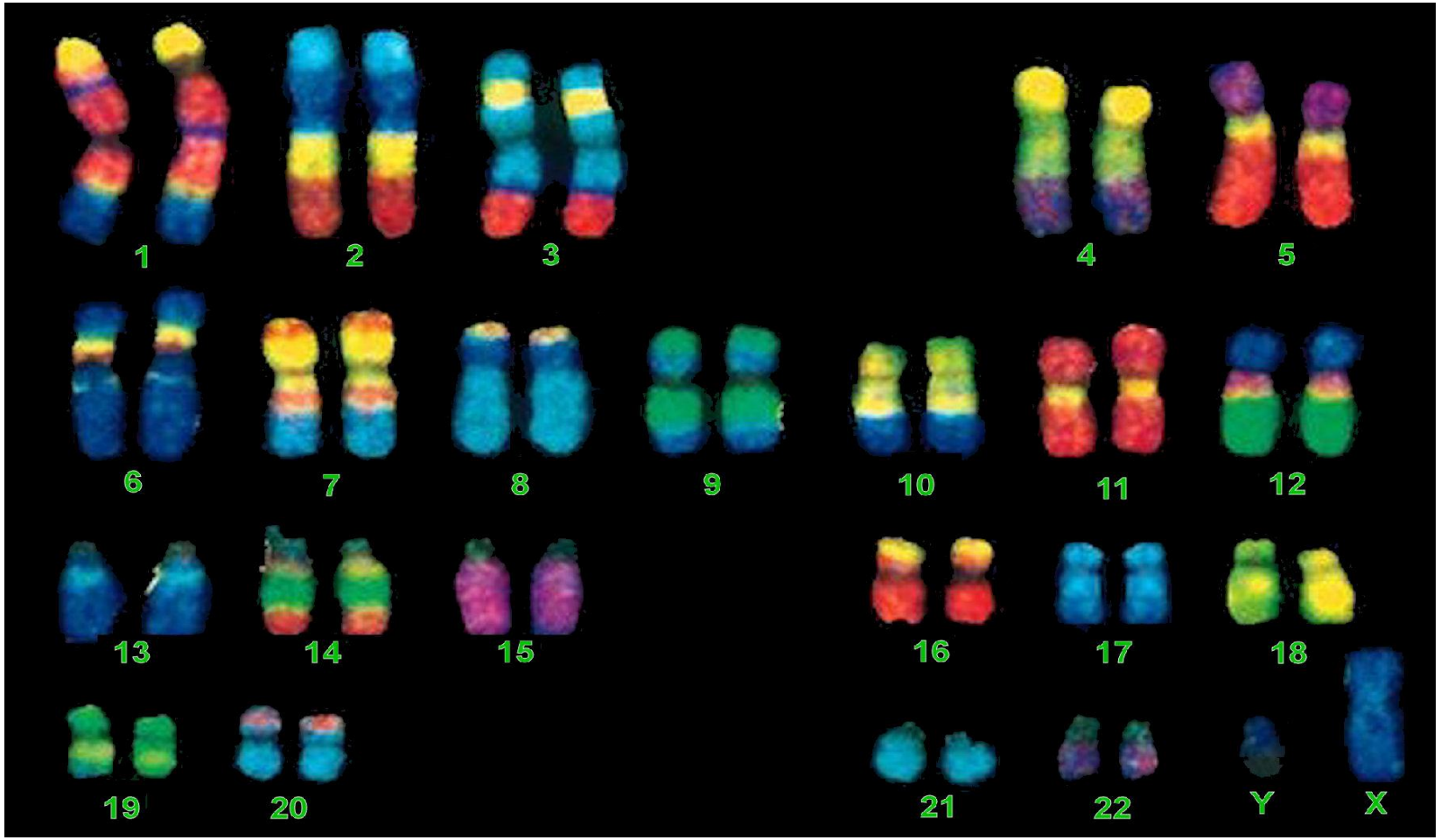


MTT reduction is measured by the formation of a colored product, and this is indicative of biochemical activity.

Advantages: Many tests can be performed rapidly in 96-well array in automatic plate readers.

Disadvantages: Some inhibited cells show a low MTT reduction value that is not necessarily related to cell viability.

