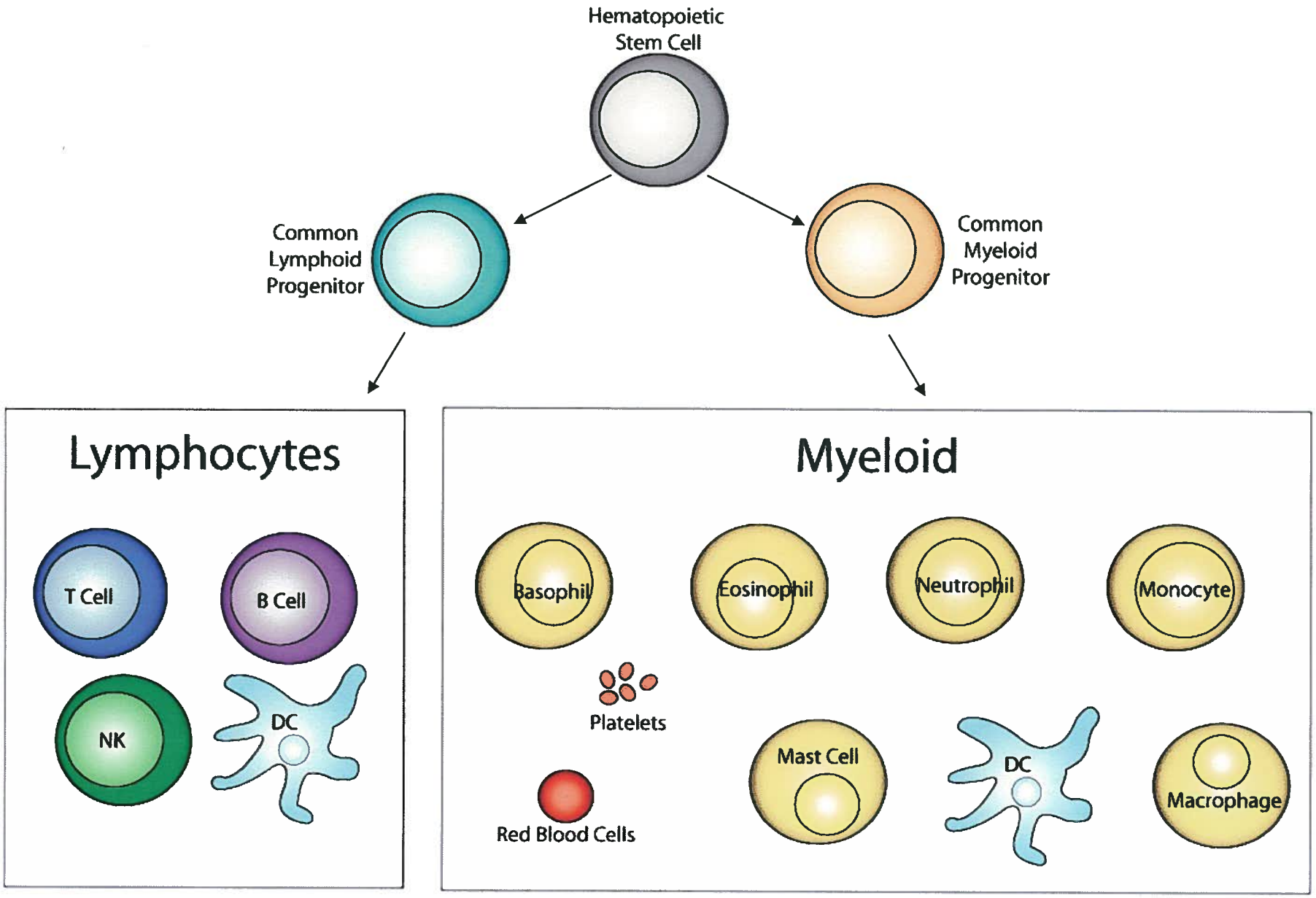
Bone marrow transplantation

Xuguang Tai, Terry Guinter and Alfred Singer

Experimental Immunology Branch
Phone: 301-402-0534
Email: taix@mail.nih.gov

Radiation chimera are prepared by subjecting recipient mice to sublethal or lethal dose of X-ray or γ-irradiation and injecting them with bone marrow cells from donor mice.

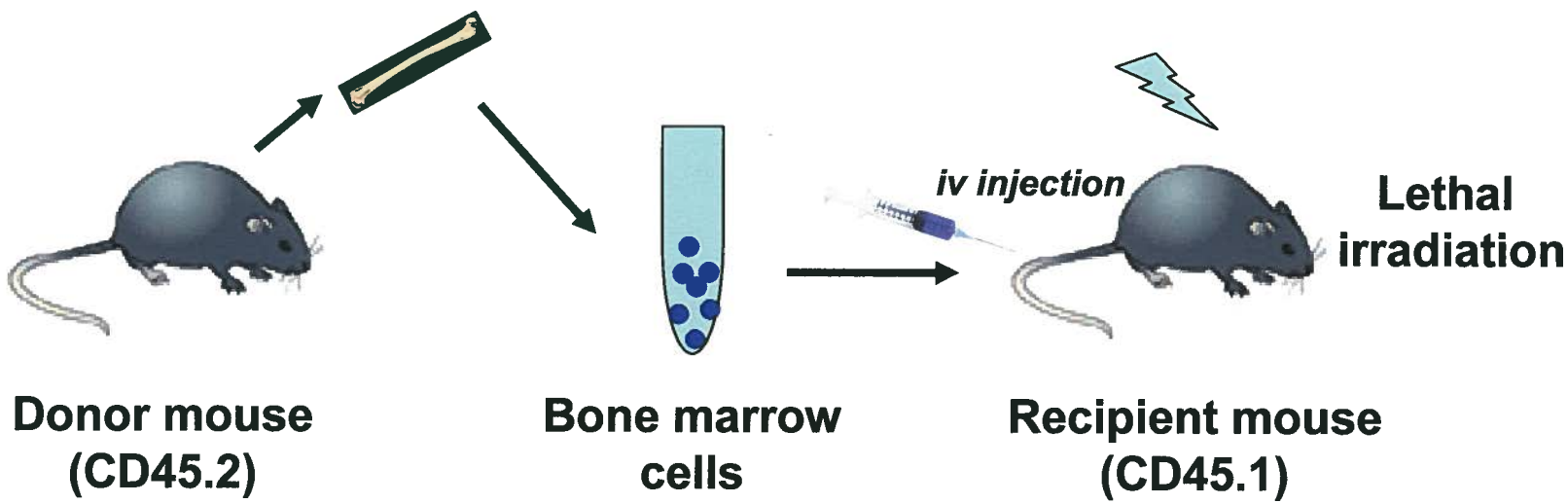


By Tom McCaughtry

Mice needed for bone marrow chimera:

Recipient mice.
Donor mice.

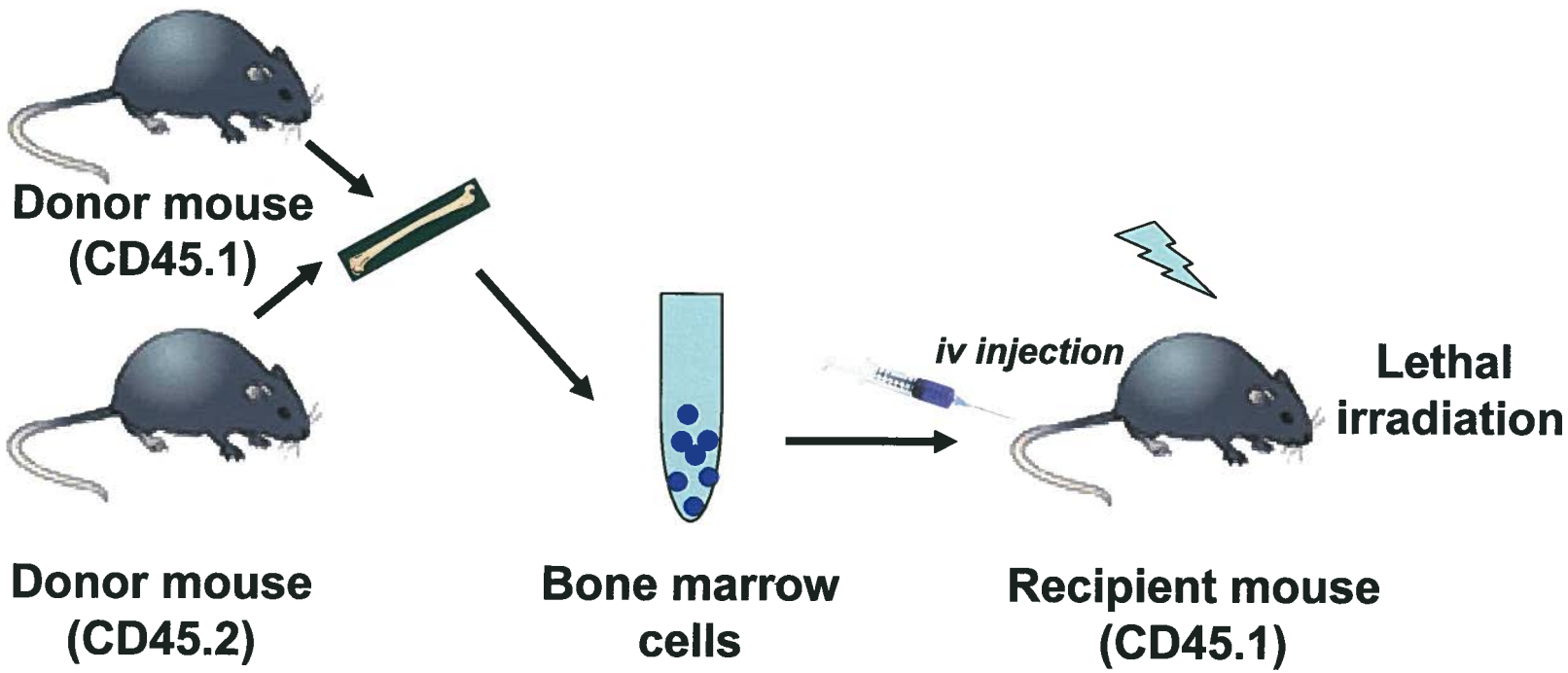
1. ***One donor strain***



Mice needed for bone marrow chimera:

Recipient mice.
Donor mice.

2. ***Two or even three different donor strains.***
   Transplantation of mixed bone marrow
   are often done with two different mouse strains with congenic marker
   (such as CD45.1 versus CD45.2; Thy1.1 versus Thy1.2).



Recipients:

Materials needed for preparation of recipient mice:

1. **X-ray machine or cesium source for γ-irradiation.**

   These procedures must be performed only by personnel trained in the proper use of radiation

2. **Medicated water.**

   Sulfamethoxazole and trimethoprim oral suspension (200mg/ml/40mg/ml) added to 8 gallon acidified water bottle (3.4ml per bottle) given every other week for dose of 40mg/8mg per kg. 1mg/0.2mg per 25g mouse.

   Animals are always maintained on water acidified by sulfuric acid to pH 2.4 to 3.1.

   To prevent *Pneumocystis carinii* and *Pseudomonas aeruginosa* infection.

Lung from Pneumocystis carinii infected mice

Recipients:

1. Choose recipient C57BL/6 mice that are at least 8-10 weeks old.
2. One week before irradiation, feed the mice with medicated water.
3. In the morning of the experiment, at least six hours before the injection of bone marrow cells, irradiate recipient mice using an X-ray machine or cesium source.
4. Irradiate recipient mice at a time at 950 Rad if C57BL/6 mice are used. When immuno-deficient mice such as RAG KO mice are used, make sure only irradiate recipient mice at a time at 600 Rad.

   The lowest permissible dose for thymic repopulation is 400 Rad, however, significant recovery of host cells will occur at this radiation level. Lethal dose of radiation (900 to 1200 Rad) will prevent endogenous recovery of host cells, but require that the animal colony be free of specific pathogens. Radiation can be delivered in two equal doses given 3hr apart to enhance the animal survival even at higher cumulative irradiation doses. BALBc mice are sensitive to radiation, and radiation should be delivered in two equal doses (450Rad) given 2-3hr apart.
5. Take the mice back to colony and maintain the animals with medicated water.

Container for recipient mice radiation

Preparation of donor cells:

Euthanasia of donor mouse:

Euthanasia of laboratory animals must be performed by trained personnel using appropriate techniques, equipment, and reagents in order to effect a death that is humane and satisfies research requirement.

Acceptable methods of euthanasia are painless, and are quick and easy to perform.

Carbon dioxide asphyxiation or cervical dislocation are often used techniques.

Preparation of donor cells:

Materials

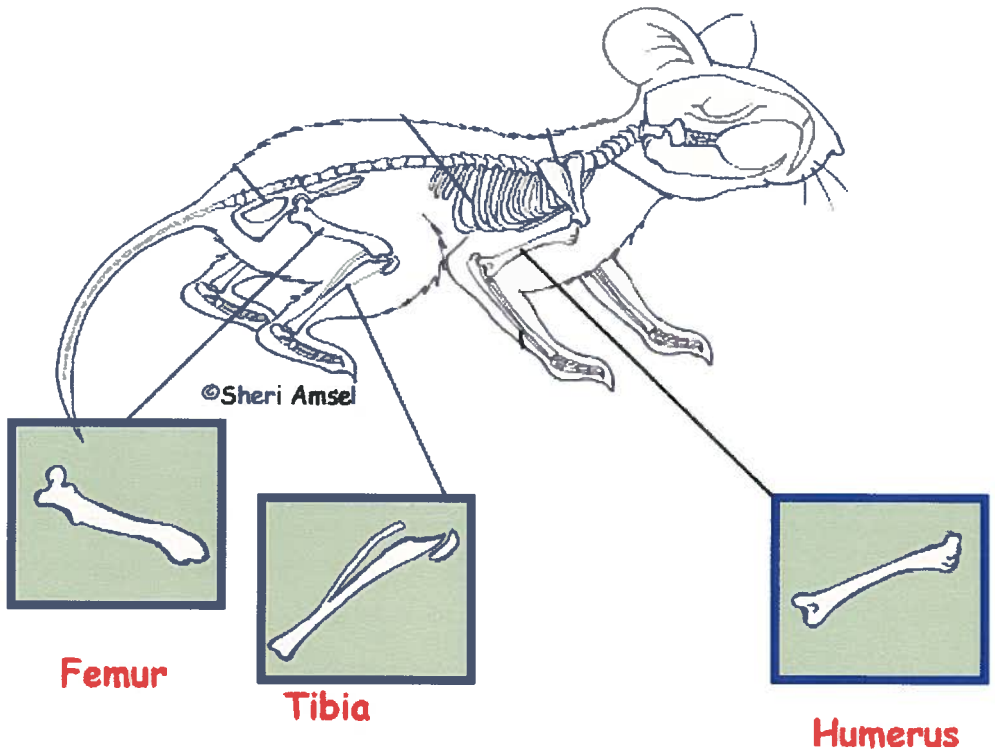
1. **Hanks' balanced salt solution (HBSS)**
2. **ACK lysing buffer**
3. **Scissors**
4. **Forceps**
5. **70% ethanol**
6. **Tissue culture plate**

Preparation of donor cells:

1. Prepare bone marrow from donors.

Harvest two femurs & two tibias from one mouse. If necessary, you can also harvest humerus, radius and sternum to get more donor cells.

Bones for isolation of bone marrow



Preparation of donor cells:

1. Prepare bone marrow from donors.

Put bones in a dish of sterile HBSS. You can have antibiotics in your buffer but without fetal calf serum.

Flush marrow out of the bones with a syringe/27g needle into another dish of HBSS. Pass marrow through a 19g needle to break up the clumps.

