細胞產品安全議題---實例說明

財團法人台灣動物科技研究所
王仕蓉

2008/8/19

Outline

- Biosafety issue
  - Autologous/Allogeneic/Xenogeneic
- Biosafety evaluation
  - Donor's Screening test
  - Cell line characterization/final product test
- Sample collection and handling requirement

Cellular or Tissue-based Products

- **Biological components**
  - Viable cell, tissue, cellular products
- **Biomolecule**
  - Nature materials
  - Synthetic materials

Manufacturing Concerns

- No terminal sterilization possible
- No sterile filtration possible
- No viral inactivation possible
- Potential for endogenous contaminants
- Potential for exogenous contaminants
- Potential biohazard

Specific Concern

- Transmission of communicable disease
- Processing control to prevent cross-contamination
- Isolated cells or tissues
  - characterization/function

Transmission of communicable disease

- Autologous ---recommend donor testing and screening
- Allogeneic ---required donor testing and screening
- Xenogeneic---required animal testing and screening

Cross contamination

- **Processing control**
  - IF HCT/P product is regulated solely under section 361 of PHS Act--**GTP**
  - IF HCT/P product is regulated under FDC Act and/or section 351 of PHS Act--**GTP and GMP/QS**

Autologus Applications

- Patient screen for potential hazards - HIV, HBV, HCV, etc.
- Sterility testing of primary cultures and quarantine procedures
- Process design to prevent mix-up
- Facility design focused on small multiple workstations

Allogeneic Applications

- Two-tier cell banking system
- Extensive testing for endogenous contaminants
- prevent exogenous contamination during manufacturing operations
- Final sterility testing to the extent possible

Donor suitability (I)

- Certain HCT/Ps recovered from donor who were tested for communicable diseases using pooled specimens or diagnostic tests【April 2008】
- Use of Nucleic acid tests to reduce the risk of transmission of West Nile Virus from donors of whole blood and blood components intended for transfusion and donors of HCT/Ps 【April 2008】
- Eligibility Determination for donors of human cells, tissues, and cellular and tissue-based products (HCT/Ps) 【Aug 2007】

Donor suitability (I)

"FDA believes that the following meet the standards for identification of relevant communicable disease agent"---

- West Nile Virus
- Sepsis
- CJD/vCJD（Creutzfeldt-Jakob Disease）
- Vaccinia (Smallpox vaccination)
- Severe Acute Respiratory Syndrome (SARS)

Possible CJD risk

- CJD/vCJD（Creutzfeldt-Jakob Disease）
- BSE（Bovine Spongiform Encephalopathy）
  - 1980/UK
  - vCJD
- Cornea & dura mater

Recommendation for Donor ineligibility

- Diagnosed with CJD/vCJD/other neurological disease of CNS
- > three months in the UK
- >5 years in Europe
- Military member >6 months in Europe
- Received any transfusion of blood & blood component in the UK
- Injected bovine insulin from the UK

Tissue Engineering Medical Product 【TEMP】Standard

- What technology do we have and how is it doing?
- What standards exist and what standards are needed?
- What standards are used globally?

ASTM Committee F04 /FDA

Standards

- F2027 - 08 Standard Guide for Characterization and Testing of Raw or Starting Biomaterials for Tissue-Engineered Medical Products
- F2211 - 04 Standard Classification for Tissue Engineered Medical Products (TEMPs)
- F2386 - 04 Standard Guide for Preservation of Tissue Engineered Medical Products (TEMPs)
- F2383 - 05 Standard Guide for Assessment of Adventitious Agents in Tissue Engineered Medical Products (TEMPs)
- E1873 - 06 Standard Guide for Detection of Nucleic Acid Sequences by the Polymerase Chain Reaction Technique

TEMP standardization meeting
27 & 28 Oct 2008 (Teddington, Middlesex, UK)

- WK13589 New Standard Guide for Quantitating Cell Viability Within Biomaterial Scaffolds
- WK8031 New Standard Guide for assessing immunogicity of Tissue Engineered Medical Products
- WK11468 Standard guide to the in situ visualization/quantification of mineralized matrix formed by mesenchymal cell culture
- WK3936 Standard Guide for Assessing the Attachment of Cells to Biomaterial Surfaces by Physical Methods