2. Visualization of the cell's chromosomes (karyotypic analysis)



2. Karyology recognize the appearance of transformed cells, which are often aneuploid (having chromosome loss or duplication, or aberrant chromosomes with translocations, deletions, inversions, etc) and heteroploid (having a wide range of chromosome numbers per cell around or, more often, above the normal number).

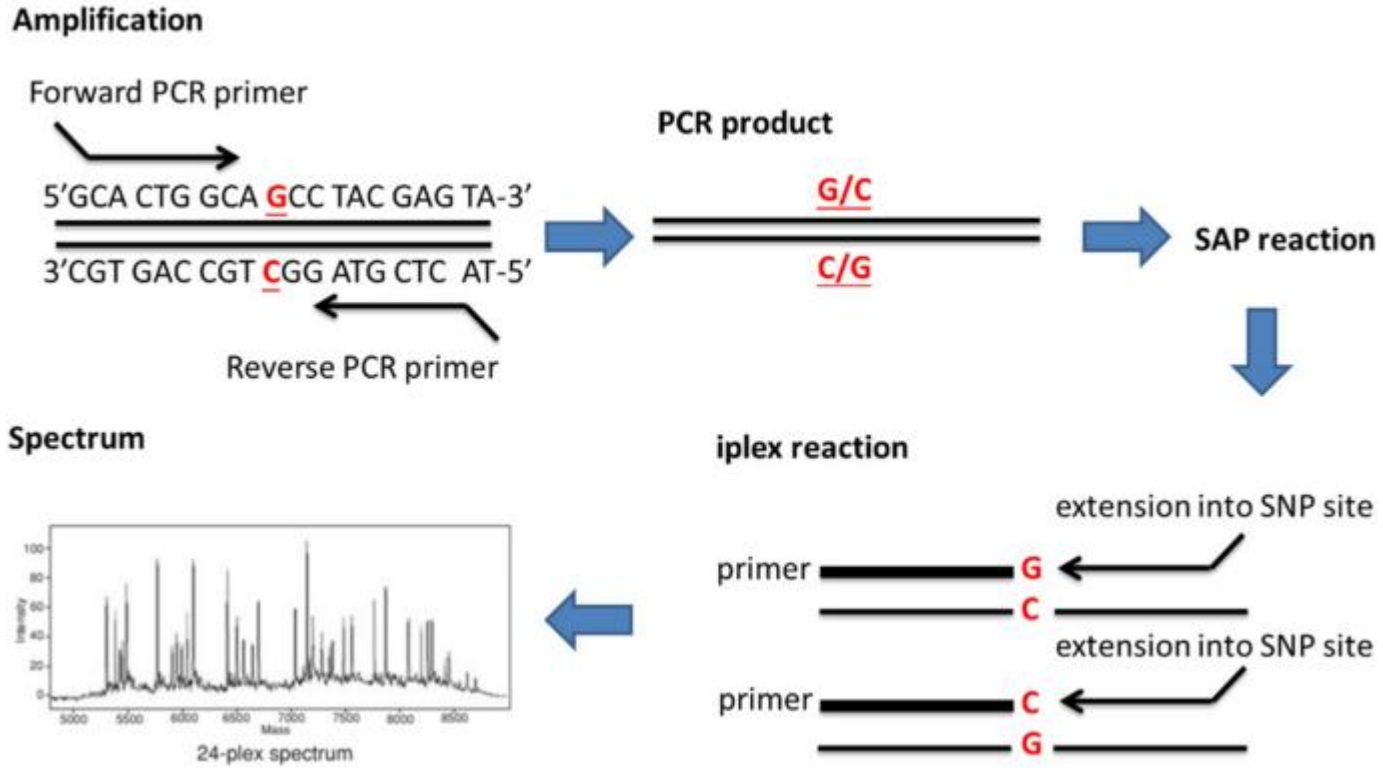
The method of visualization of chromosomes most commonly used today employs Colchicine or a similar compound to block cell division at metaphase when the individual chromosomes are separate and condensed and thus most readily visualized.

More recently, studies of hES cell cultures have revealed that they are prone to karyological changes, and a major challenge has emerged in maintaining the cells in the undifferentiated state while preserving a diploid karyotype.

