

VIDEO

AUDIO

OPEN ON TFL LOGO AND TITLE.  
DISSOLVE TO:

INT. TFL HALLWAY AS  
RESEARCHERS COMPARE NOTES  
(STOCK SHOT 02.01.32.01)

NARRATOR (VO): The Terry Fox Lab in  
Vancouver B.C. has an international  
reputation for standards of excellence in  
research and training. A staff of one  
hundred make up a multidisciplinary unit at  
the forefront of research to improve  
diagnosis and treatment of cancer.

INT. CRYOGENICS LAB --  
EQUIPMENT AND RESEARCHER  
PUNCHING IN NUMBERS (STOCK  
SHOTS 02.09.43.07 AND  
02.10.15.28)

New technologies are developed at Terry Fox  
to address basic questions in a wide range  
of disciplines...

MONTAGE OF STOCK SHOTS FROM  
MOLECULAR BIOLOGY LAB, GENE  
THERAPY LAB, AND MOLECULAR  
GENETICS LAB

...biochemistry, cell biology, molecular  
biology, immunology, genetics ...

RESEARCHER AT MICROSCOPE IN  
CELL CULTURE LAB (STOCK SHOT  
01.02.56.13)

...and in particular -- hematology.  
Long experience has produced an optimized  
and standardized system for studying  
hematopoiesis.

BRING UP TITLE, SUPERIMPOSED:

"How to use the methylcellulose  
system to assay human  
hematopoietic cells"

DISSOLVE TO:  
SAMPLES OF MARROW AND BLOOD  
ARRIVING IN THE LAB

Marrow and blood. The red blood cells will  
be removed, and the scarce hematopoietic  
progenitor cells will be incubated in  
culture as an assay for numerical changes.

PHOTOMICROGRAPHS OF COLONIES

Colonies, each descended from a single  
progenitor, allow counts to be made of

VIDEO

PAN SLOWLY ACROSS AN ARRAY  
OF CULTURING PRODUCTS - LTC  
AND CONDITIONED MEDIA, SERA,  
AND SUPPLIES, COMING TO REST  
ON THE MC MEDIA.

THE MARROW/BLOOD FLOWCHART

HIGHLIGHT THE FIRST MODULE -  
"PREPARATION"

CUT TO TECHNICIAN ALIQUOTTING  
MC MEDIUM INTO TUBES

CUT TO PLACING IN FREEZER

CUT TO TECHNICIAN MIXING  
FCS/ISCOVES INTO TEST TUBES,  
PLACING IN RACK

NOW ALIQUOTTING FICOLL  
INTO CONICAL TUBES

CU OF MC TUBES, FCS/ISCOVES  
TUBES, AND FICOLL TUBES IN  
FLOW HOOD

AUDIO

committed and pluripotent progenitors of  
erythrocytes, granulocytes, and  
macrophages, identified by colony  
morphology.

The Terry Fox Lab's Media Preparation  
Service offers these reagents to any  
investigator to conduct hematopoietic  
assays. The methylcellulose medium promotes  
longer lasting cultures, easier harvesting,  
and growth of both erythrocyte and  
granulocyte colonies together. The required  
protocol for the methylcellulose assay will  
now be demonstrated.

These are the steps of the protocol, which  
will be demonstrated one at a time.  
Appropriate sterile technique and biohazard  
precautions should be upheld throughout.  
All technical details are included in the  
corresponding manual.

To maintain stability of the ready-mix  
methylcellulose culture medium, store in  
the required volumes at minus twenty,  
for no more than a year.

Prepare Iscoves medium with fetal calf  
serum prior to arrival of the marrow or  
blood samples.

This is Ficoll-paque, ready to be used for  
enhanced separation of red blood cells.

All three must equilibrate to room  
temperature.

VIDEO

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BACK TO THE MARROW/BLOOD  
FLOWCHART. HIGHLIGHT "SAMPLE  
MARROW/BLOOD"

CUT TO SAMPLES BEING PLACED  
IN RACK - TWO MARROW...

... AND TWO BLOOD.

THE MARROW/BLOOD FLOWCHART.  
HIGHLIGHT "INITIAL CELL COUNT"

CUT TO ADDING SMALL AMOUNT OF  
MARROW SAMPLE TO FCS/ISCOVES  
TUBE.

ADDING ACETIC ACID

CUT TO TECHNICIAN NOW AT  
MICROSCOPE

VORTEXING SAMPLES

CU OF FILLING THE  
HEMOCYTOMETER

CU OF GRID (PHOTOMICROGRAPH)

TECHNICIAN LOOKING INTO  
MICROSCOPE, CLICKING HAND-  
COUNTER, DUMPING THE FINISHED  
HEMOCYTOMETER IN ALCOHOL,  
AND WRITING DOWN THE NUMBER

THE MARROW/BLOOD FLOWCHART.  
HIGHLIGHT "FICOLL-PAQUE  
SEPARATION" -OR- "RBC LYSIS"

Marrow is collected in these tissue culture tubes with heparin and Iscoves to avoid clotting.

These heparin vacutainer tubes are for the blood samples.

The small sample for counting is prediluted in the Iscoves medium to prevent clotting when acetic acid is added.

Acetic acid with methylene blue is added to lyse the red blood cells and stain for nucleated cells. For counting blood samples, use only half the dilution used for marrow.

If the white blood cell count is abnormally high, repeat the counting with a more dilute sample.

VIDEO

AUDIO

TECHNICIAN HOLDING FICOLL  
TUBE

Generally, enhanced separation in Ficoll is used for both marrow and blood samples, but a simpler lysing method may be used for marrow.

ADDING MARROW TO FCS/ISCOVES.  
RECORDING VOLUME OF MARROW.

This Ficoll is used for density centrifugation of samples. Erythrocytes and granulocytes will end up below the Ficoll. The progenitors will stay on top.

SLOWLY LAYERING MARROW/ISCOVES  
ONTO FICOLL IN CONICAL TUBE.  
SETTING IN RACK.

Marrow is first diluted in the Iscoves and serum solution. Record the marrow volume.

NOW ADDING BLOOD SAMPLE TO  
SECOND FICOLL TUBE.

Blood samples are not diluted.

HOLDING UP SECOND MARROW  
SAMPLE TUBE.

For marrow samples that are known to have elevated levels of progenitors, a simpler method may be used.

MEASURING THAT MARROW VOLUME  
AND TRANSFERRING IT ALL TO A  
TEST TUBE. ADDING AMMONIUM  
CHLORIDE SOLUTION. VORTEX.  
PUTTING ON ICE.

The full measured specimen is combined with buffered ammonium chloride. After ten minutes on ice, all of the red blood cells should be lysed. The lysed cells will remain in the supernatant upon centrifuging.

PICKING UP THE TWO BLOOD AND  
MARROW FICOLL TUBES

The Ficoll samples should not be mixed or disturbed.

CUT TO PUTTING FICOLL TUBES  
IN BECKMAN. CU OF BRAKE BEING  
TURNED OFF. START SPINNING.

Spin for thirty minutes at four hundred g, at room temperature. Do not use the brake.

DISSOLVE TO:  
LATER AT BECKMAN, REMOVING  
FICOLL TUBES.