Xenogeneic Applications

- Public health issues posed by the use of nonhuman primate xenografts in humans
【April 1999】
- PHS guideline on infectious disease issues in xenotransplantion
【Jan 2001】
- Precautionary measure to reduce the possible risk of transmission of zoonoses by blood and blood products from xenotransplantation product recipients and their intimate contacts
【Feb 2002】
- Human Cells or Tissues Intended for Transplant Into a Human Recipient That Have Ex-vivo Contact With Live Nonhuman Animal Cells, Tissues, or Organs
【Feb 2002】
- Source animal, product, preclinical, and clinical issues concerning the use of xenotransplantation products in human
【April 2003】

Definition

- **Transplantation** of xenogeneic heart, kidney, pancreatic tissue
- **Implantation** of neural cells
- Ex vivo cultured human cells with live non-human **feeder cells**
- **Extracoporeal perfusion**

Public health risks

- Pathogenic transmissible organisms
- Immunosuppressed individuals
- Recombinant organisms

Source animal characterization

- Animal welfare concerns
- Animal origin
- Animal history
- Animal health and husbandry
- Tissue harvesting and transportation
- Disposal of animal

Individual Source animal qualification

- Test for infectious agents
  - You should perform all tests at a time as close as possible to the date of harvest.
  - If more than 3 months have elapsed since the initial testing, you should repeat tests before harvest.
- Quarantine
  - you should generally quarantine individual source animal for a minimum of three weeks before harvest of their live cells, tissues, or organs.

Samples to be archived and storage condition

- At least 10 aliquots of citrated- or EDTA-anticoagulated plasma (0.5 mL/aliquot)
- At least 5 aliquots of viable leukocytes ($1x10^7$ cells/aliquot)
- Archived samples
  - stored at -70C (animal tissue & body fluid)
  - Stored at room temp (fixed tissue)
- You should be stored for 50 years from the time of sample acquisition.

Microbiological testing of xenotransplantation products

- Whole organs
  - Testing of the source animal
  - Appropriate relevant biological surrogates
- Xenogeneic cells or tissues
  - You should perform testing periodically during the culture period.(the initial of culture, lot release of the product…)
- Combination products
  - Physical barriers might prevent or reduce transmission of infectious agent
  - Provide the data of validation study.

Epicel ® /Genzyme

Cultured epidermal autografts

- Autologous human keratinocytes
- Co-culture with murine fibroblast

Epicel ® /Genzyme

- **Biosafety test**
  - Sterility test
  - Bact/fungi
  - Mycoplasma
  - Adventitious Virus and retrovirus

✓**Warning ! ! !**

✓Bovine serum

✓Vancomycin

✓Amikacin

Xenogeneic Feeder Cell

- Human embryos co-cultured with living nonhuman animal feeder layer, such as mouse cell line.
  - The history of cell line (Animal species, tissue of derivation…)
  - Free from infectious agents, including murine infectious agents

Dermagraft™/ATS

Living dermal equivalent

- Allogeneic Fibroblast
- Bovine collagen

Dermagraft™/ATS

- **Biosafety test**
  - Donor blood
    - HIV, CMV, HTLV, HBV, HCV, HSV
  - Cell strain
    - Sterility
    - Mycoplasma
    - Adventitous virus

- **Biocompatibility test**
  【 ISO10993 】
  - dermal irritation
  - dermal sensitization
  - hemocompatibility
  - pyrogenicity
  - mutagenecity
  - cytotoxicity
  - systemic toxicity

Apligraf®/Organogenesis Inc.

Living Human Skin Cells

- Human keratinocytes and fibroblasts are derived from neonatal foreskins.
- Cell stocks are frozen and stored in master cell banks for later use.

Apligraf®/Organogenesis Inc.

- **Biosafety test**
  - Donor blood
  - Cell bank characterization
  - In vitro/in vivo adventitious viral detection

- **Biocompatibility test**
  - 【 ISO10993 】
    - dermal irritation
    - dermal sensitization
    - hemocompatibility
    - pyrogenicity
    - mutagenecity
    - cytotoxicity
    - systemic toxicity

Carticel®/ Genzyme

- Autologous cultured chondrocytes
  - Orthopedic cartilage repair

Carticel®

- **Biosafety test**
  - Sterility test
- **Biocompatibility test**
  - Pyrogen test
  - Toxicity test