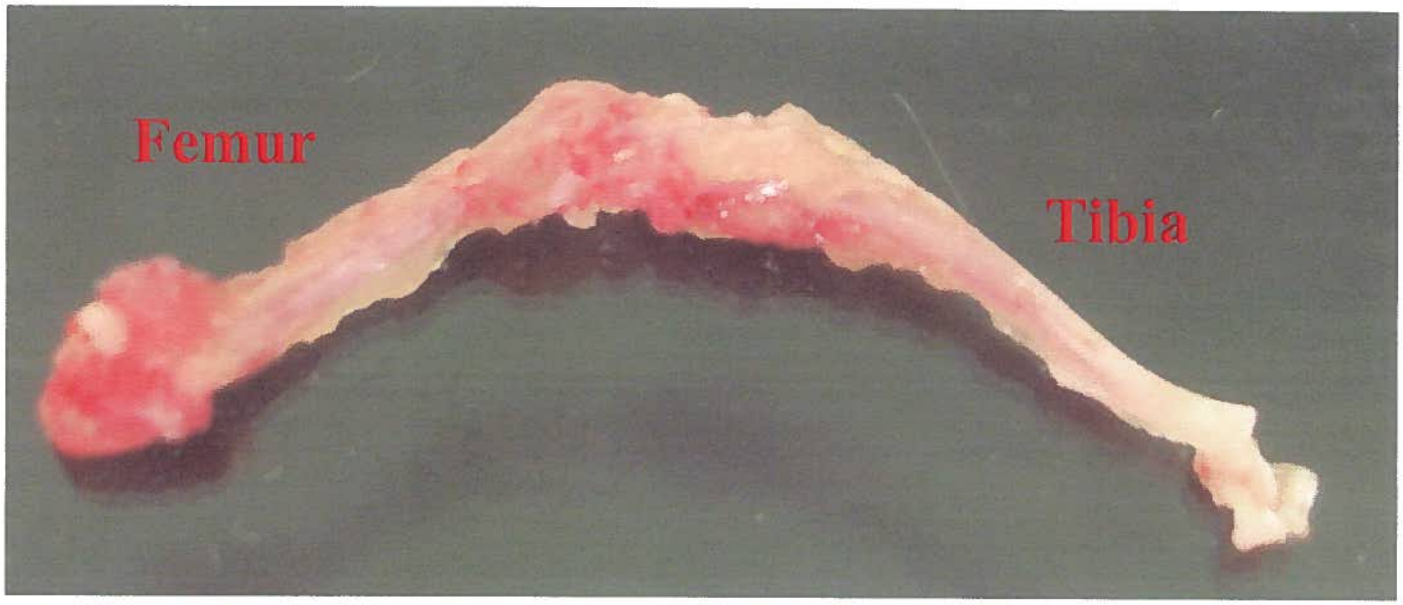
Filter the cell suspension through nylon mesh (Cell Strainer) to make single cell suspension.

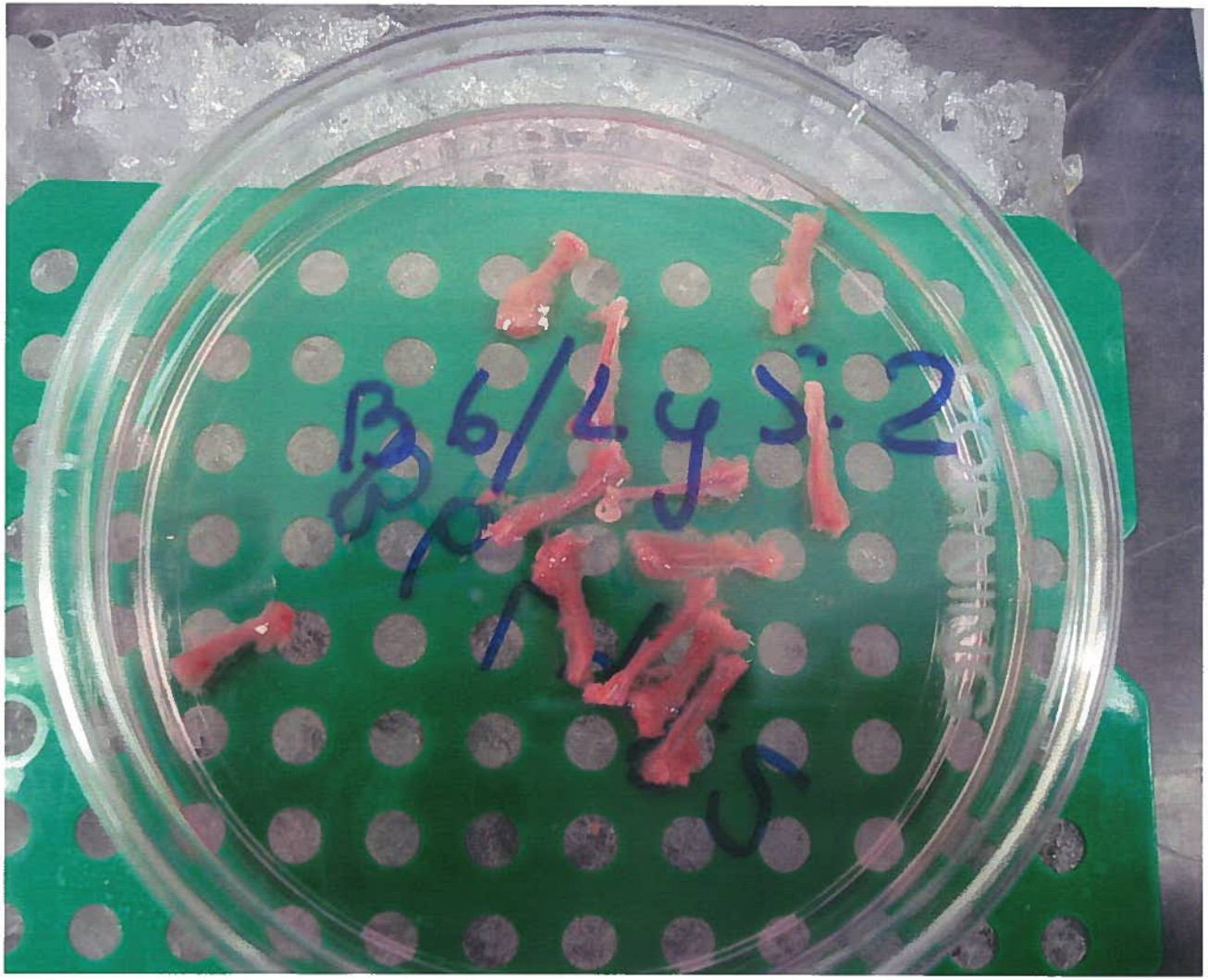
In general, at this step, you should have about 30-40 million but no more than 50 million bone marrow cells from one donor.

Spin down the cells and re-suspend in media.

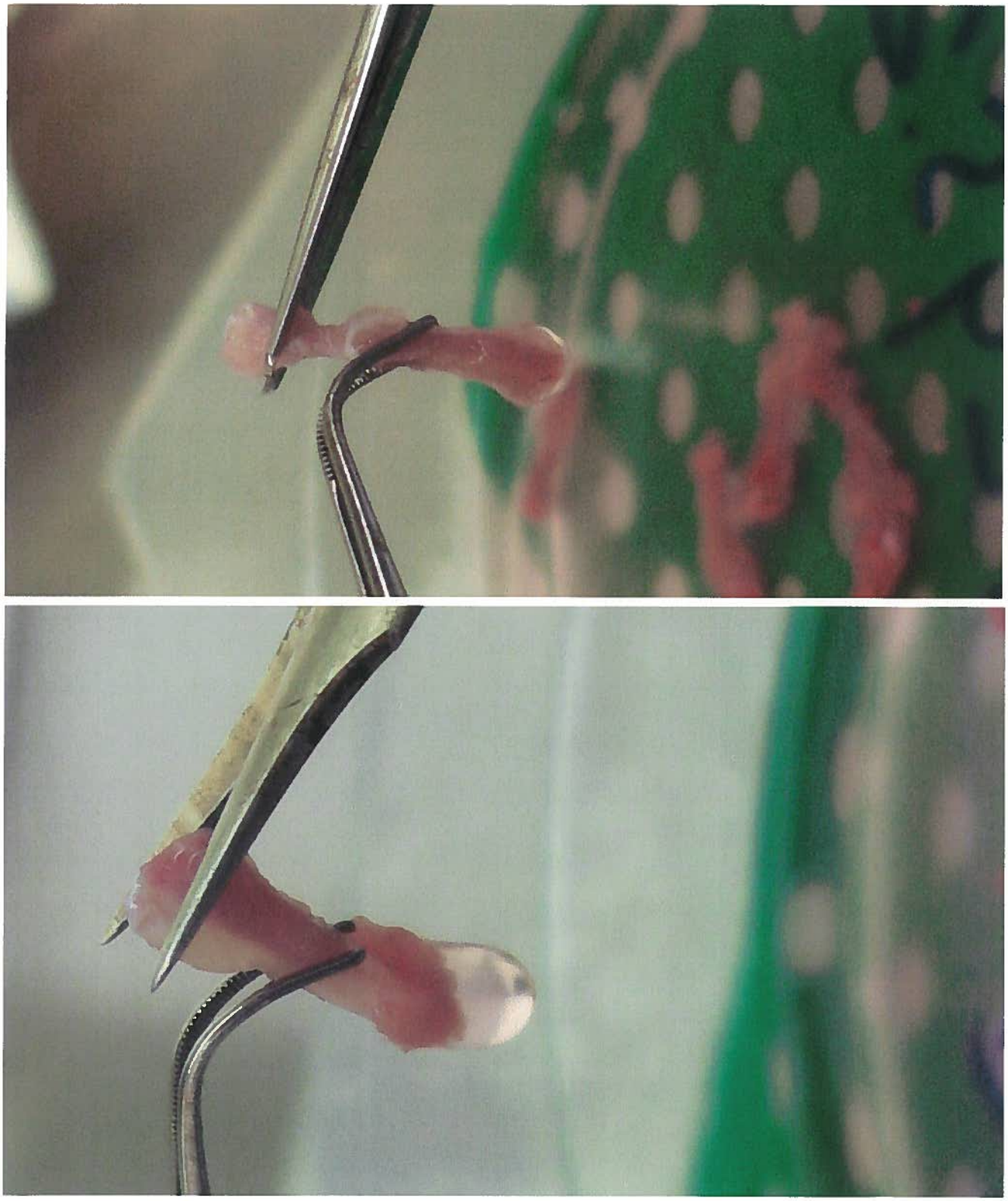
CAUTION
DO NOT USE
HOOD WITH
LIGHT ON

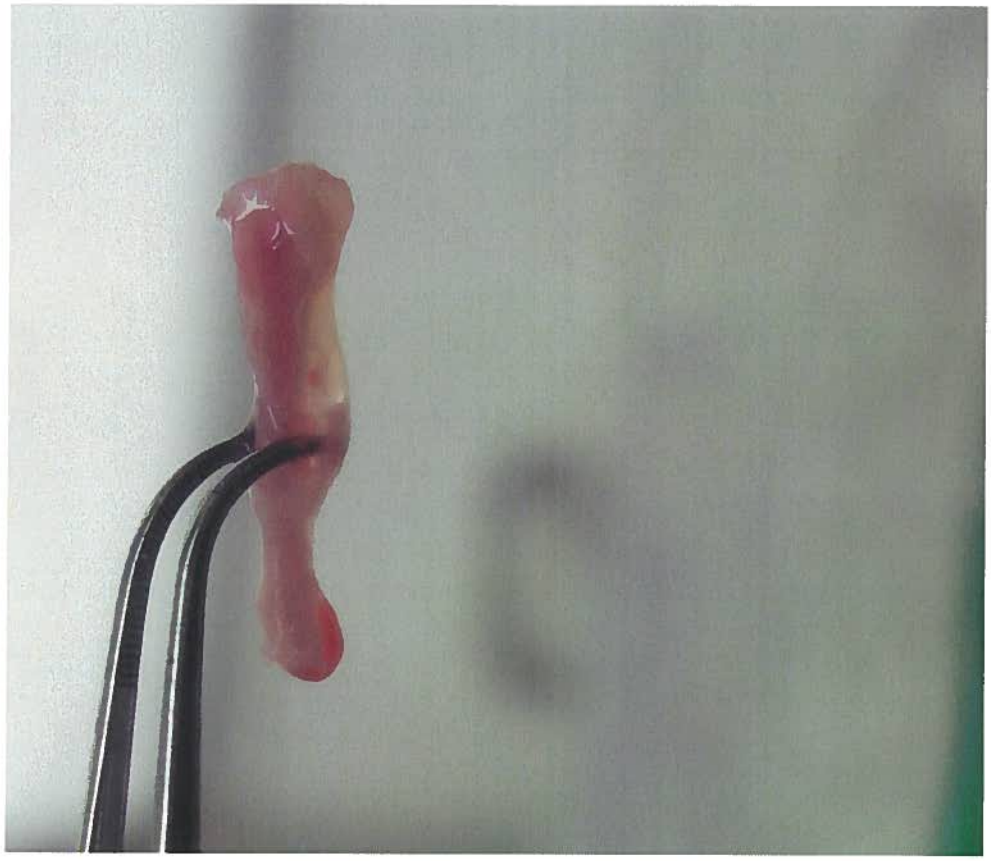
10-28-09
GETINGE STEAM MONITOR STRIP Catalog No. 61301600018
For use in pressure steam autoclaves, place a Steam Monitor Strip in every pack. If the white line across
the strip turns uniformly black it is an indication of attainment of steam penetration into the pack