For hES cells there appear to be common patterns of chromosome alteration representing "adaptation" of these cells to *in vitro* culture conditions, notably changes involving chromosomes 12 and 17.

There are ongoing efforts to develop a chip-based or molecular assay for the karyotypic stability of hES cells in culture.

One of these methods is based on single nucleotide polymorphism (SNP) genotyping.

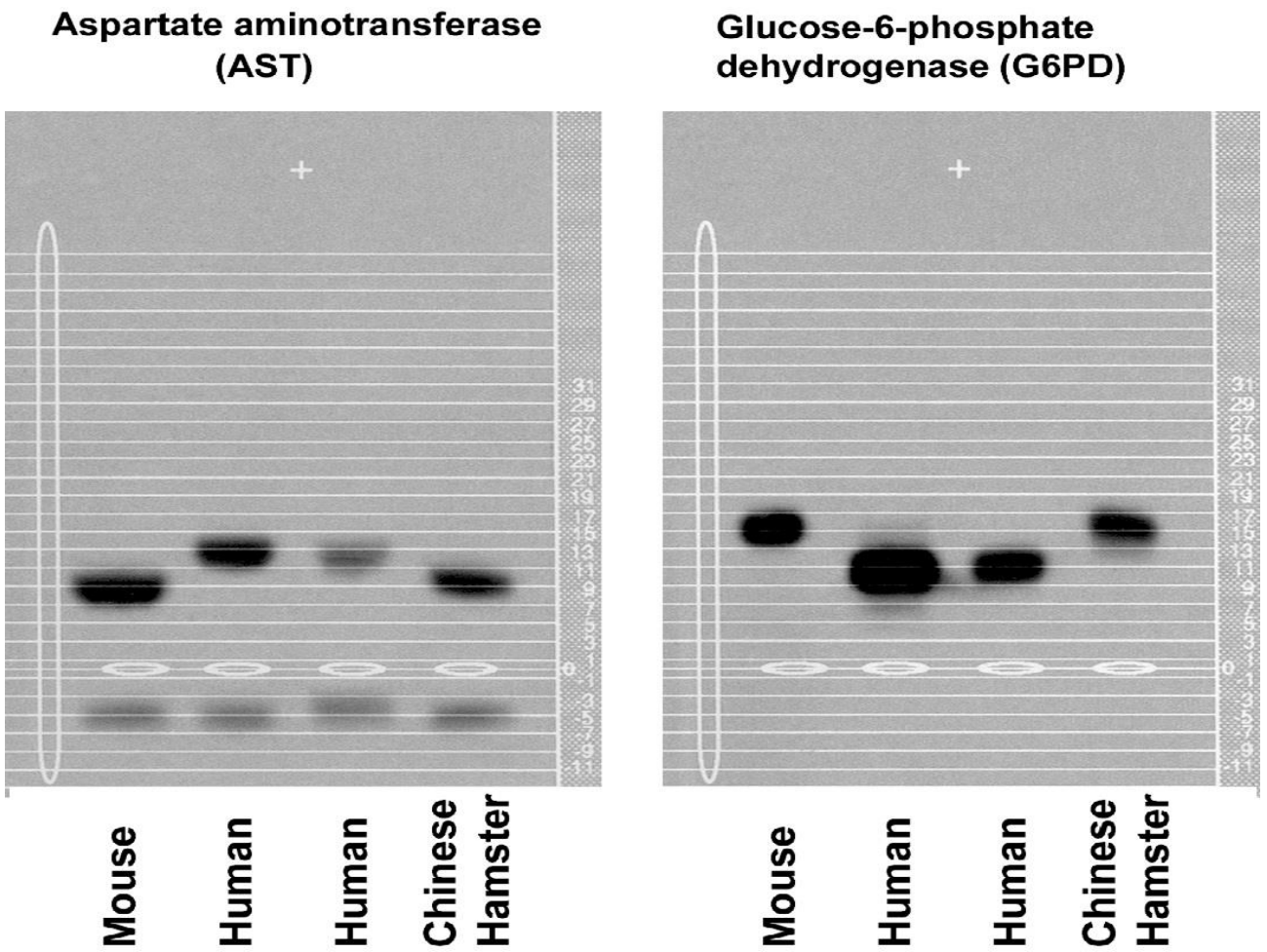


3. Identity Testing

3.1. Confirmation of Species of Origin

Numerous molecular methods are now available for confirming the species of origin based on the amplification by the polymerase chain reaction (PCR) of conserved sequences *and sequencing of specific genes such as cytochrome* oxidase.