✓**Warning ! ! !**

- ✓Biohazard
- ✓Bovine serum
- ✓Gentamycin

HepatAssist®/Circe

- **Porcine hepatocyte**
- **Microporous hollow fiber**

HepatAssist®/Circe

- **Animal source**

15~20 Kg

- **Biosafety test**
  - Sterility test
  - Mycoplasma
  - Pyrogen test
  - Human/Animal adventitious virus
  - PERV (porcine endogenous retrovirus)

Test panel

	Test category
Whole animal	PERV, HEV, PCV I/II, porcine CMV, PRRS, PRV, porcine parvovirus, TGEV, swine influenza H1/H3, EMCV, porcine adenovirus, BVDV I/II, VSV
	Leptospira, Brucella, Mycoplasma, Trichinella, Toxoplasma, Cryptosporidium, Campylobacter, Coccidia, Ascaris, Mycobacterium, Actinobacillus, Coccidia, E.Coli, hemophilus parasuis, Salmonella, Streptococcus I/II, Mycobacterium, Erysipelothirx
	Complete blood count, hemoglobulin, parasitology, pathology
Hepatocyte lot	HEV, PCV I/II, porcine CMV, PRRS, PRV, porcine parvovirus, TGEV, swine influenza H1/H3, EMCV, porcine adenovirus, BVDV I/II, VSV, Reovirus, Rabies, Techen/Enterovirus, SVD, poxvirus, porcine rotavirus, Vaccinia,
	Sterility test, mycoplasma, endotoxin test, bioburden test, Gram stain, Fungal stain
patient	PERV, porcine CMV, HEV, Leptospira, Toxoplasma, Brucella

Donor's Screening test

HCT/P Donor Testing

- For all HCT/Ps
  - HIV, types 1 and 2
  - HBV
  - HCV
  - Human TSE, including CJD
  - Treponema pallidum (agent of syphilis)
- For viable, leukocyte-rich HCT/Ps
  - HTLV, types I and II
- For reproductive HCT/Ps
  - Chlamydia trachomatis
  - Neisseria gonorrhea

Donor Testing - General

- Donor testing must be performed at lab
  - Registered with FDA
  - CLIA certified or equivalent
- Donor tests
  - FDA-licensed, approved donor SCREENING tests (not diagnostic)
  - Used in accordance with the instruction
  - Should be labeled for use for cadaveric donors if such a test is available
  - Recommendations for specific tests may change with time and increasing technology

Screening vs Diagnostic test kits

- Clinical trials to support donor screening test kits perform testing in a "pre-screened", low prevalence population (emphasis on sensitivity)
- Clinical trials to support diagnostic test kits generally perform testing in a symptomatic population with suspicion of having a particular disease before the test is performed (more emphasis on specificity)

FDA licensed screening tests

- Specifically recommended tests include for
  - HIV types 1 and 2 (anti-HIV-1 *and* anti-HIV-2 or licensed combination test)
  - HBV (HBsAg *and* anti-HBcore)
  - HCV (anti-HCV)
  - *Treponema pallidum* serological test for syphilis (Donor with reactive non-Treponemal screening test and nonreactive specific Treponemal confirmatory test is permitted to donate)

Licensed Donor Screening Tests ---HBV

- **HBsAg Assays**

  (Detects Hepatitis B surface Antigen)

- **Anti-HBc Assays**

  (Detects antibodies to Hepatitis B core antigen)

- **Hepatitis B Virus Nucleic Acid Tests**

  (Direct detection of HBV DNA)

ChLIA: Qualitative chemiluminescent

EIA: Enzyme-Linked Immunosorbent Assay

HBsAg Assays (Detects Hepatitis B surface Antigen)

Tradename(s)	Format	Specimen Collection	Donors	Manufacturer	Approval Date
Genetic Systems HBsAg EIA 3.0	EIA	Living: Serum, plasma, Cadaveric: serum	Living, Cadaveric	Bio-Rad Laboratories	1/23/ 2003
ORTHO Antibody to HBsAg ELISA Test System 3	EIA	Living: Serum, plasma	Living	Ortho-Clinical Diagnostics, Inc	4/23/ 2003
Abbott Prism HBsAg Assay (Screening Test)	ChLIA	Living: Serum, plasma Cadaveric: serum	Living, Cadaveric	Abbott Laboratories	7/18/ 2006

Anti-HBc Assays

(Detects total IgG+IgM antibodies to Hepatitis B core antigen)