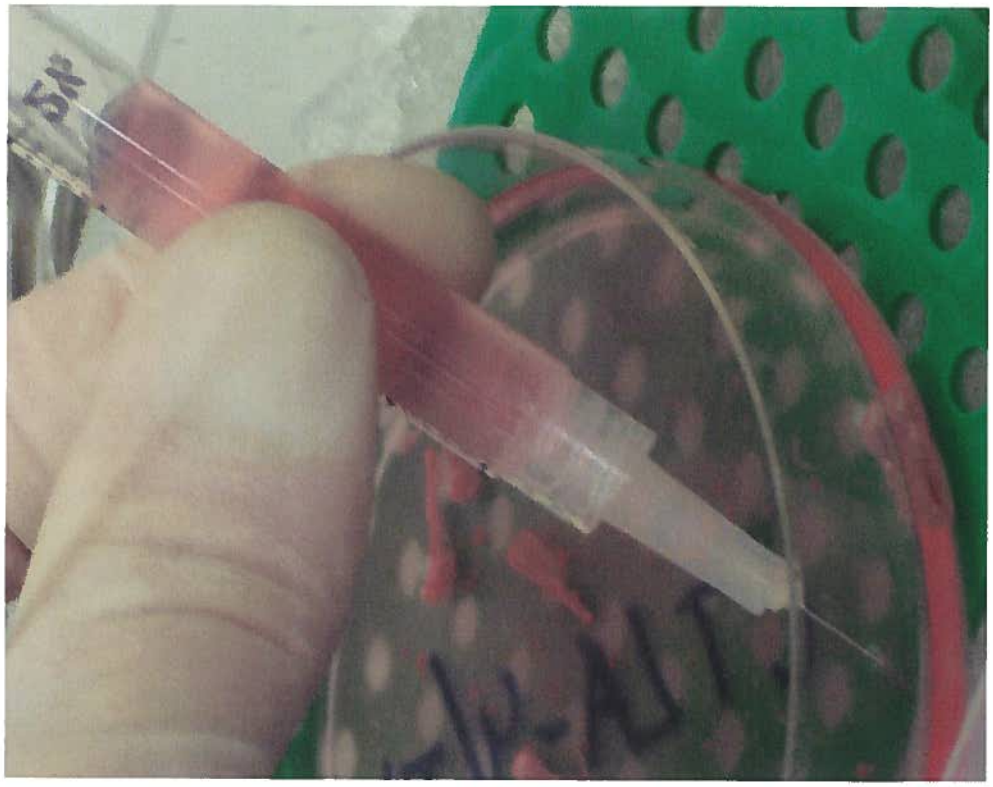

Femur
Tibia

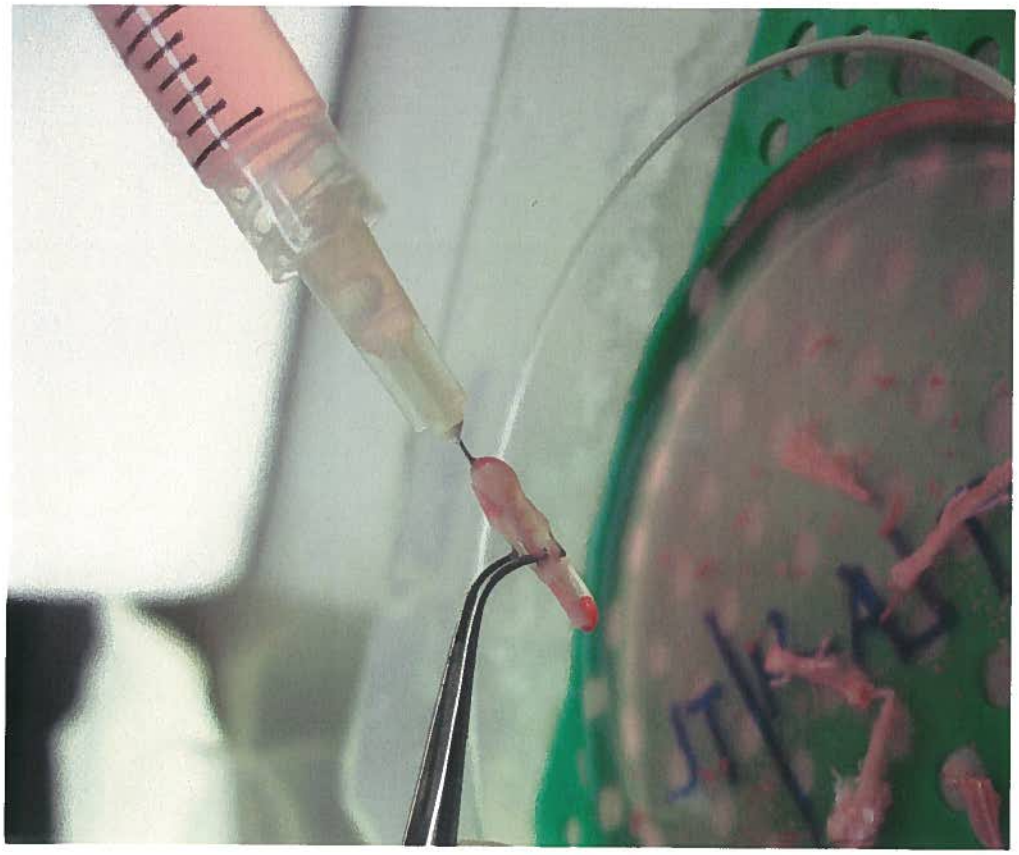

B6/49.2

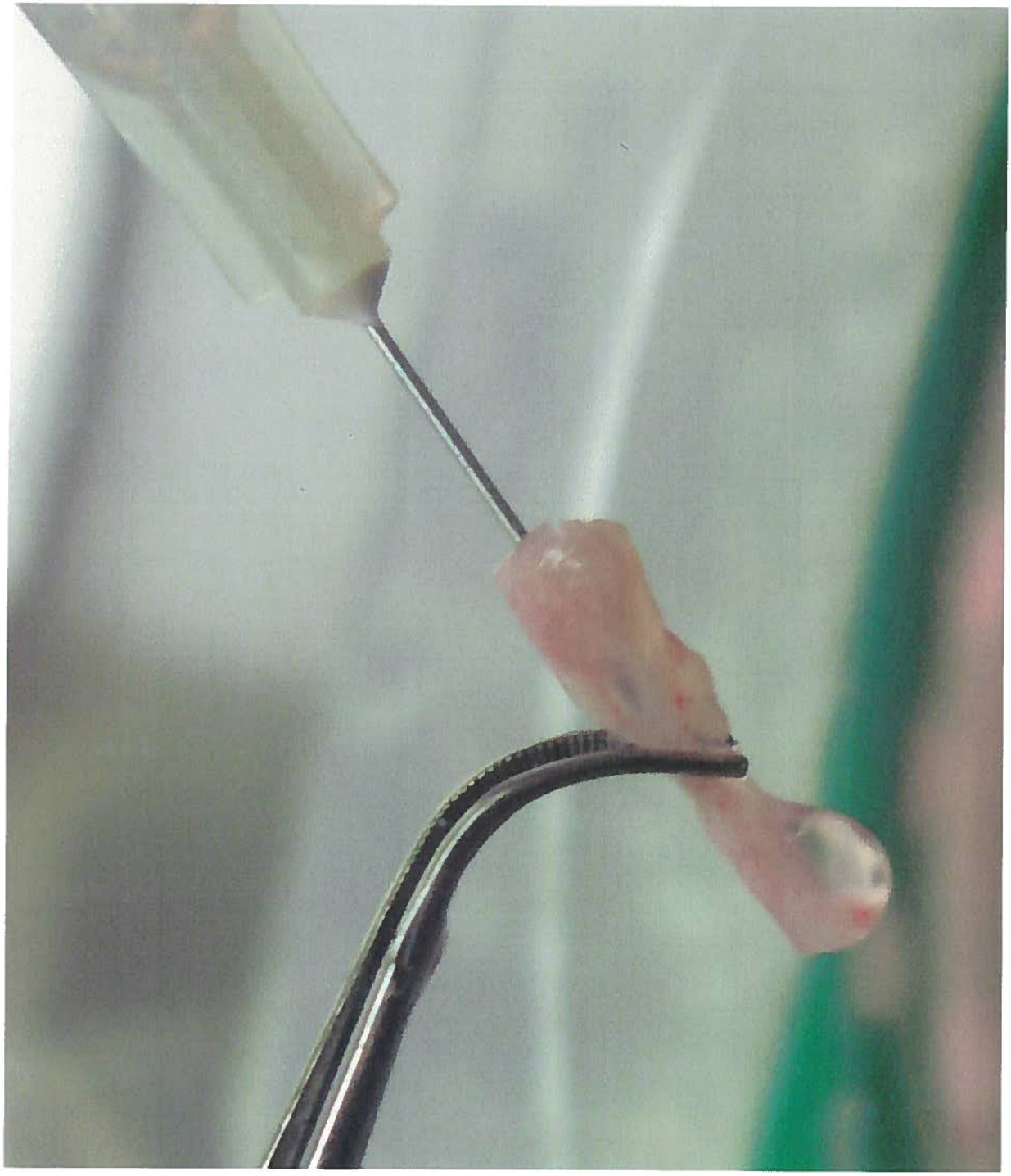
Syringe/27Gauge needle

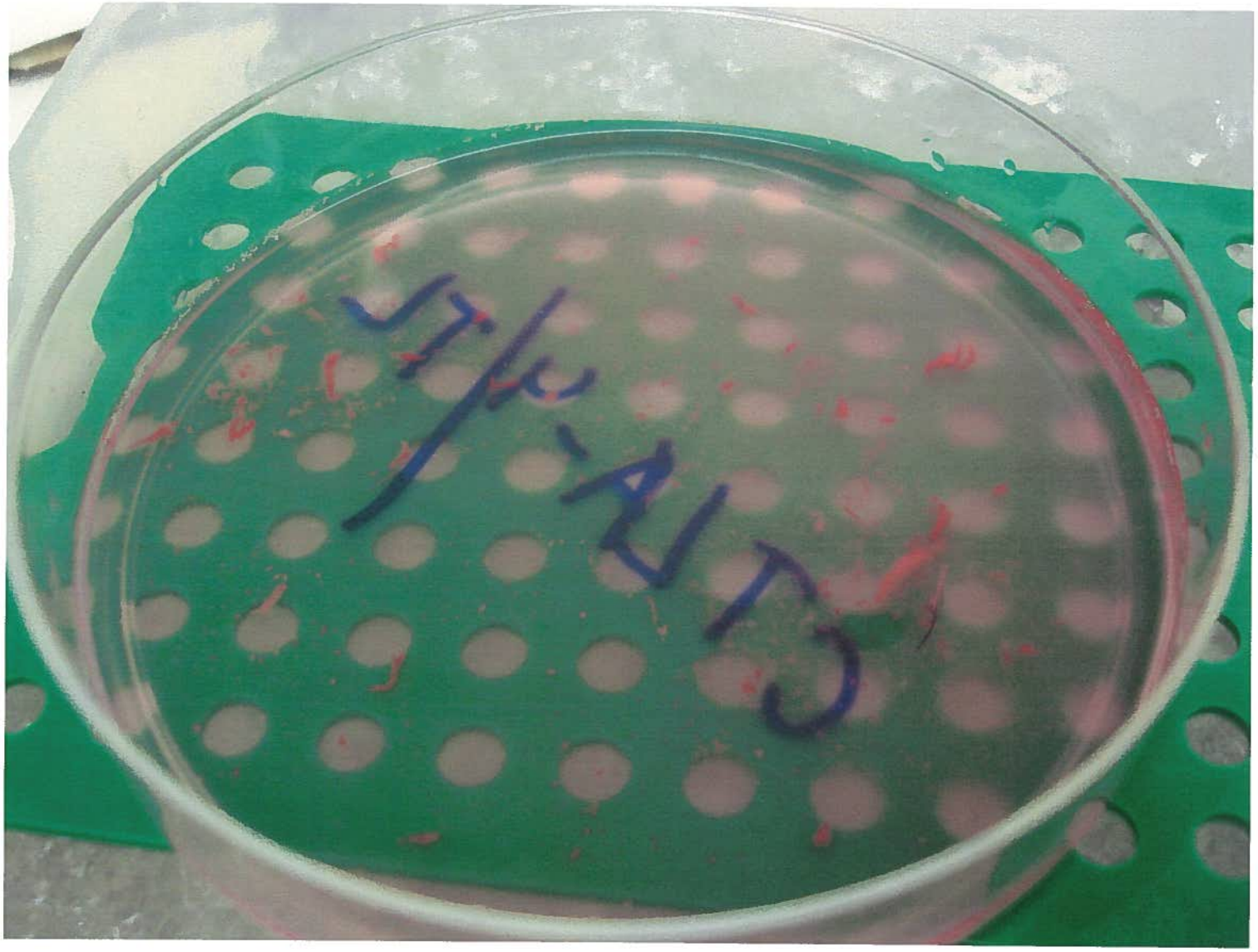


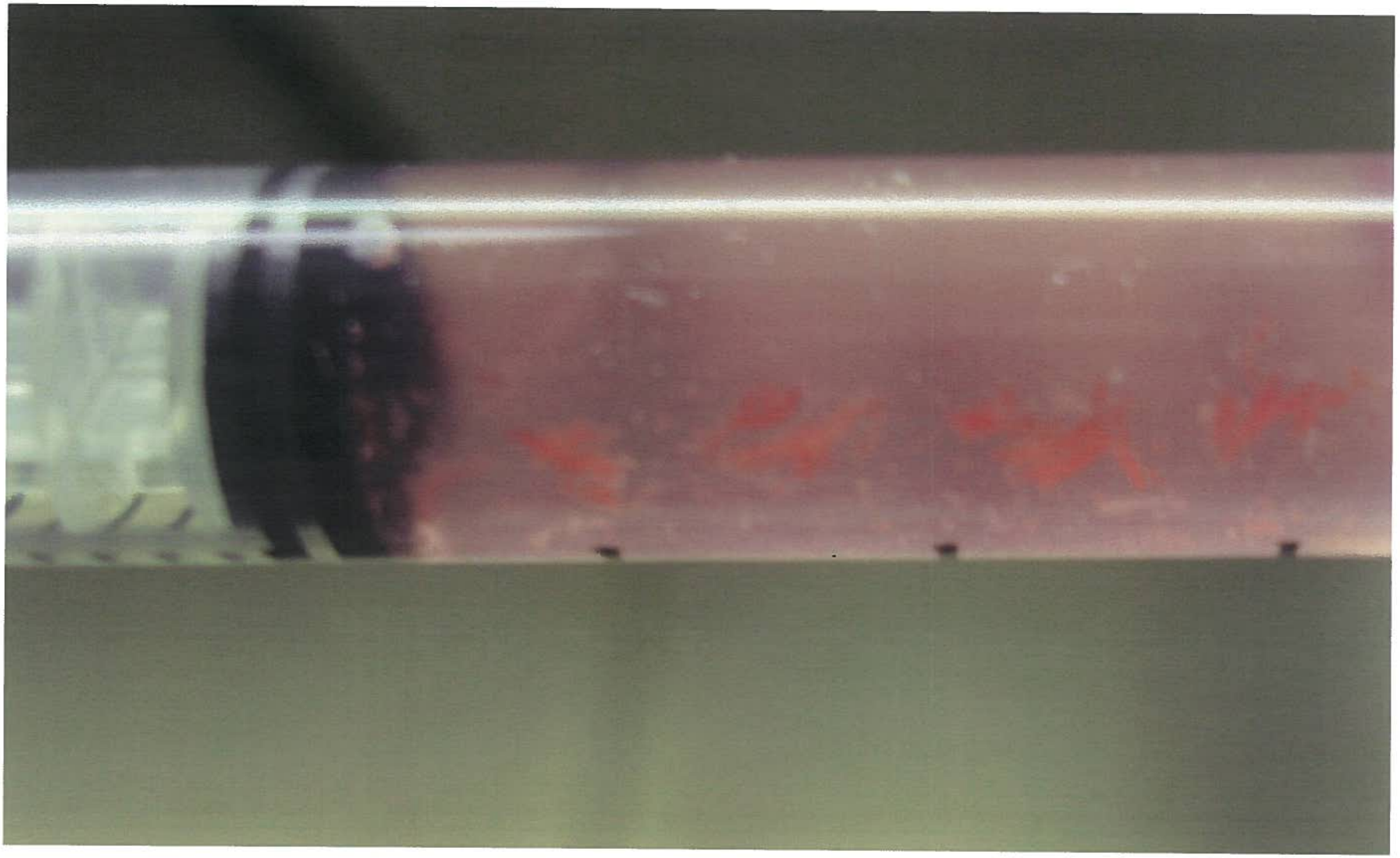




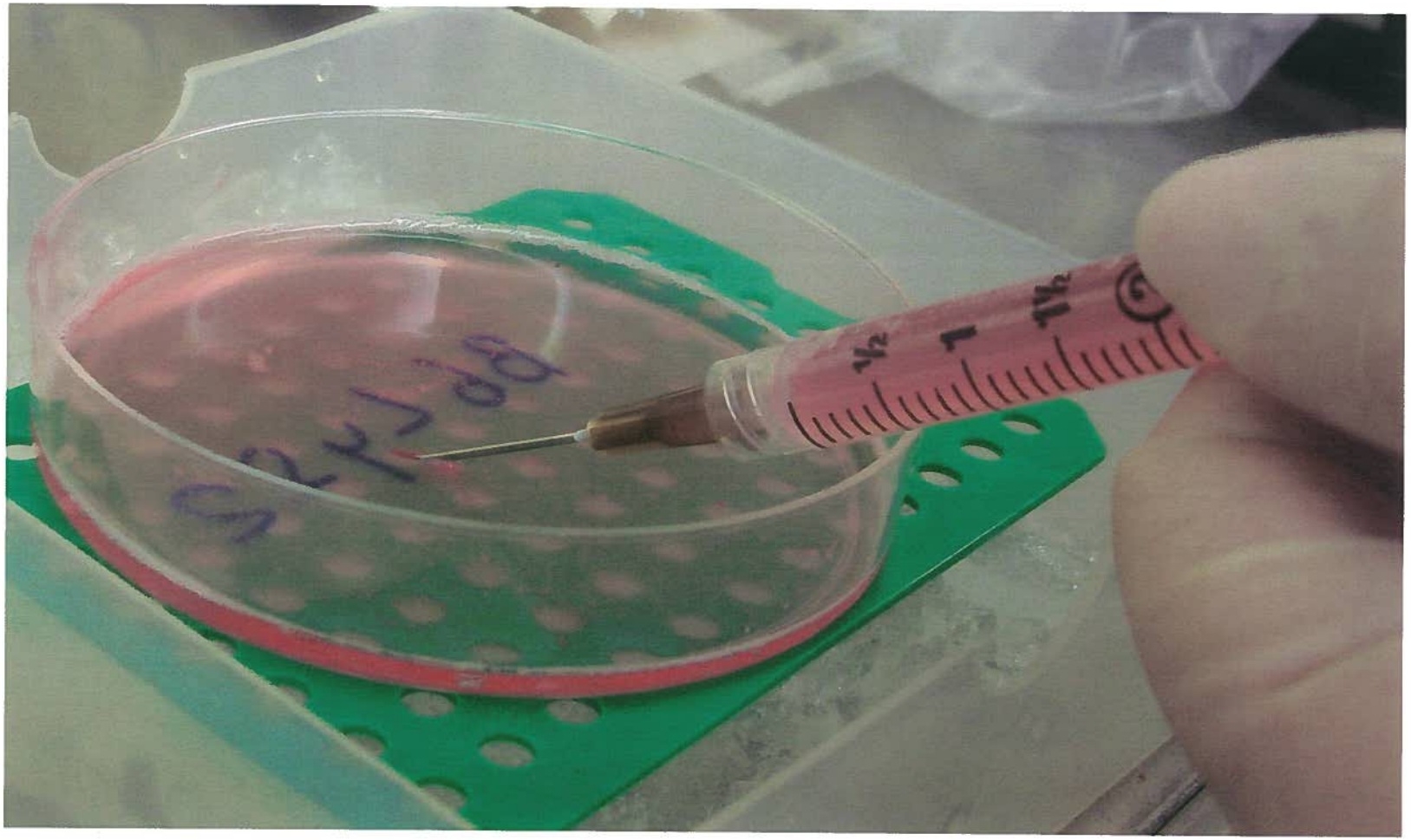

JT/LAST




7/4/2013

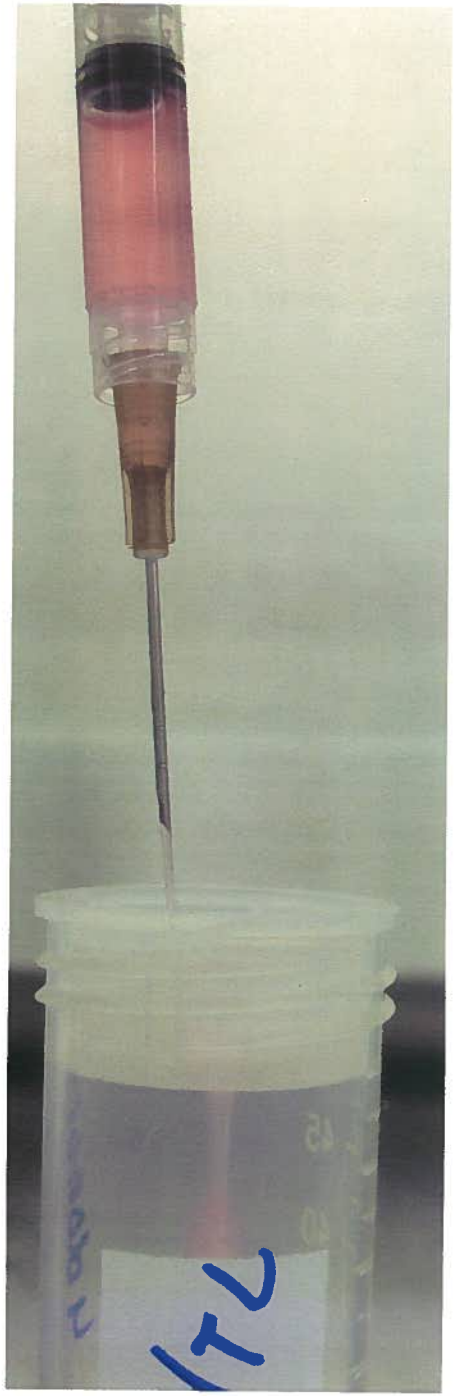


Syringe/19g needle

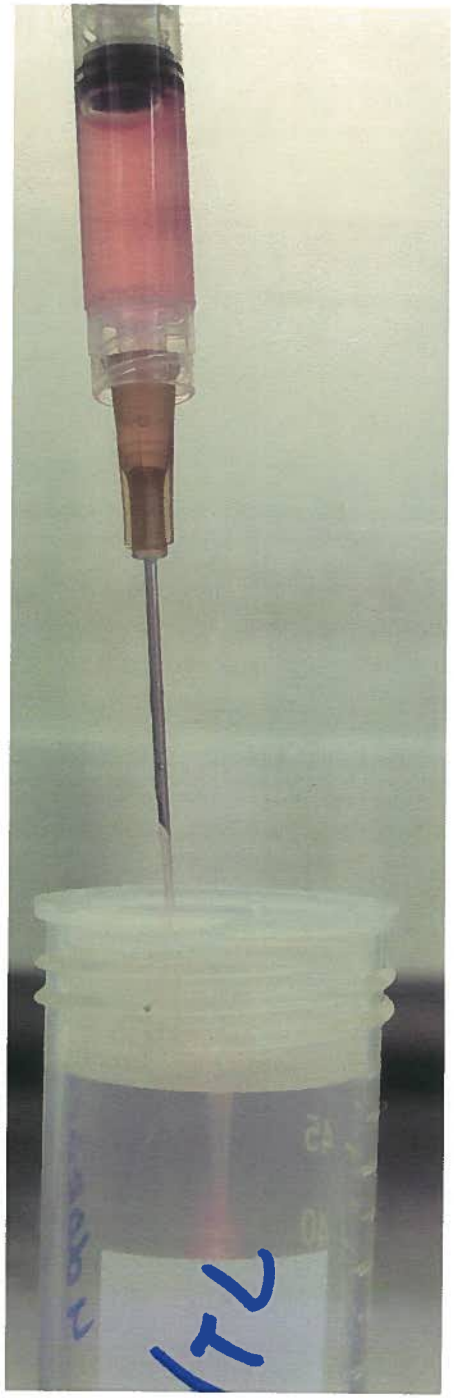


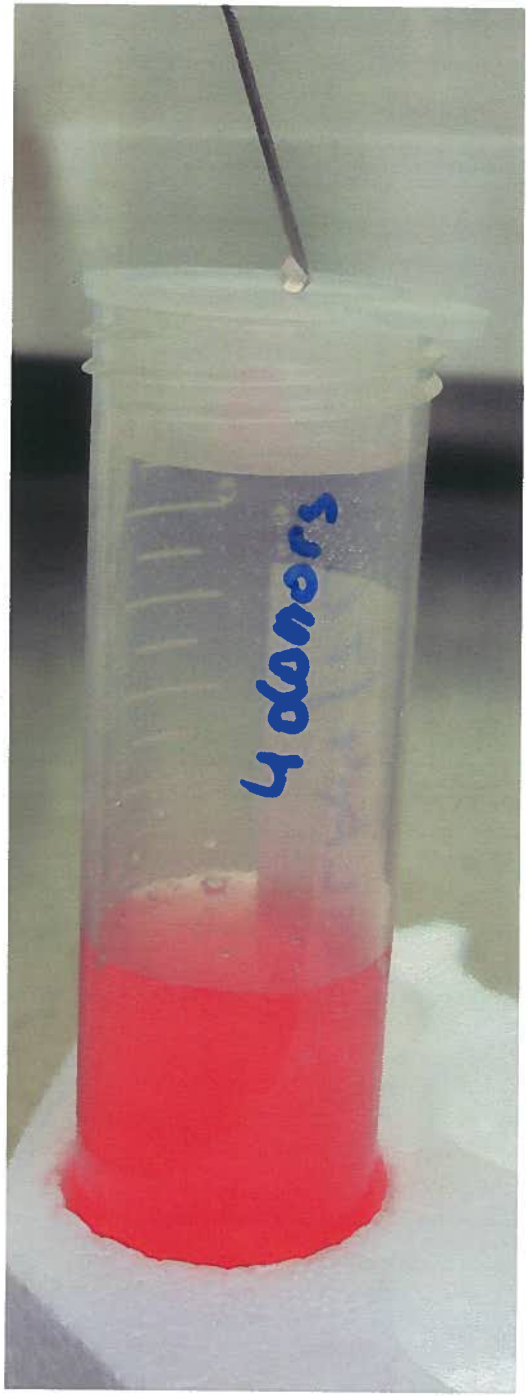
Cell Strainer

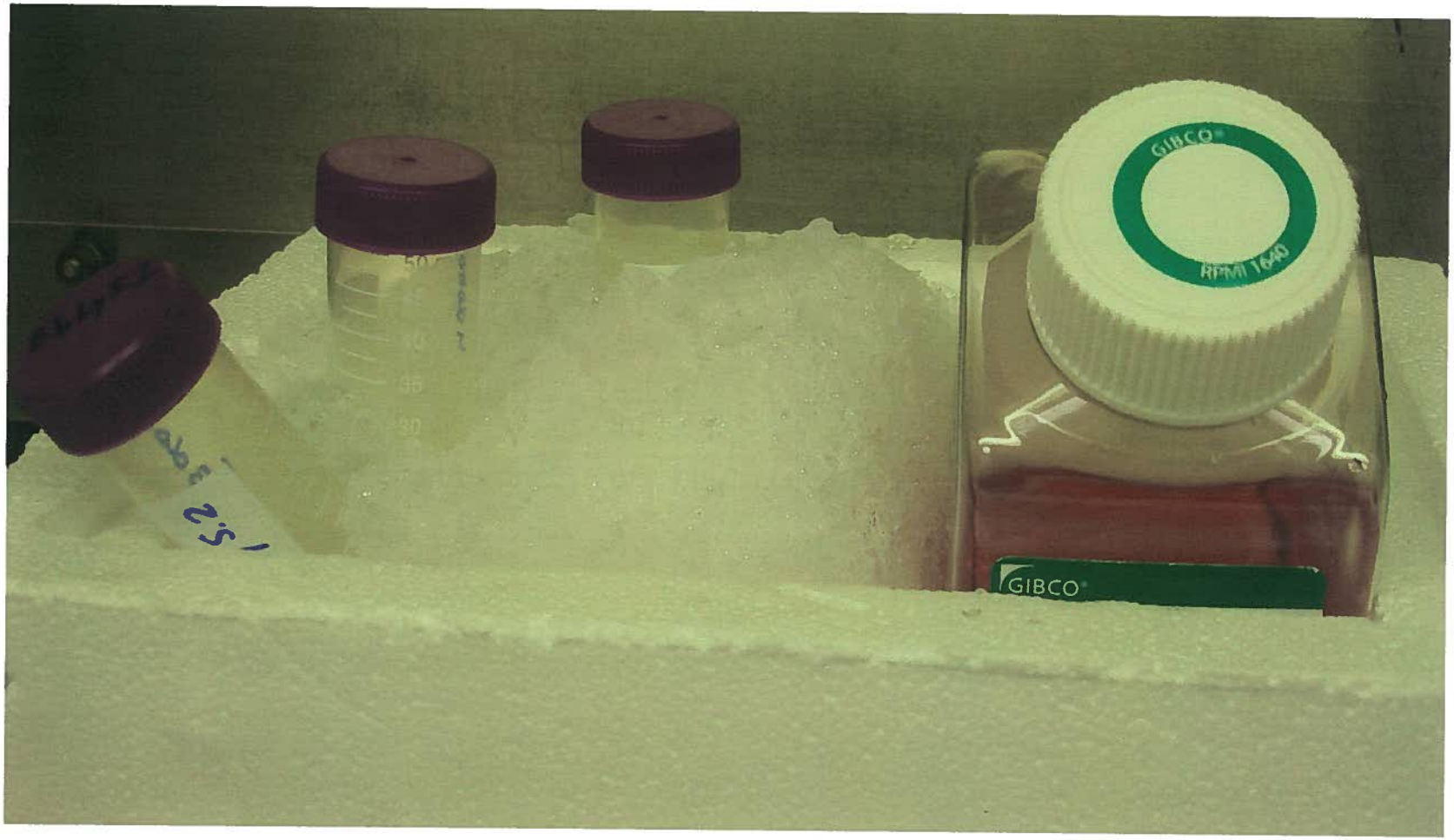
Cell Strainer



Cell Strainer




4 donors


GIBCO
RPMI 1640
GIBCO

Preparation of donor cells:

2. **Kill mature T cells - either by anti-Thy1 + complement, or by removing $CD4^+$ and $CD8^+$ cells with magnetic beads. Estimate 5% T cells in the bone marrow for your calculations.**

   When anti-Thy1 + complement are used to delete T cells, One need to test the dilution of anti-Thy1 antibody and complement. Incubate bone marrow with