Species identification is a useful part of cell authentication, and which method is used will be a decision based on the types of cell lines used in the laboratory, staff time available to carry out in-house testing, and access to appropriate facilities and equipment.

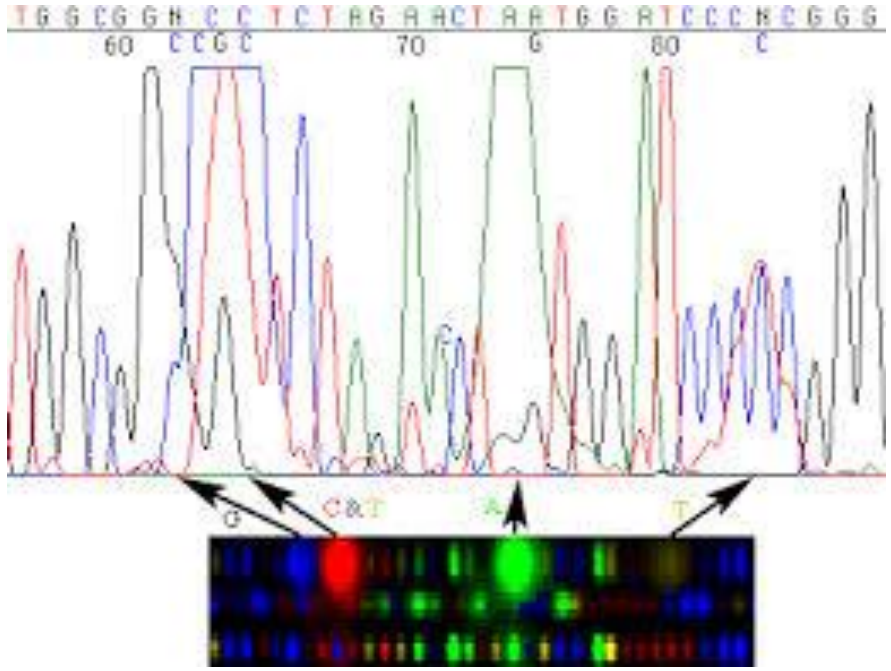


Isoenzyme profiles for cells from mouse, human, and Chinese hamster cell lines.

3. Identity Testing

3.2. DNA Profiling for Cell-Specific Identification

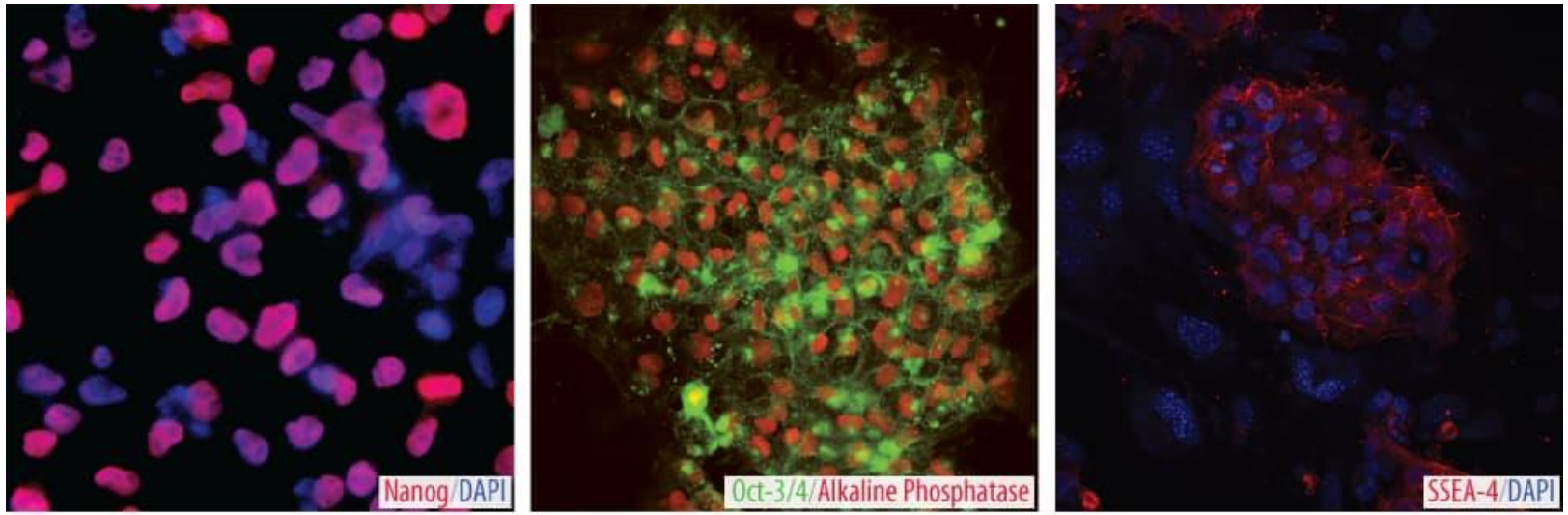
Variable number tandem repeats (VNTRs) and short tandem repeats (STRs) are interesting sequences in the human genome that are comprised of repeated core units of sequences, some of which, when excised from the human genome with certain restriction enzymes, show polymorphism between individuals in the number of repeat units at a particular genomic locus.