Tradename(s)	Format	Specimen Collection	Donors	Manufacturer	Approval Date
Ortho HBc ELISA Test System	EIA	Living: Serum, plasma	Living,	Ortho-Clinical Diagnostics, Inc	4/18/1991
ABBOTT PRISM HBcore	ChLIA	Living: Serum, plasma,	Living,	Abbott Laboratories	10/13/2005

*Currently, there is no donor screening test for Anti-HBc specifically licensed for use in testing cadaveric specimens from nonliving donors of HCT/Ps

Hepatitis B Virus Nucleic Acid Tests (Direct detection of HBV DNA)

Tradename(s)	Format	Specimen Collection	Donors	Manufacturer	Approval Date
COBAS AmpliScreen HBV Test	PCR	*Living:* Plasma *Cadaveric:* Serum, EDTA plasma	Living, Cadaveric	Roche Molecular Systems Inc	8/16/ 2007

1. The COBAS AmpliScreen HBV Test is a qualitative *in vitro* test for the direct detection of Hepatitis B Virus (HBV) DNA in human plasma.
2. This product is intended for use as a donor screening test to detect HBV DNA in plasma samples from individual human donors, including donors of whole blood and blood components, source plasma and other living donors. It is also intended for use to screen organ donors when specimens are obtained while the donor's heart is still beating.
3. This test is not intended for use on specimens from cadaveric (non-heart-beating) donors. This test is not intended for use on samples of cord blood.

Licensed Donor Screening Tests ---HCV

- Hepatitis C Virus (HCV)

  (Detects antibody to HCV)

- Hepatitis C Virus Nucleic Acid Testing

  (Direct detection of HCV RNA)

Anti-HCV Assays
(Detects antibody to HCV)

Tradename(s)	Format	Specimen Collection	Donors	Manufacturer	Approval Date
Ortho HCV Version 3.0 ELISA Test System	EIA	*Living:* Serum, Plasma	Living	Ortho-Clinical Diagnostics, Inc	5/20/ 1996
Abbott HCV EIA 2.0	EIA	Living: Serum, plasma, Cadaveric: serum	Living, Cadaveric	Abbott Laboratories	7/22/ 2004
ABBOTT PRISM HCV	ChLIA	*Living:* Serum, Plasma *Cadaveric:* Serum	Living Cadaveric	Abbott Laboratories	7/11/ 2007

*** Abbott Laboratories**
Hepatitis C Virus Encoded Antigens (Recombinant c100-3, HCr43, NS5)

Hepatitis C Virus Nucleic Acid Testing (Direct detection of HCV RNA)

Tradename(s)	Format	Specimen Collection	Donors	Manufacturer	Approval Date
Procleix HIV-1/HCV Assay	TMA	*Living:* Plasma *Cadaveric:* Serum, EDTA Plasma	Living Cadaveric	Gen-Probe, Inc	6/4/ 2004
COBAS Ampliscreen HCV Test, version 2.0	PCR	*Living:* Plasma *Cadaveric:* Serum, EDTA Plasma	Living Cadaveric	Roche Molecular Systems	5/22 /2007

1. The PROCLEIX® HIV-1/HCV Assay involves three main steps which take place in a single tube
2. HIV-1 and HCV RNA target amplification by Transcription-Mediated Amplification (TMA); and detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA).

Licensed Donor Screening Tests
Human Immunodeficiency Virus (HIV)-1 AND 2

- **Anti-HIV-1/2 Assays**

  (Detects antibodies to HIV Types 1 & 2)

- **HIV-1 Nucleic Acid Tests**

  (Direct detection of HIV-1 RNA)

HIV-1 Nucleic Acid Tests (Direct detection of HIV-1 RNA)

Tradename(s)	Format	Specimen Collection	Donors	Manufacturer	Approval Date
COBAS AmpliScreen HIV-1 Test, version 1.5	PCR	*Living:* Plasma *Cadaveric:* Serum, EDTA Plasma	Living, Cadaveric	Roche Molecular Systems	5/23/ 2007
Procleix Ultrio Assay	TMA	*Living:* Plasma, Serum *Cadaveric:* Serum, EDTA Plasma	Living, Cadaveric	Gen-Probe, Inc.	10/3/ 2006

Anti-HIV-1 & -2 Assays (Detects antibody to HIV-1 & -2)

Tradename(s)	Format	Specimen Collection	Donors	Manufacturer	Approval Date
Genetic Systems HIV-1/HIV-2 Plus	EIA	*Living:* Serum, Plasma *Cadaveric:* Serum	Living, Cadaveric	Bio-Rad Laboratories	8/5/ 2003
HIVAB HIV-1 / HIV-2 (rDNA) EIA	EIA	*Living:* Serum, Plasma *Cadaveric:* Serum	Living, Cadaveric	Abbott Laboratories	7/22/ 2004