anti-Thy1 antibody at 4°C for 30 mins, wash once with sterile HBSS and then suspend the cell pellet with diluted complement and incubate at 37°C for 30mins.

3. **Fill up the tubes with cold HBSS and spin down the cells; and resuspend in HBSS with no additions to a concentration of 20-30million cells per ml. Store on ice until injection.**

   In general, you should harvest 20-30 million bone marrow cells from one donor mouse after Thy1 killing.

Intravenous injection of the mouse:

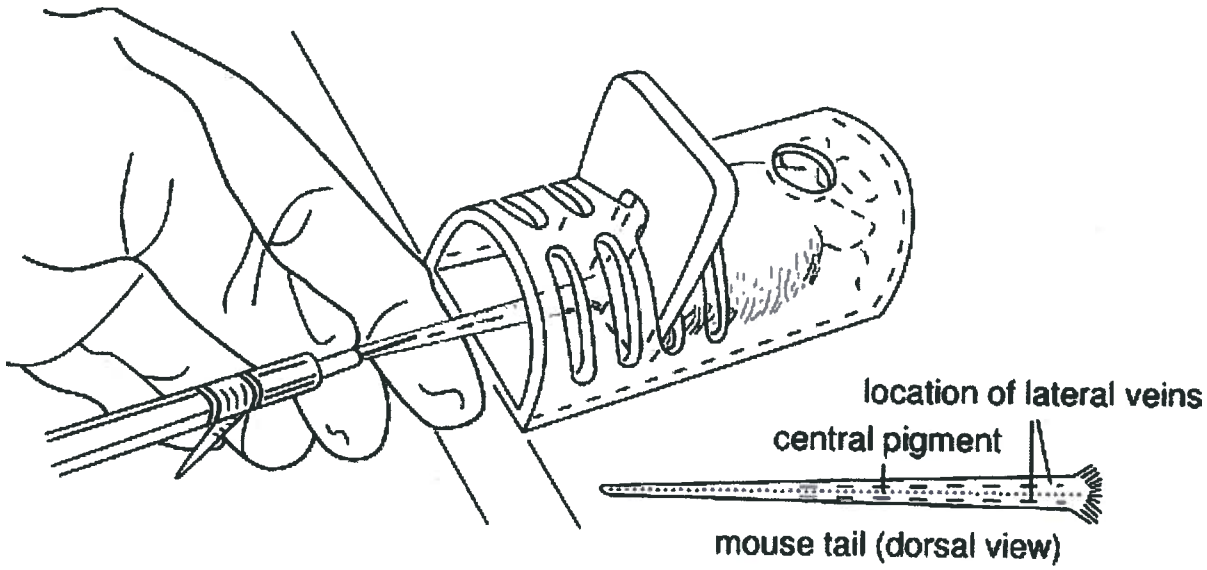
Intravenous injection of the recipient mouse is a difficult procedure which requires practice and patient.

The inexperienced investigator should take the trouble to gain this skill in several practice sessions with PBS as the injectate.

Intravenous injection of the mouse:

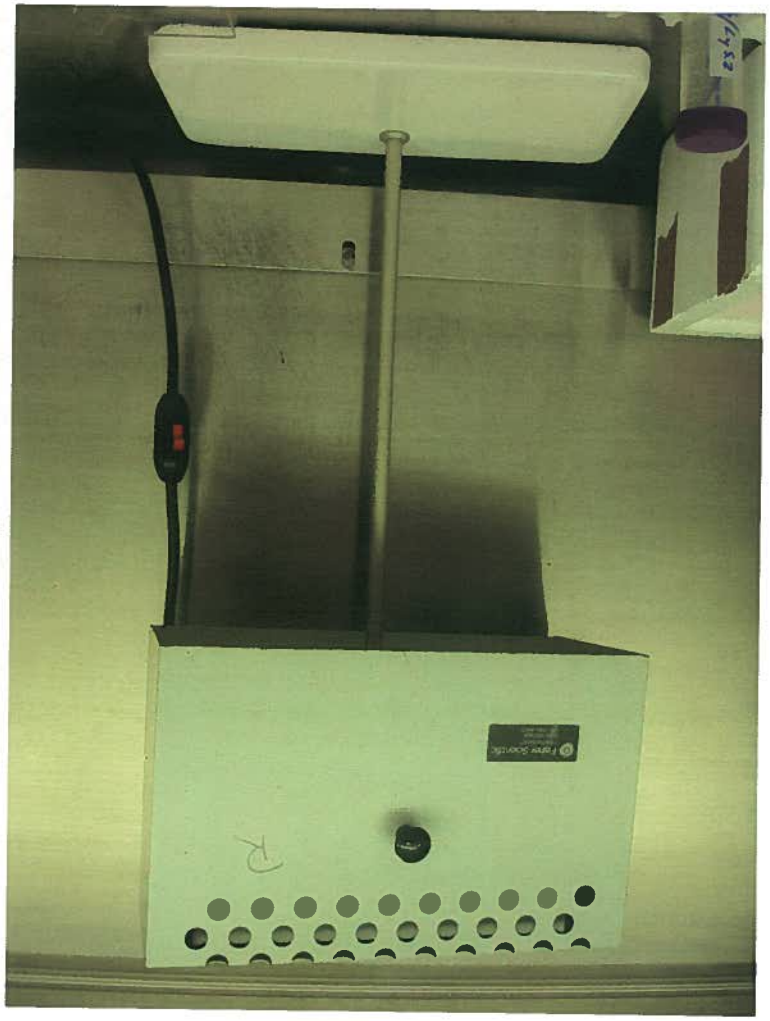
Material:

70% ethanol
1-ml syringe with 25G needle
Restrainer
Heat lamp or beaker containing warm water
Gauze sponge or swab