STR electropherograms.

3. Identity Testing

3.3. Antibody Markers



An important characteristic of any cell is its profile of antigen expression.
A panel of antibodies has been commonly used to characterize hES cell antigens and show typical patterns of reactivity in such cultures.

Several current markers are largely based on a single precursor (lactosylceramide) that undergoes biochemical modification including glycosylation to create the different epitopes representing the stage-specific early antigens (SSEAs).

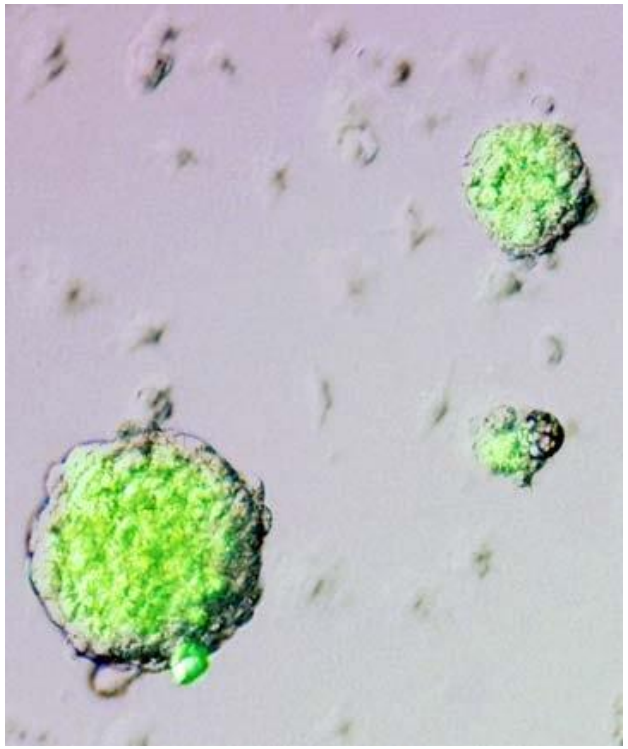
3. Identity Testing

3.4. Pluripotency

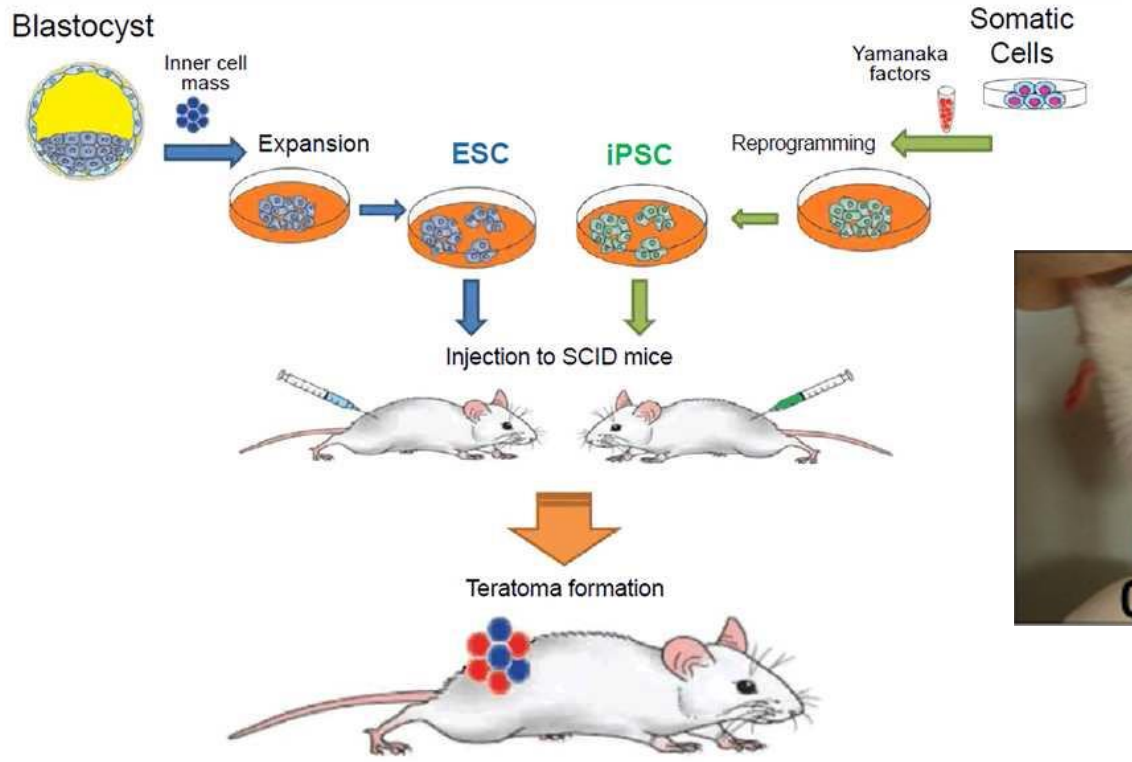