Cell line characterization
Final product test

Primary cells or Cell lines

- Points to consider in the characterization of cell line used to produce biologicals. 【FDA/CBER/1993】
- Q5A Viral safety evaluation of Biotechnology products derived from cell lines of human or animal origin. 【ICH/1998】
- Q5D Quality of Biotechnological/Biological products: Derivation and characterization of cell substrates used for production of Biotechnological/Biological products. 【ICH/1998】

Sterility test

- Bacteria and Fungi
  - Direct inoculation method
  - Bioburden assay
  - Endotoxin detection

§ 9 CFR 113.26

§ 21CFR 610.12/610.13

Endotoxin test

- In vivo pyrogen test (rabbit)
- LAL (Limulus Amebocyte Lysate) assay
  - Gel clot
  - Absorbance
  - Kinetics

Mycoplasma Detection

- **Cultivation assay**
- **Fluorochrome staining**
- **PCR**

§ Point to Consider in the characterization of cell lines used to produce biologicals, 1993

§ 21CFR 610.30

§ 9 CFR 113.28

Adventitious virus

- Human viruses
  - § Regulation of cellular and tissue-based products, 1997
  - § Human tissue intended for transplantation (21CFR1270)
- Animal viruses
  - § PHS Guidance on infectious diseases issues in xenotransplantation, 1997
  - § Medical devices containing materials derived from animal sources, 1998

Sources of viral contamination (I)

- **Source material may be contaminated with a virus indigenous to the species of origin.**
  - Blood
  - Feeder cell
- **Cell may have a latent or persistent infection.**
  - Herpes virus
  - retrovirus

Sources of viral contamination (II)

- The process of construction of a production cell line may introduce a contaminant virus indigenous to another species.
  - Murine retrovirus
- Adventitious viruses may be introduced by the use of contaminated animal products in the production process.
  - Bovine serum
- Other sources of contamination
  - operating personnel

Detection of viral contamination

- Co-culture assay
- Immunoassay
- Nucleic acid detection
- Reverse transcriptase activity
- Transmission electron microscopy
- In vivo assay (mice, guinea pigs, embryo chicken eggs)

Co-culture assay

- MRC-5 cells
- 3T3 cells
- Vero cells

Q-PCR assays for human viruses

Human virus	Target gene/region
Human immunodeficiency virus	gag gene (HIV-1) /env gene (HIV-2)
Epstein-Barr virus	BALF5 gene
Hepatitis type A	5’ NCR
Hepatitis type B	core gene
Hepatitis type C	5’ NCR
Enteroviruses	5’ Untranslated region
Human T-cell leukaemia virus	tat gene
Human adenovirus	E1
Adeno-associated virus	Cap

Q-PCR assays for animal viruses

Animal virus	Target gene/region
Xenotropic murine leukemia virus	U3 LTR region
Porcine endogenous retrovirus	Gag gene
Bovine viral diarrhoea virus	5'UTR
SV40	Large T-antigen
Porcine cytomegalovirus	OF-1 gene
Bovine polyomavirus	VP1 gene
Rat endogenous retrovirus	Gag gene
Porcine / bovine circovirus	Rep gene
Bovine herpes 1 and 4	TK gene

Retrovirus testing

- Reverse transcriptase assay
- PG-4 S+L- focus assay
- XC plaque assay
- Transmission electron microscopy

In vivo assays for the detection of viral contaminants (I)

- **Detection of viruses by using embryonated chicken eggs**
  - virus detected: Arboviruses, Herpes simplex type 1, Herpes simplex type 2, Influenza, Mumps Newcastle disease, Parainfluenza 1(sendai), Parainfluenza 2, Rabies, Vaccinia
- **Detection of viruses by using suckling mice**
  - virus detected: Arboviruses, B Virus, Coxsackie A Virus, Coxsackie B Virus, Foot and mouth disease virus, Herpes simplex type 1, Herpes simplex type 2, Junin, Machupo, Variola, Rabies, Vaccinia

In vivo assays for the detection of viral contaminants (II)