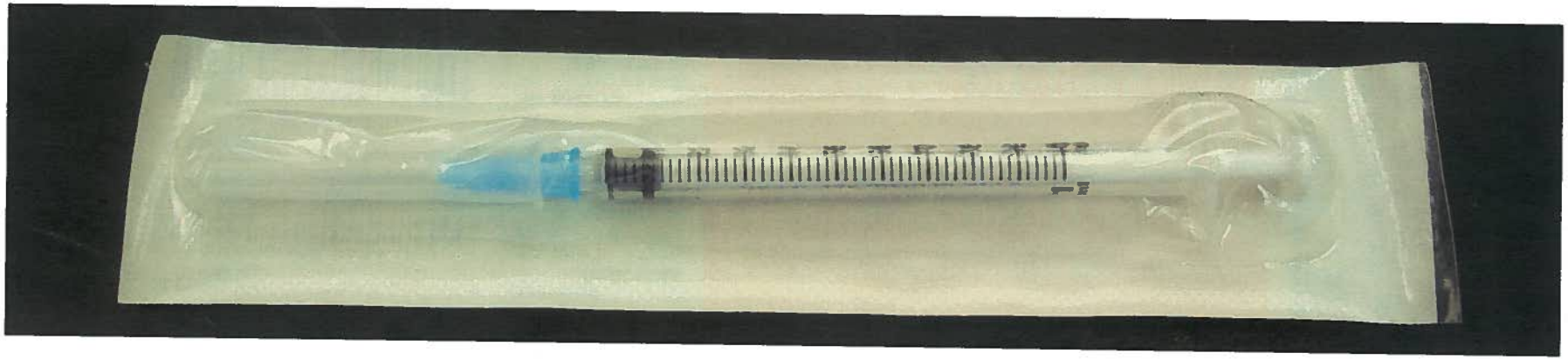
Intravenous injection of the mouse:

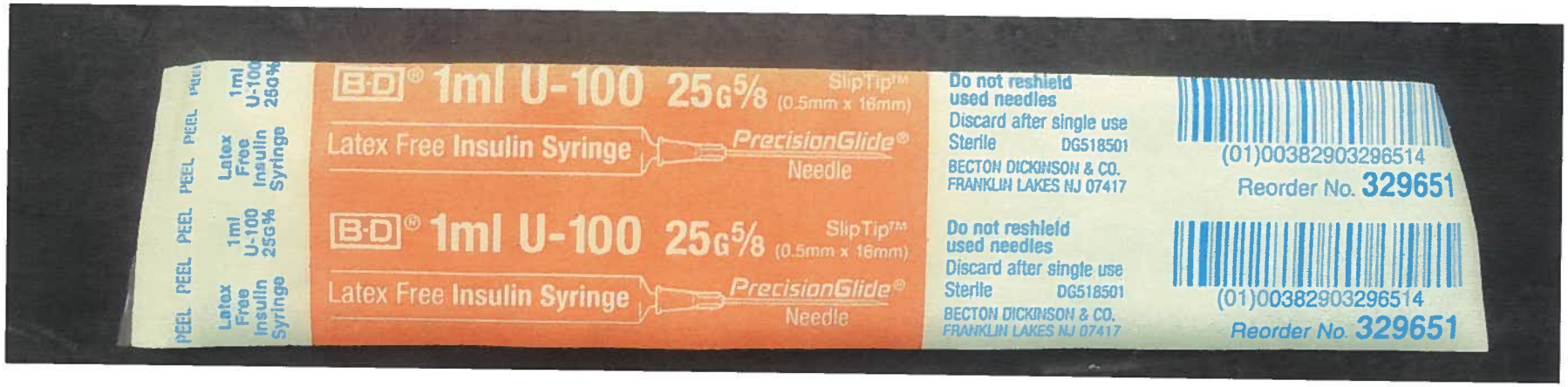
1. Fill syringe with cell suspension and remove air bubbles.
   Removal of air bubbles is critical to avoid air embolism.
2. Place mouse in a restrainer.
3. Warm the tail with a heat lamp for about one minute
   or by immersing in warm water to dilate vessels.
3. Swab the tail with 70% ethanol on gauze sponge or swab.
4. Immobilize the tail with gentle traction.
5. Visualize the lateral tail vein and insert needle parallel to the vein 2 to 5 mm
   into to the lumen.
7. Inject slowly. No bleb should form if needle was properly located.
   Inject 10-15 million in 0.5ml per mouse.
8. Withdraw the needle and apply digital pressure to achieve hemostasis.
9. Maintain the mice on medicated water.