This is clearly a key measure of stem cell line performance in which the expected outcomes may vary depending on the cell type (hES, mesenchymal stem cells, etc.).

There are a number of ways of measuring pluripotency including the following:

- **Teratoma formation in immunocompromised mice**
- **Generation of embryoid bodies with the three germ layers represented**
- **Differentiation in vitro into cell types representing the three germ layers**



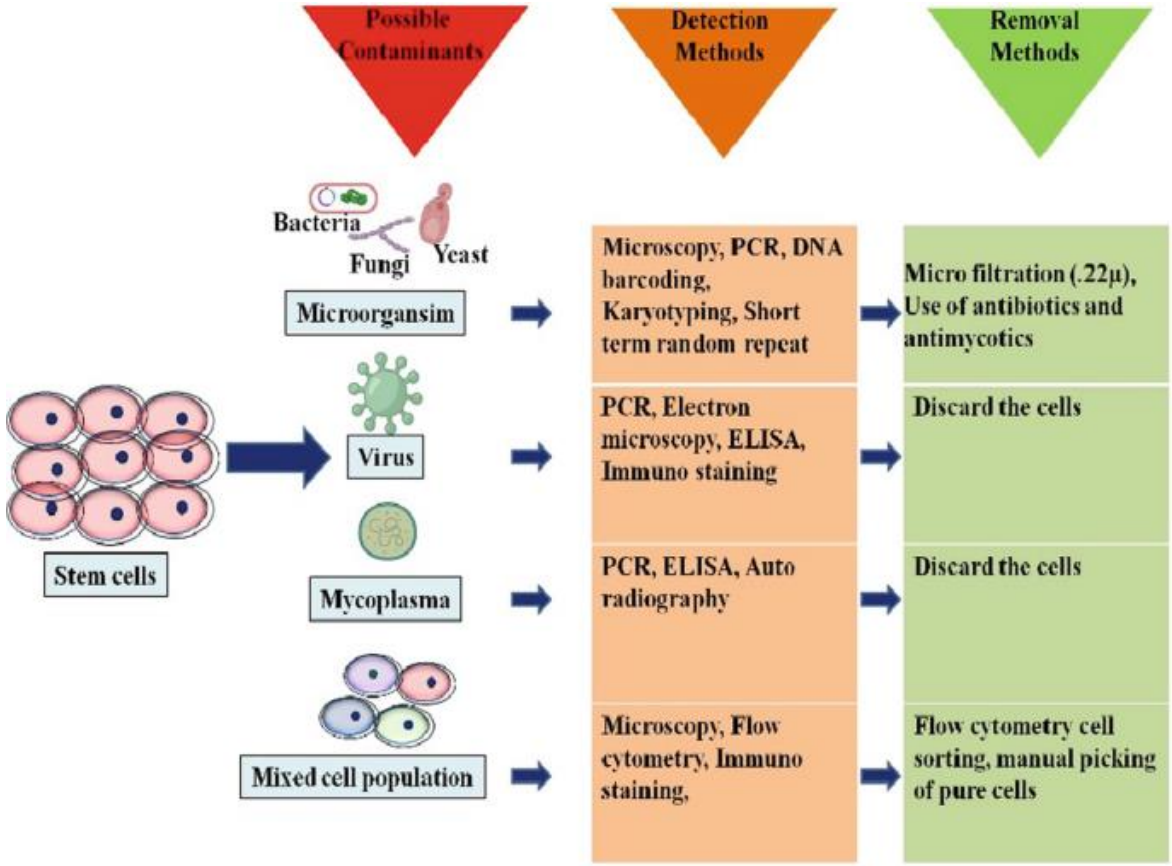
After teratoma growth and explantation, the tissue samples are fixed and embedded in paraffin or cryopreserved. Paraffin embedding followed by sectioning and hematoxylin and eosin (H&E) staining is the standard for verifying the formation of the three germ layers in the explanted teratoma tissue.