- Detection of viruses by using adult mice
  - Arboviruses, Encephalomyocarditis, Herpes simplex type 1, Herpes simplex type 2, Lassa, LCMV, Rabies
- Detection of viruses by using guinea pigs
  - Arboviruses, B Virus, Ebola, Encephalomyocarditis, Junin, Lassa, Marburg, Rabies

Rodent viruses detection (I)

- **Mouse Antibody Production (MAP)**
  - Ectromelia Virus, Hantaan Virus, K Virus, Lactic Dehydrogenase Virus (LDM), K Virus, Lactic Dehydrogenase Virus (LDM), Lymphocytic Choriomeningitis Virus (LCM), Minute Virus of Mice, Mouse Adenovirus (MAV), Mouse Cytomegalovirus (MCMV), Mouse Encephalomyelitis Virus (Theilers, GDVII), Mouse Hepatitis Virus (MHV), Mouse Rotavirus (EDIM), Pneumonia Virus of Mice (PVM), Polyoma Virus, Reovirus Type 3 (Reo 3), Sendai Virus Rat Antibody Production (RAP)

Rodent viruses detection (II)

- Hamster Antibody Production (HAP)
  - Lymphocytic Choriomeningitis Virus (LCM), Pneumonia Virus of Mice (PVM), Reovirus Type 3 (Reo3), Sendai Virus (SV5))

Rodent viruses detection (III)

- Rat Antibody Production (RAP)
  - virus detected:Hantaan Virus, Kilham Rat Virus (KRV), Mouse Encephalomyelitis Virus (Theilers, GDVII), Pneumonia Virus of Mice (PVM), Pneumonia Virus of Mice (PVM)Rat Coronavirus (RCV), Reovirus Type 3 (Reo3), Sendai Virus, Sialodacryoadenitis Virus (SDA V), Toolan Virus (HI)

Cytogenetics

- Confirm cell identity
- Assess heterogeneity

§ 21 CFR 610.14

§ cGMP cell banking program

§ Points to considers in the characterization of cell lines used to produce biologicals, 1993.

Assays

- DNA Fingerprinting
- Isoenzyme analysis
- Karyology

Sample collection & handling requirement

Donor's samples

- Serum or plasma specimens may be stored for **up to 14 days at 2 to 8˚C**. However, if storage periods of greater than 14 days are anticipated, the specimens should be stored frozen at -10 ˚C or colder.
- Cadaveric serum specimens may be stored for **up to five days at 2 to 8˚C**. However, if storage periods of greater than five days are anticipated, the specimens should be stored frozen at -20˚C or colder

HCV EIA 2.0 (Abbott Laboratories)

Individual sample vs pool samples

- **Procleix® WNV NAT Assay**

  "…The assay is intended for use in testing individual donor samples. It is also intended for use in testing pools of human plasma comprised of equal aliquots of not more than 16 individual donations from volunteer donors of whole blood and blood components."

- **COBAS AmpliScreen HIV-1 Test, version 1.5**

  - **…Plasma from all donors may be screened as individual sample. For donations of Whole blood and blood components, plasma may be tested in pools comprised of equal aliquots of not more than 24 individual donations. For donations of Source Plasma, plasma may be tested in pools comprised of equal aliquots of not more than 96 individual donations.**

In-process and final products (I)

Test	Sample Requirements	Shipment Temp
Sterility test	1.For cell testing, cells must be grown in the absence of antibiotics for at least two passages prior to testing. 2.Sterility testing will be performed on Individual containers. 3.1% of the bank or no less than 2 containers will be tested. 4. Sample requirements for 20 mL is recommended for sterility test.	Cold
Isoenzyme assay	1.Minimum of $2x10^6$ cells 2.Cell viability > 10%	Ambient

In-process and final products (II)

Test	Sample Requirements	Shipment Temp
Mycoplasma test (culture & Hoechst stain)	1. If the test article includes cells, passage twice in media free of antibiotics before sample submission 2. Cell culture should be confluent at the time of harvest. It is at least 3-4 days since the last medium change. 3. The cells should be harvested by scraping into conditional medium. (trypsin should not be used) 4. Freeze in polypropylene screw-capped vials, allowing some air space for expansion.	cold

What kind of specimen does the test require?

- Is it FDA-licensed, cleared, or approved for donor screening purposes?
- Is it approved for cadaveric specimens?
- What kind of specimen does the test require?
- Should the test be performed using individual donor ?
- specimens or is the use of pooled specimens acceptable?
- What specimen storage/handling requirements should you consider ?

Thank you very much