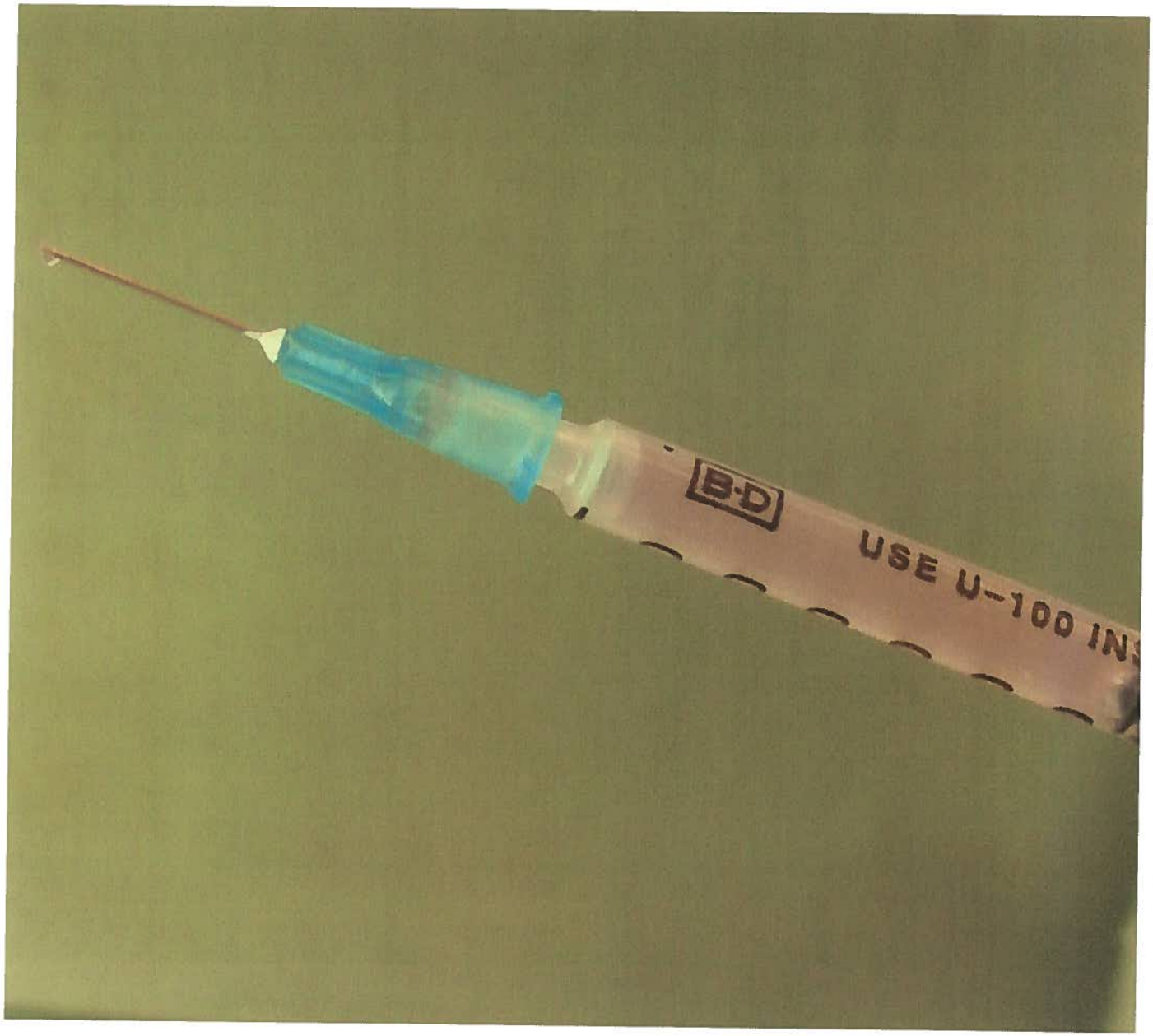
Fisher Scientific
TAI-90
TAI-90

Syringe/25g needle





Fill syringe with cell suspension and remove air bubbles

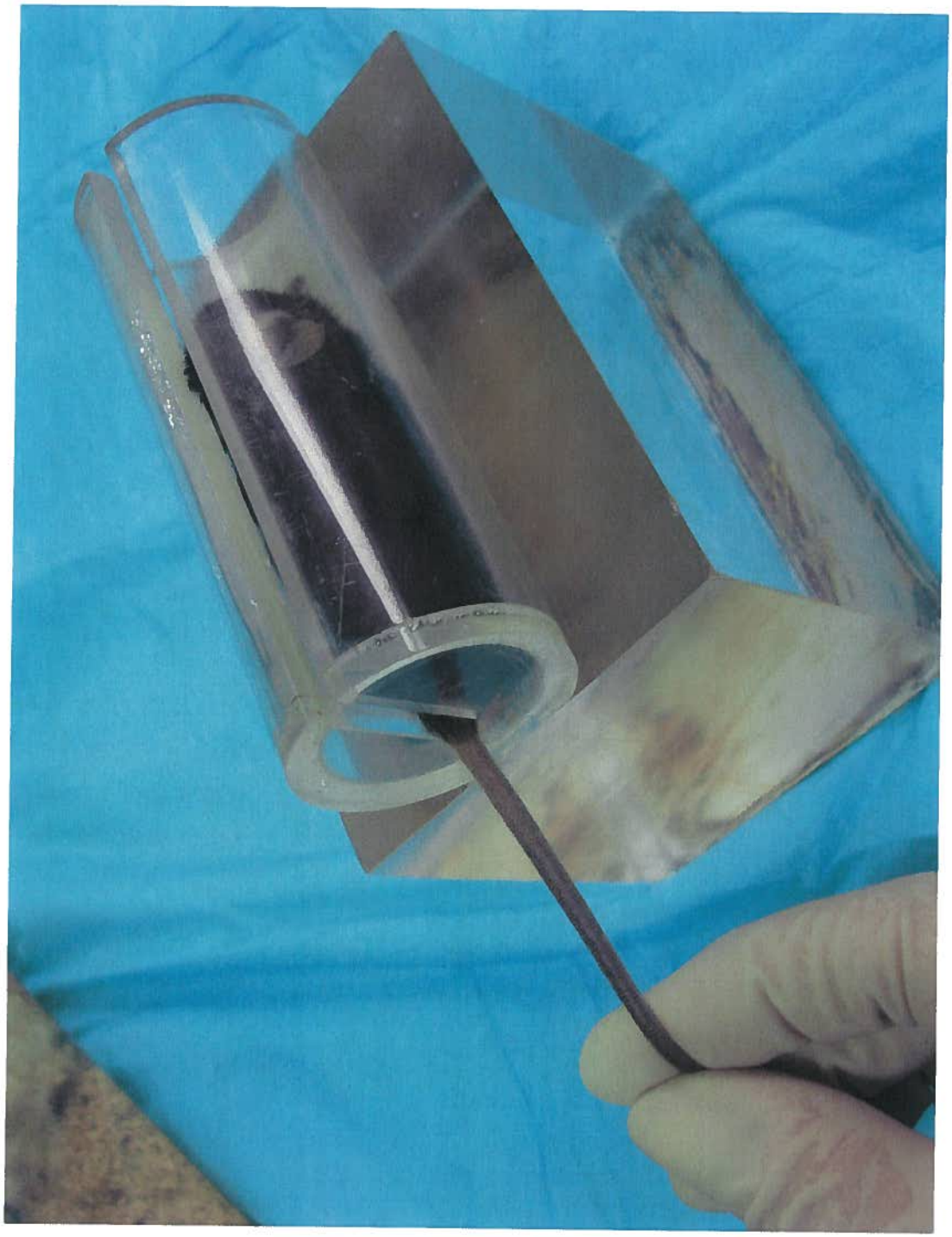

B·D
USE U-100 IN

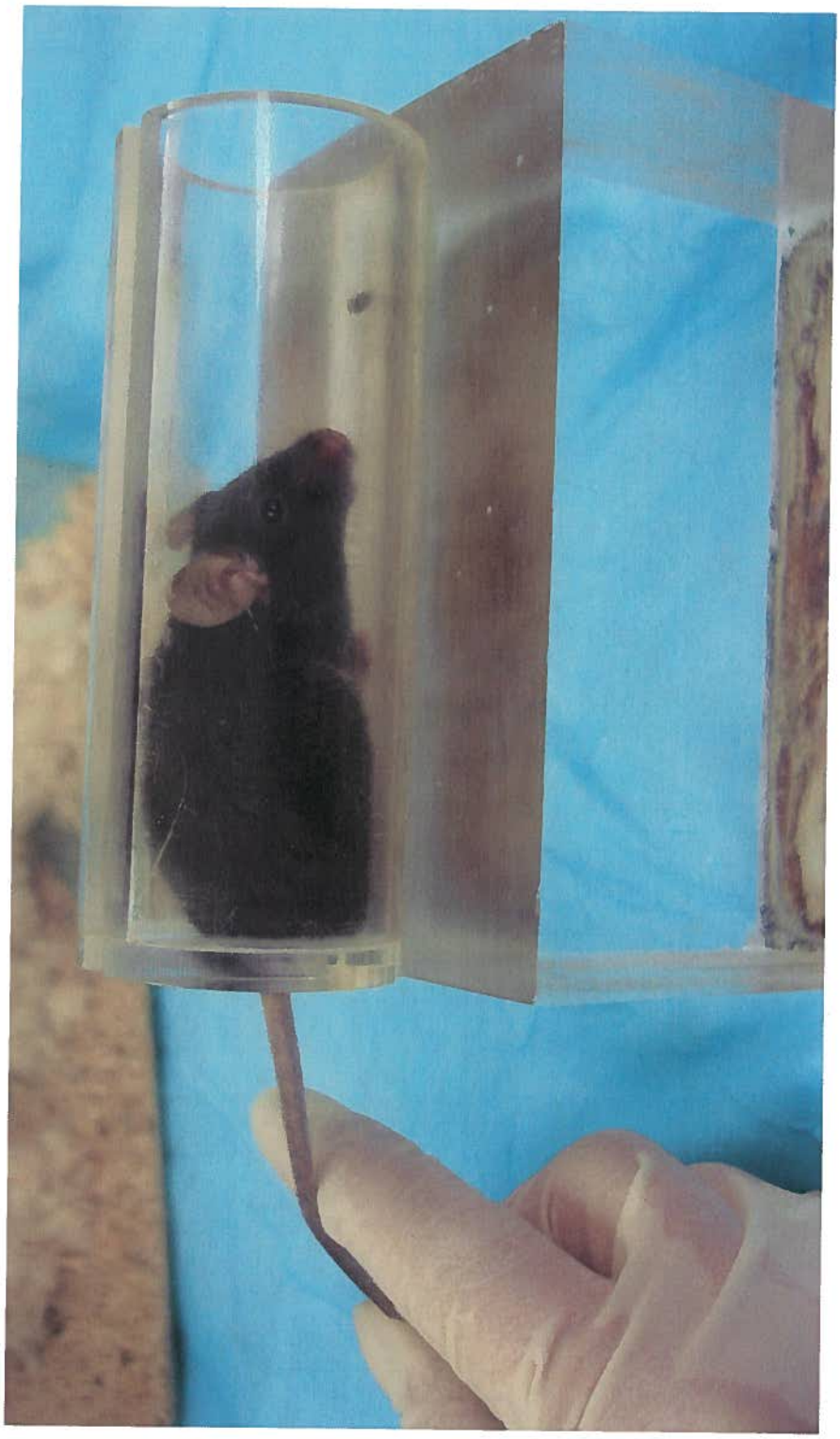
Restrainer

Restrainer

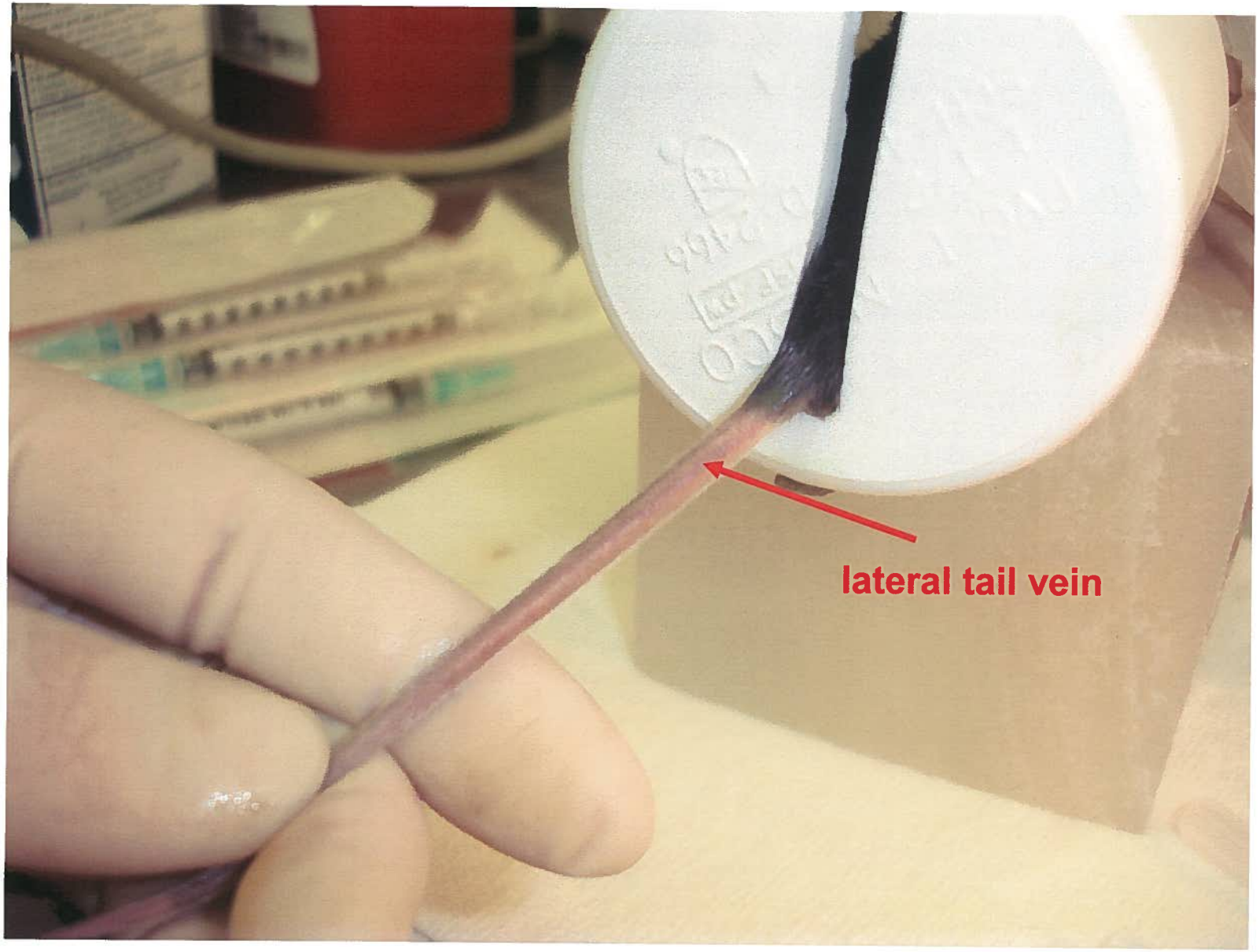
WASH WITH SOAP
AND WARM
WATER
DO NOT USE ANY
ALCOHOL BASED
PRODUCTS TO
CLEAN THE
PLASTIC.

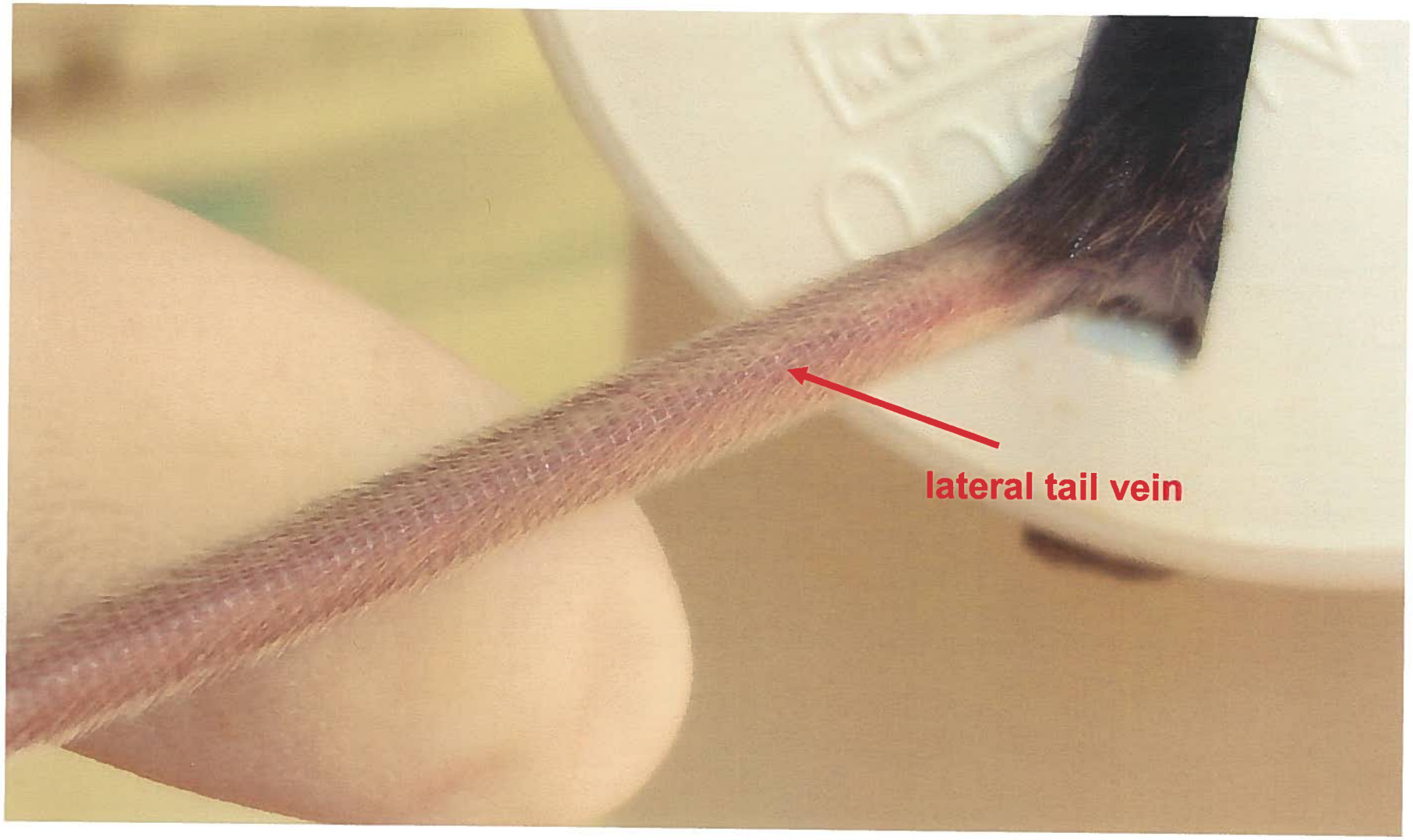
WASH WITH SOAP
AND WARM
WATER
DO NOT USE ANY
ALCOHOL BASED
PRODUCTS TO
CLEAN THE
PLASTIC.

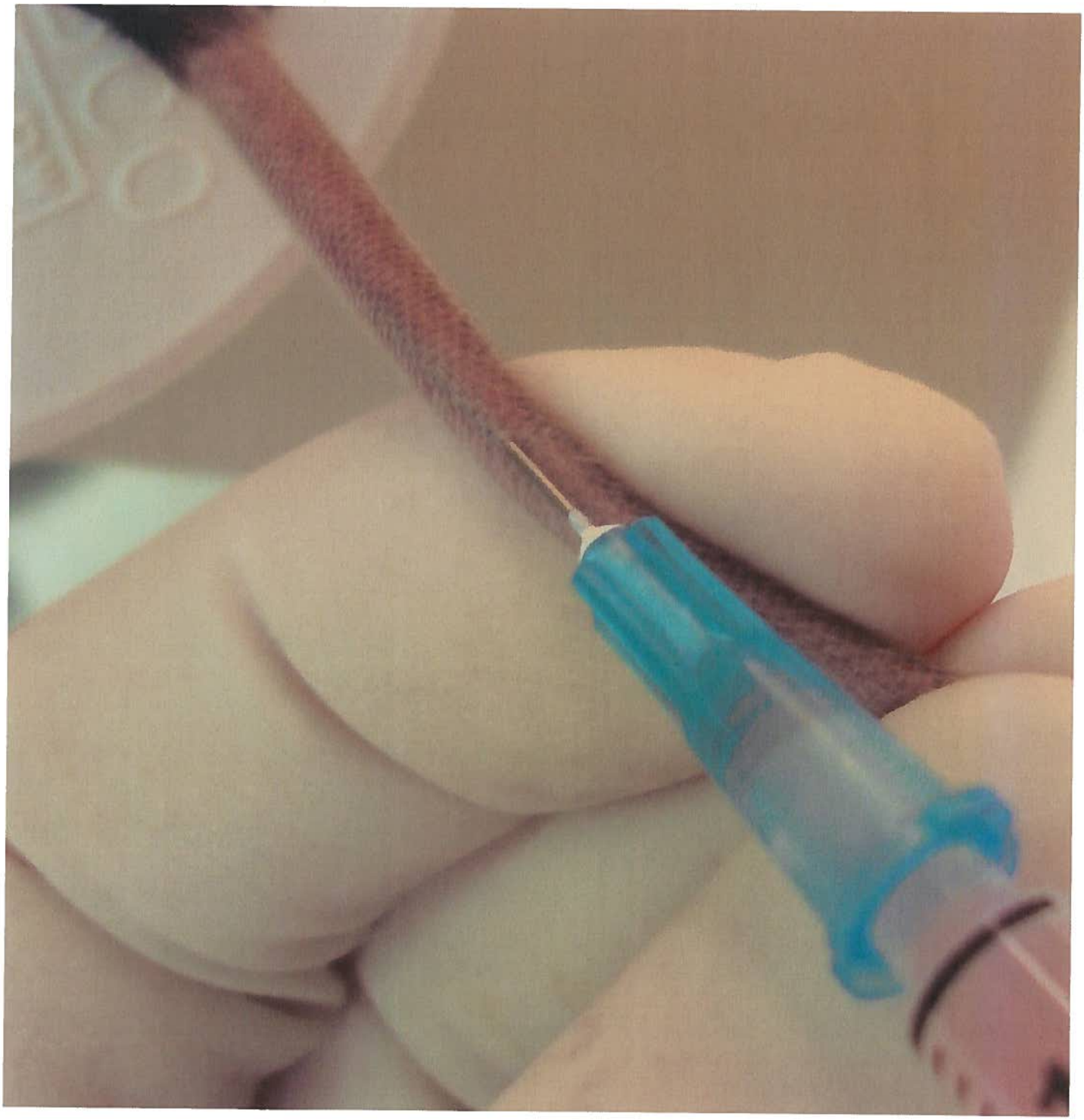




Immobilize the tail with gentle traction




lateral tail vein



Withdraw the needle and apply digital pressure to achieve hemostasis

Analyzing chimera:

Irradiated recipient mice should turn gray in spots if they were properly irradiated.

Normally, animals exposed to marrow-ablative dose of γ-irradiation should survive 12-14 days in the absence of a marrow transplant.

If given bone marrow, they should survive a normal life-span and be reconstituted with peripheral blood cells almost entirely derived from the marrow inoculums.

Analyze them after 6-8weeks.

Analyzing chimera:

Peripheral blood screening allows detection of donor-derived cells starting about 2 weeks after irradiation and reconstitution, when very low number of circulating cells are present and chimerism is just beginning to be established.

Donor-derived cells will be easily detectable at 4 weeks.

Blood collections allow the same animal to be followed over time.

Duplicate animals should be sacrificed at different time points and peripheral and central lymphoid and hematopoietic tissues can be analyzed from donor-derived cells.

Bone marrow chimera are often used to distinguish the intrinsic versus extrinsic effect of given mutations

1. To detect cell-autonomous effect (intrinsic) on lymphoid or myeloid differentiation:

   Mutant mice are often used as donor and wild type mice are used as recipient.

2. To detect non cell-autonomouse effect (extrinsic) on lymphoid or myeloid differentiation (such as effects on the lymphoid microenvironment).

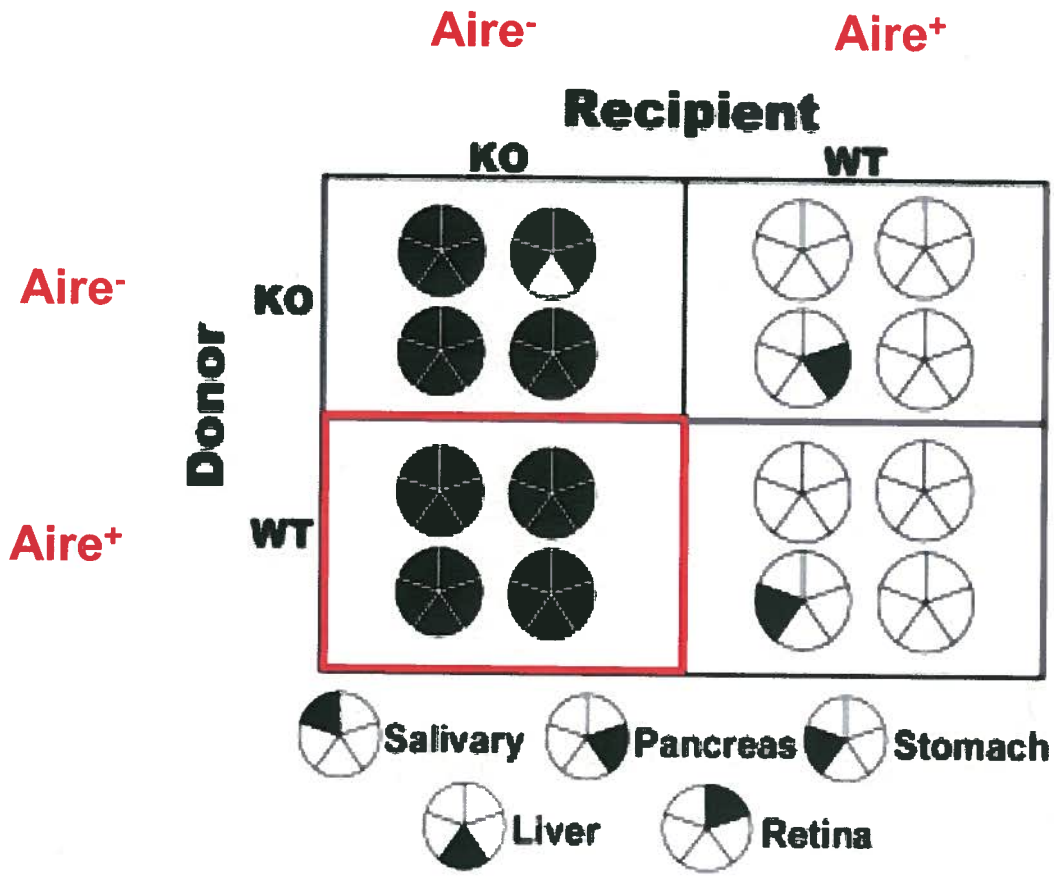
   Wild type mice are used as donor and mutant mice are used as recipient.

Projection of an Immunological Self Shadow Within the Thymus by the Aire Protein

Mark S. Anderson,[1] Emily S. Venanzi,[1] Ludger Klein,[2]
Zhibin Chen,[1] Stuart P. Berzins,[1] Shannon J. Turley,[1]
Harald von Boehmer,[2] Roderick Bronson,[3] Andrée Dierich,[4]
Christophe Benoist,[1*] Diane Mathis[1*]

Humans expressing a defective form of the transcription factor AIRE (autoimmune regulator) develop multiorgan autoimmune disease. We used *aire*-deficient mice to test the hypothesis that this transcription factor regulates autoimmunity by promoting the ectopic expression of peripheral tissue–restricted antigens in medullary epithelial cells of the thymus. This hypothesis proved correct. The mutant animals exhibited a defined profile of autoimmune diseases that depended on the absence of aire in stromal cells of the thymus. Aire-deficient thymic medullary epithelial cells showed a specific reduction in ectopic transcription of genes encoding peripheral antigens. These findings highlight the importance of thymically imposed "central" tolerance in controlling autoimmunity.

Autoimmune manifestations in aire-deficient mice are attributable to its absence from radioresistant but not hematopoietic cells



Bone marrow chimera are used to identify the function of CD28 costimulation for regulatory T cell generation

- CD28 costimulation is required for Treg cell generation.

- CD28 costimulation is required for IL-2 production.

- In vivo IL-2 is required for Treg cell generation.

- CD28 costimulation is required for Treg cell generation.