4. Sterility Testing

Bacterial and fungal contamination generally prevents work with affected cultures as they become turbid with organisms that completely overwhelm and kill the cells.

The use of antibiotics may be helpful to avoid loss of cells in circumstances where the risk of contamination is high, for example, in primary mouse embryonic feeder cultures or in routine experimental work where environmental contamination is very high.

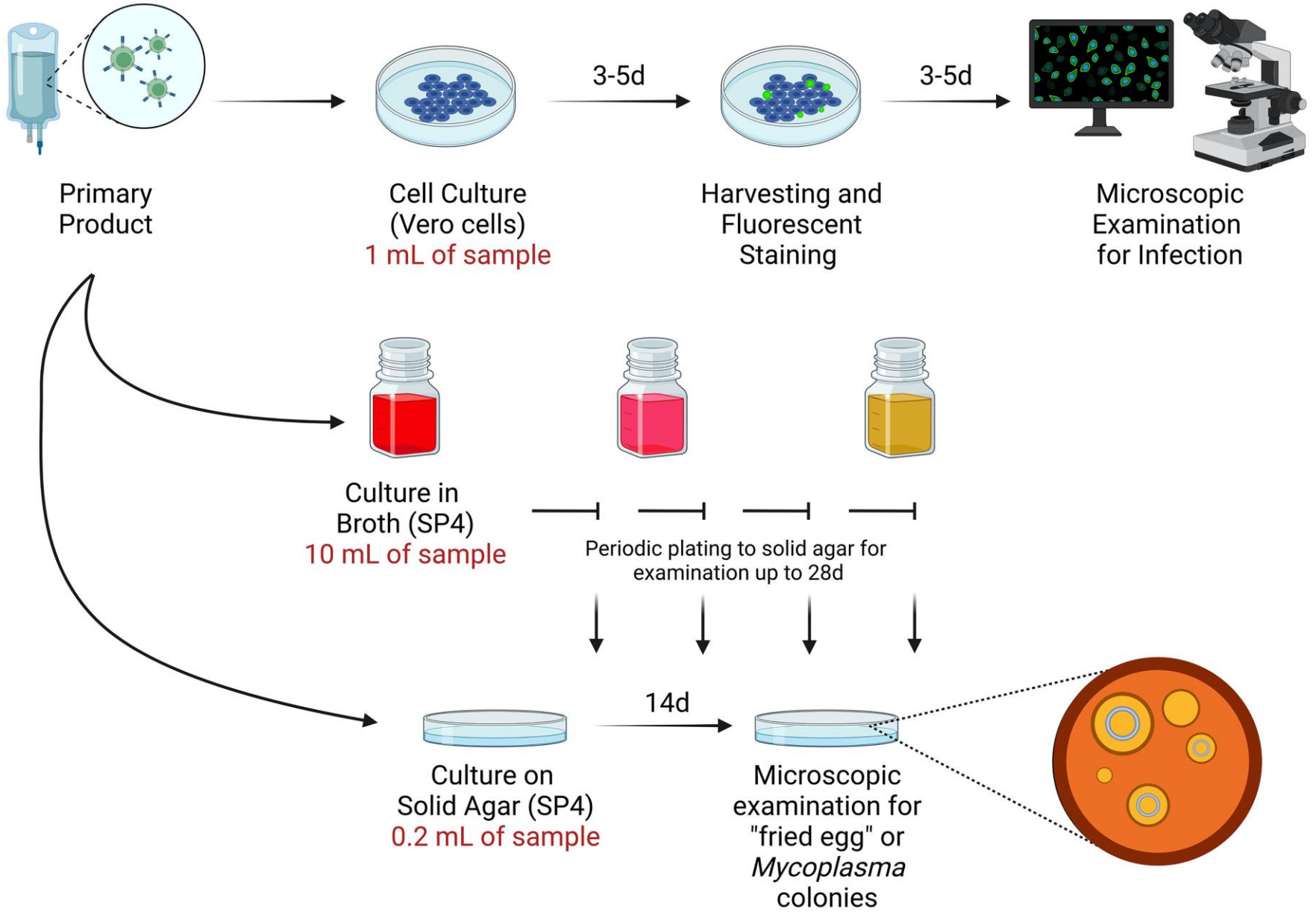


4.1. Mycoplasma detection

There are a number of techniques for mycoplasma detection:

For routine screening, direct PCR or Hoechst 33258 staining *are* useful, but these methods are generally not as sensitive in routine use as culture

Technique	Advantages	Disadvantages
Broth and agar subculture	Highly sensitive	Long incubation periods (approx. 50 days total) Will not detect nonculturable strains
Vero cell culture inoculation and DNA stain	Results in 3 days	Vero test cells must be maintained and prepared
PCR	Large numbers of samples readily screened	Nested PCR may give rise to false positives
Mycoplasma RNA hybridization	Sensitivity is high but may vary	Difficult to discriminate between negative and low positive results


Primary Product
Cell Culture (Vero cells)
1 mL of sample
3-5d
Harvesting and Fluorescent Staining
3-5d
Microscopic Examination for Infection
Culture in Broth (SP4)
10 mL of sample
Periodic plating to solid agar for examination up to 28d
Culture on Solid Agar (SP4)
0.2 mL of sample
14d
Microscopic examination for "fried egg" or *Mycoplasma* colonies

4.2. Endotoxin detection

