

VIDEO

AUDIO

OPEN ON TFL LOGO AND TITLE.
DISSOLVE TO:

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

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

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

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

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

PHOTOMICROGRAPHS OF COLONIES

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.

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

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

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

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.

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

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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.