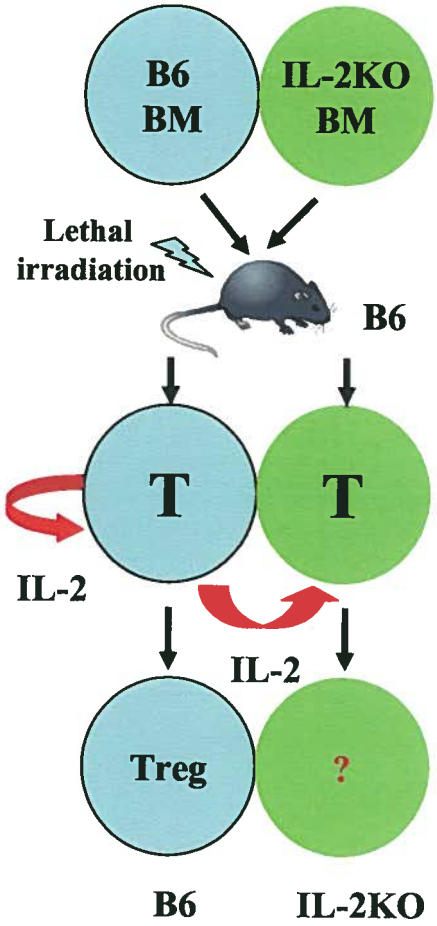
- CD28 costimulation is required for IL-2 production.

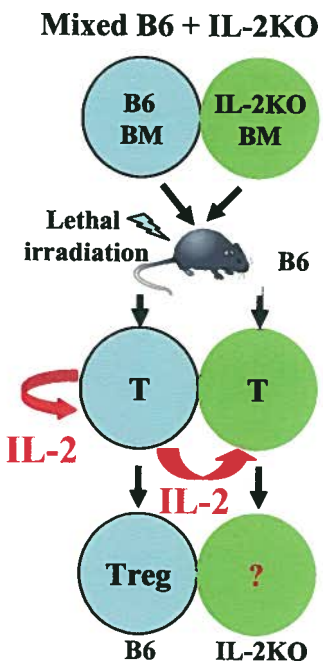
- In vivo IL-2 is required for Treg cell generation.

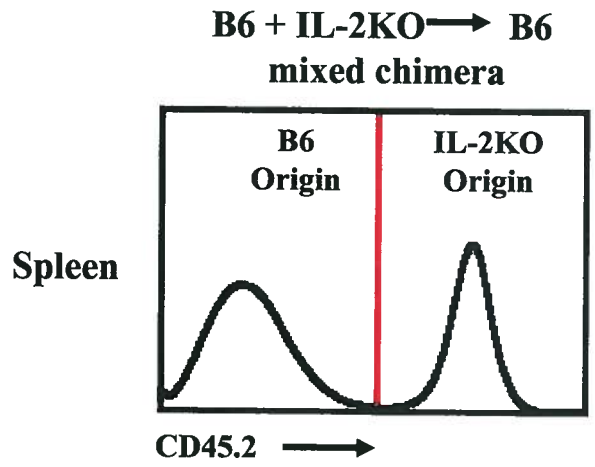
Does CD28 costimulation simply provide in vivo IL-2 for Treg cell generation ?

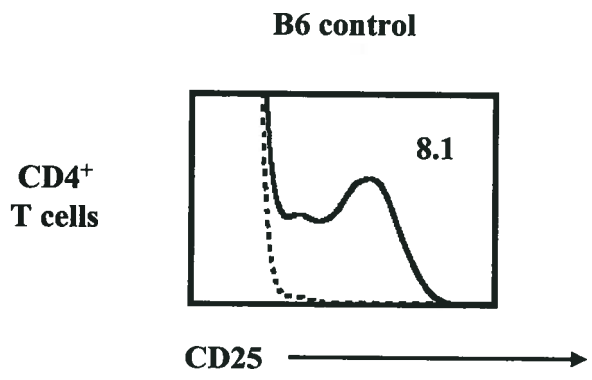
Experimental system for assessing in vivo IL-2 dependent Treg cell generation:

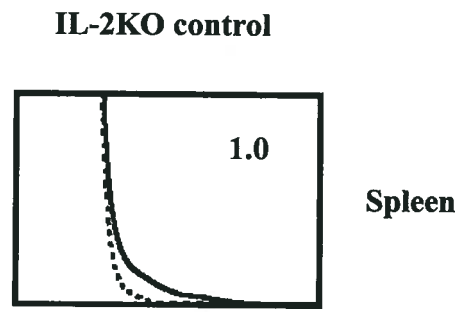
Mixed bone marrow chimera

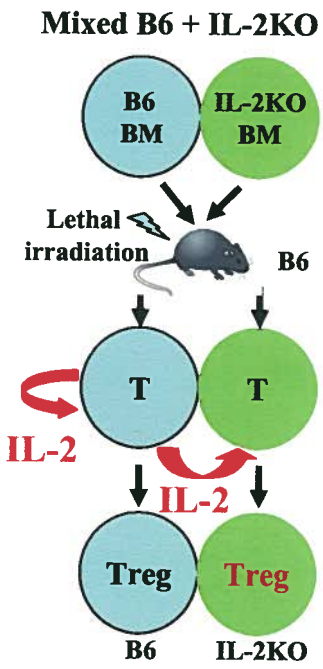


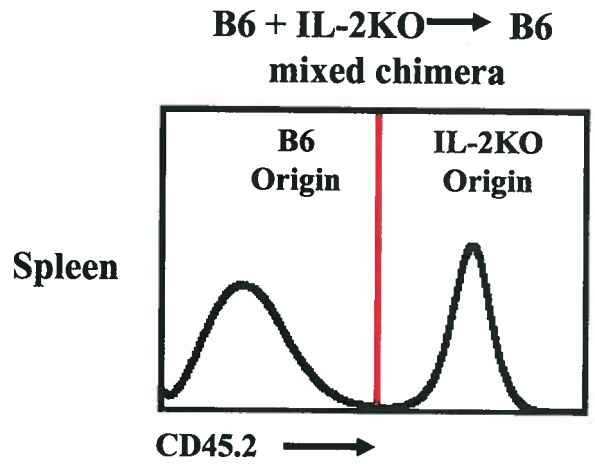

Mixed B6 + IL-2KO
B6 BM
IL-2KO BM
Lethal irradiation
B6
T
T
IL-2
IL-2
Treg
?
B6
IL-2KO

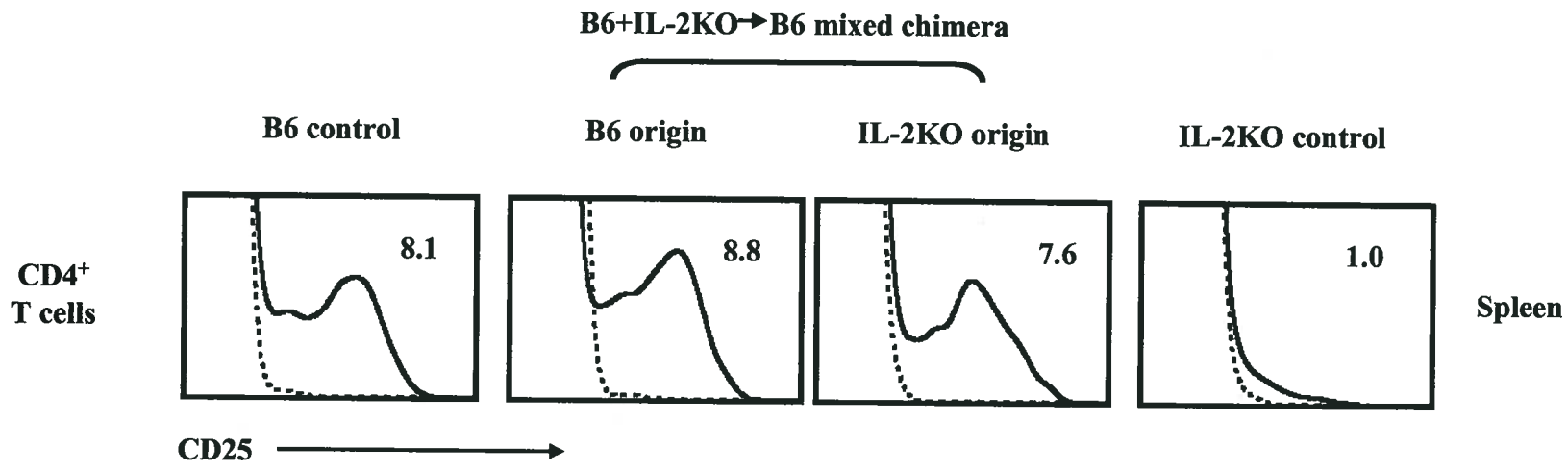

B6 + IL-2KO → B6
mixed chimera
Spleen
B6 Origin
IL-2KO Origin
CD45.2


B6 control
CD4+ T cells
8.1
CD25


IL-2KO control
1.0
Spleen


Mixed B6 + IL-2KO
B6 BM
IL-2KO BM
Lethal irradiation
B6
T
T
IL-2
IL-2
Treg
Treg
B6
IL-2KO

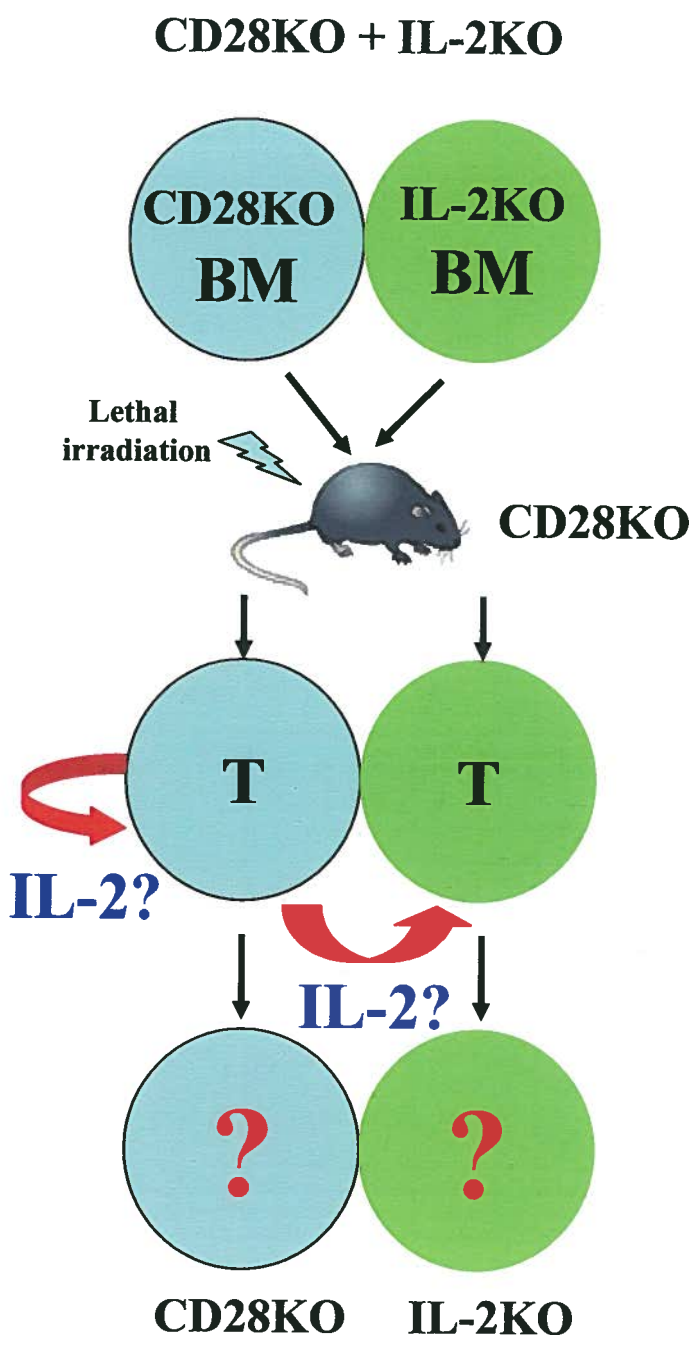

B6 + IL-2KO → B6
mixed chimera
Spleen
B6 Origin
IL-2KO Origin
CD45.2

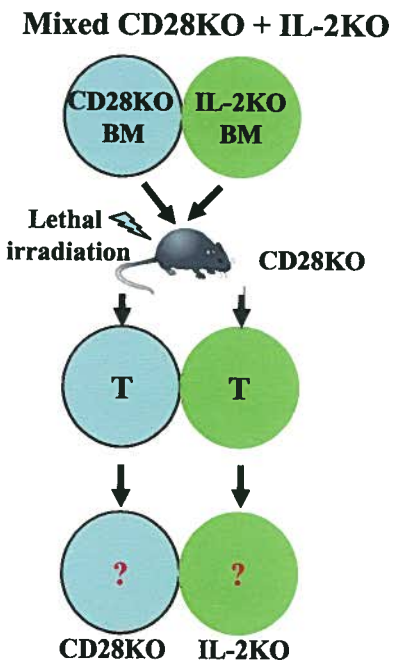

B6+IL-2KO→B6 mixed chimera
B6 control
B6 origin
IL-2KO origin
IL-2KO control
CD4+ T cells
8.1
8.8
7.6
1.0
Spleen
CD25

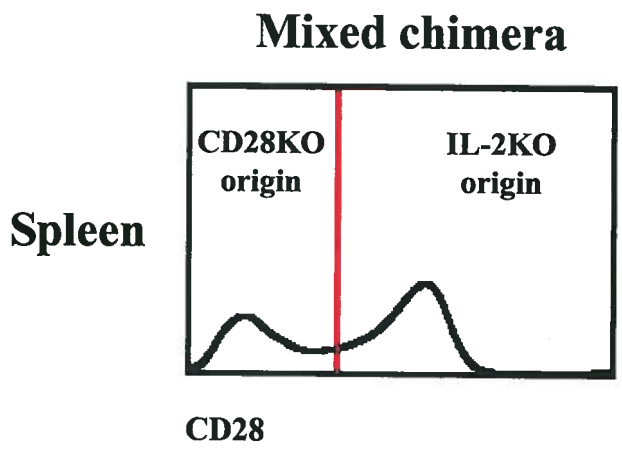
Mixed bone marrow chimeras are an experimental assay for in vivo IL-2 production and Treg cell generation.

Question:

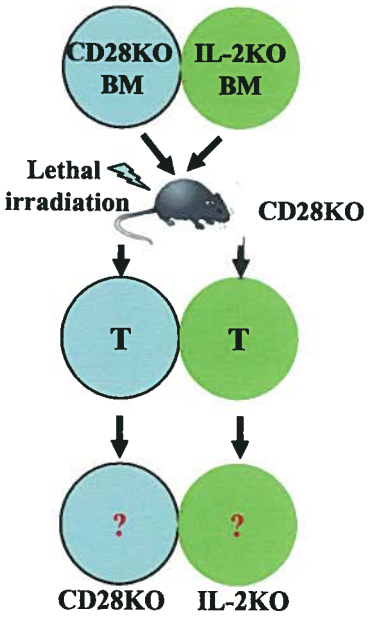
Do CD28KO T cells produce IL-2 in vivo?