Endotoxins can affect cellular functions, an endotoxin limit should be established for *in vitro* MSC cultures. For medical devices, the limit is 0.5 EU/mL or 20 EU/device for products that directly or indirectly contact the cardiovascular system and lymphatic system.

The limulus amebocyte lysate assay (LAL assay) is the most commonly used test for endotoxin. LAL (derived from the horseshoe crab) reacts with bacterial endotoxin lipopolysaccharide (LPS), which is a membrane component of gram-negative bacteria, to form a gel-clot which can be quantified.

5. Quality Control of Culture Conditions, Reagents and Media

In cultures of stem cells there is great potential for variability and instability. It is helpful in dealing with these issues to try to control the variation in the nutritional and environmental influences to which the cells are subjected.

For establishing high quality cultures, one must procure high quality culture-grade reagents from reputed manufacturers. Serum is particularly a source of mycoplasma and viruses. Every batch must be stored at appropriate temperature and tested for contaminants before use.

Lot number and date of expiry must be recorded This is also true for culture media, and other reagents such as trypsin. Laboratory grade sterile water must be used for reconstitution of reagents and preparation of buffers.

Finally

As the basic science develops, it will be important to be responsive to review and update quality control methods and establish more quantitative methods for phenotypic analysis that may also become important factors in the future development of stem cell therapy.