CD28KO + IL-2KO
CD28KO
BM
IL-2KO
BM
Lethal
irradiation
CD28KO
T
T
IL-2?
IL-2?
?
?
CD28KO
IL-2KO


Mixed CD28KO + IL-2KO
CD28KO BM
IL-2KO BM
Lethal irradiation
CD28KO
T
T
?
?
CD28KO
IL-2KO

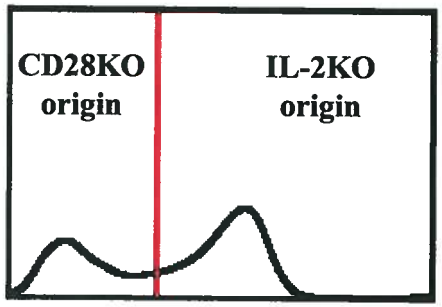

Mixed chimera
Spleen
CD28KO origin
IL-2KO origin
CD28

Mixed CD28KO + IL-2KO



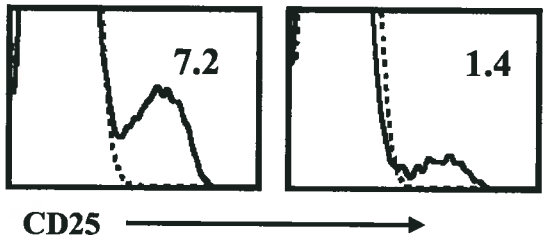
Mixed chimera

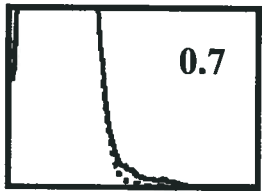
Spleen

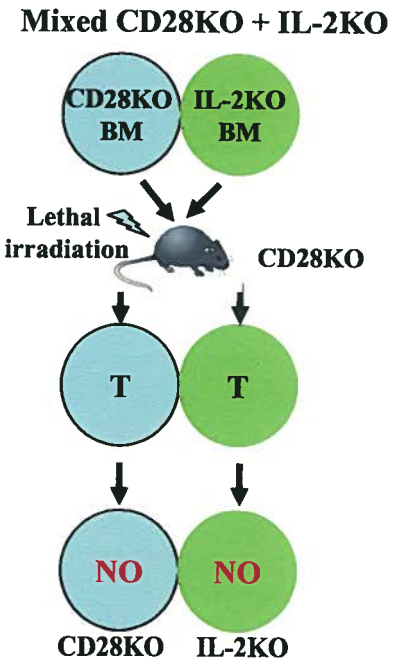


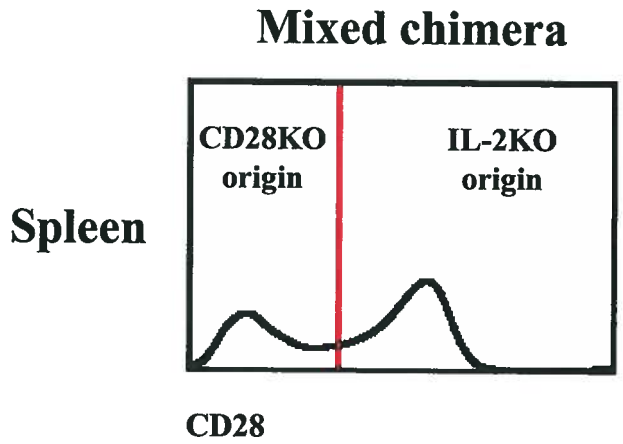
CD28

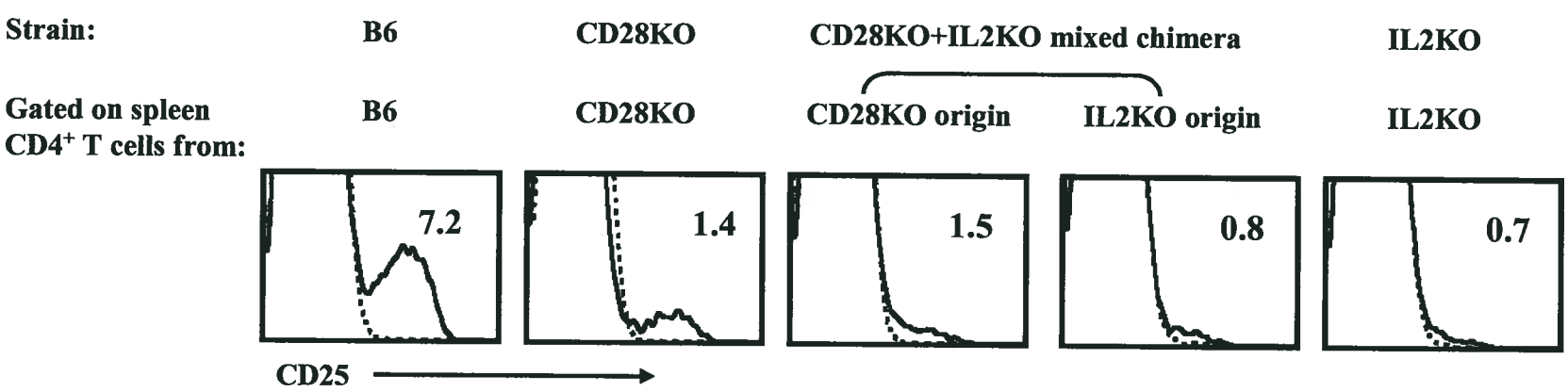
Strain:	B6	CD28KO	IL2KO
Gated on spleen $CD4^+$ T cells from:	B6	CD28KO	IL2KO







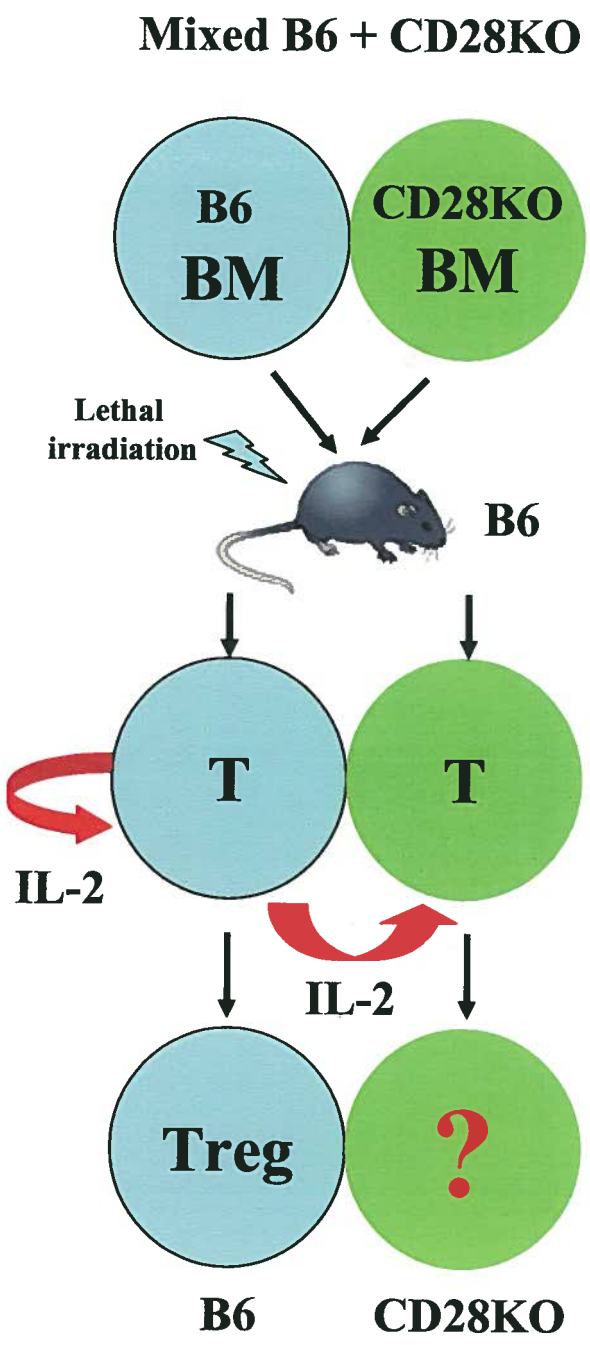


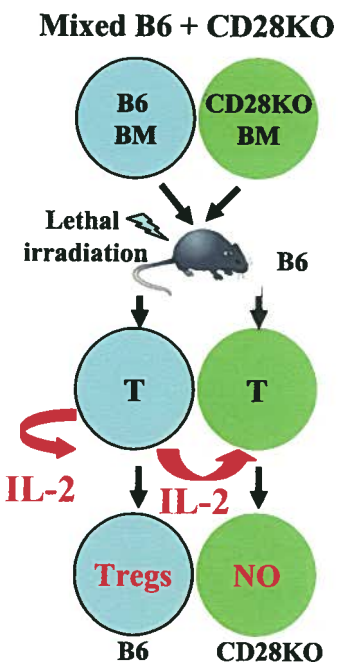


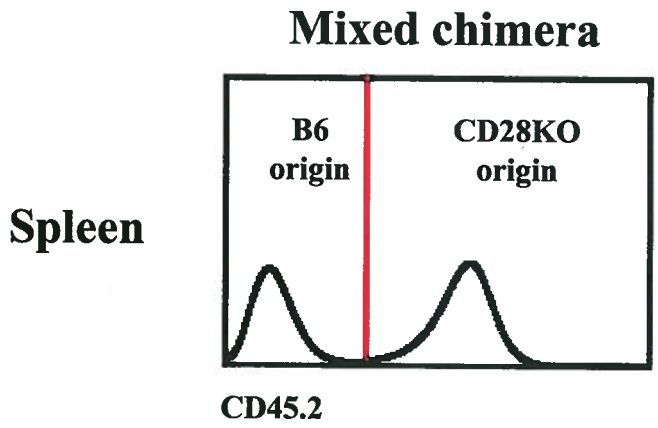
Conclusion: CD28KO T cells do not produce IL-2 in vivo for generating Treg cells.

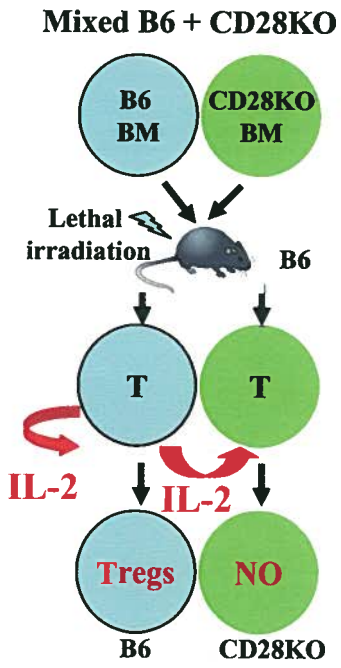
Question:

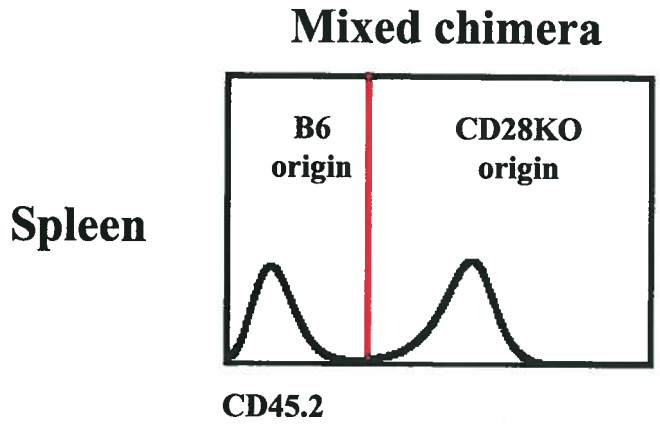
Does in vivo IL-2 overcome the requirement for CD28 signaling for Treg cell generation?

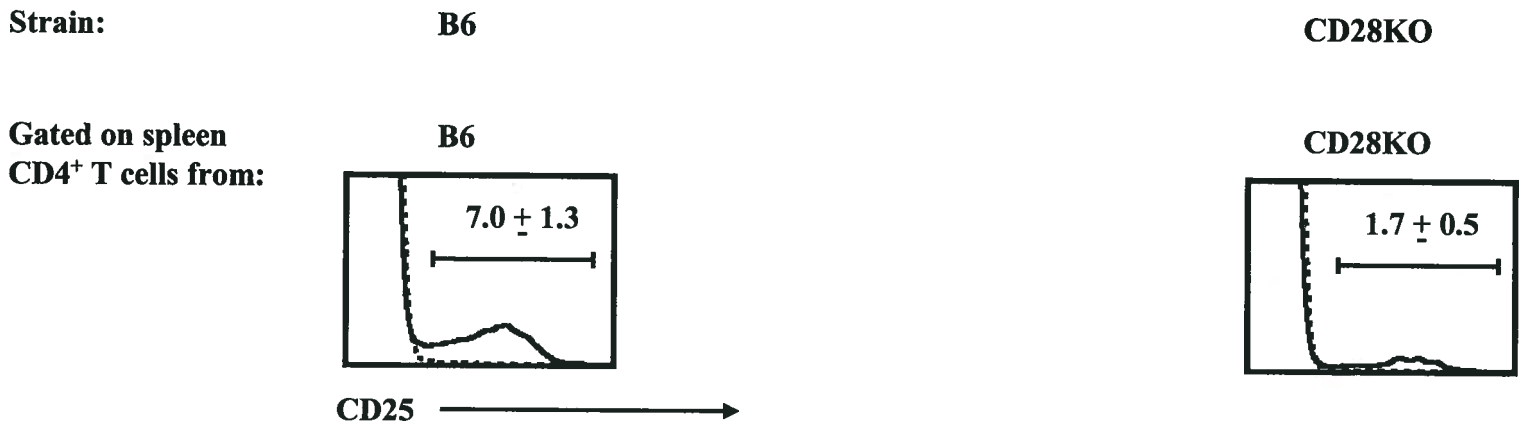

Mixed B6 + CD28KO
B6
BM
CD28KO
BM
Lethal
irradiation
B6
T
T
IL-2
IL-2
Treg
?
B6
CD28KO


Mixed B6 + CD28KO
B6 BM
CD28KO BM
Lethal irradiation
B6
T
T
IL-2
IL-2
Tregs
NO
B6
CD28KO

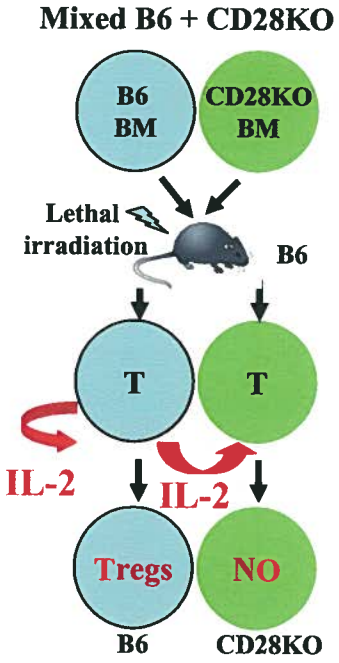

Mixed chimera
Spleen
B6 origin
CD28KO origin
CD45.2


Mixed B6 + CD28KO
B6 BM
CD28KO BM
Lethal irradiation
B6
T
T
IL-2
IL-2
Tregs
NO
B6
CD28KO


Mixed chimera
Spleen
B6 origin
CD28KO origin
CD45.2

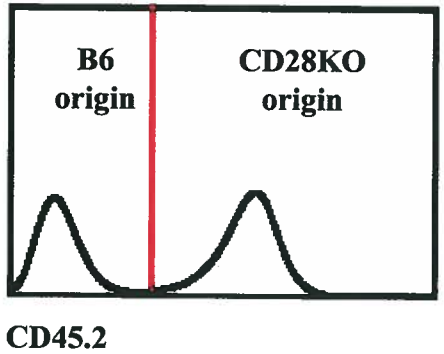

Strain:
B6
CD28KO
Gated on spleen CD4+ T cells from:
B6
7.0 ± 1.3
CD28KO
1.7 ± 0.5
CD25

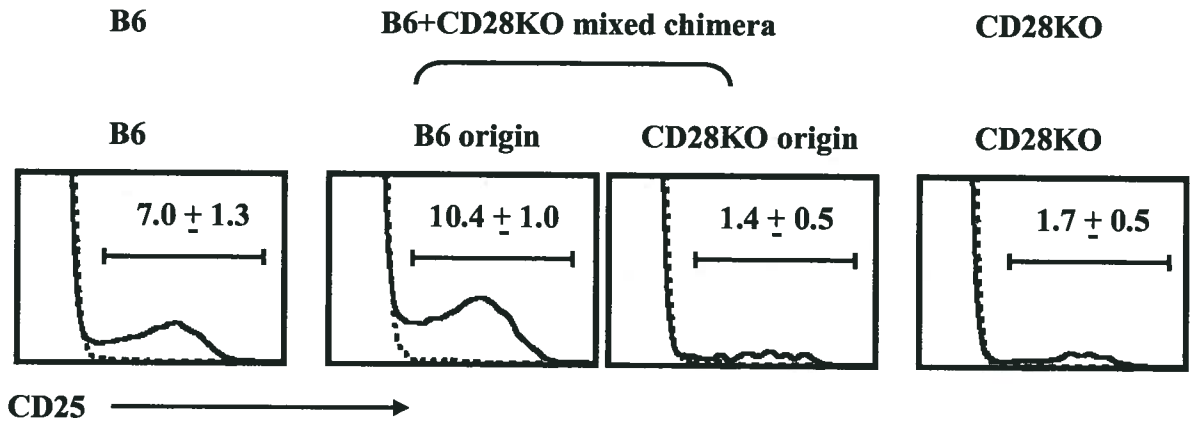
The defect of CD4+CD25+ development in CD28KO is cell autonomous



Mixed chimera

Spleen





IL-2 is required but not sufficient for Treg cell generation in CD28KO mice.

Thus CD28 signaling does more than provide IL-2 for Treg cell generation.

Conclusion:

IL-2 is required for Treg cell generation, but CD28 signaling is required even in the presence of in vivo IL-2.

References:

1. **Current Protocols In Immunology**

   UNIT 4.6 **Assessment of Lymphocyte Development in radiation Bone Marrow Chimeras**

2. **Annu. Rev. Med. 2005. 56:509-38**

Acknowledgement

Experimental Immunology Branch

Alfred Singer
Terry Guinter

Bioqual

Genevieve Sanchez-Howard
Lana Stepanian