Introductory laboratory exercises for medical technologists

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Preface

This manual is an outgrowth of a one-semester course that has been given for several years to students in medical technology at Brigham Young University. The course was designed to introduce the students to the field of medical technology by performing simple clinical laboratory procedures. Brief explanations of test principles are given so that the student may appreciate the indications for such procedures. The material in the manual may be supplemented in class by photomicrographs and demonstration materials to provide a more graphic understanding of the laboratory procedures.

The manual should be considered as an introduction to laboratory procedures and not as a complete presentation of the subject. Upon completion of the course, the student will have had adequate exposure with laboratory procedures so that he or she will be able to make a rational decision concerning a career in laboratory medicine.

I would like to thank all my students for inspiring the book and my mother for typing the manuscript.

Shauna C. Anderson

Contents

1 Medical terminology

The following work elements are commonly used to construct medical terms

Element	Definition
a-	absent or deficient
ab-	away from
abdomin-	abdomen
ac-	to (*see* af-)
acou-	hear
af-	to
a-, an-	without, not
ant(i)-,	signifying against
ante-	before in time or place
arthr-	joint
auto-	self
bi-	two
bio-	life
brachi-	arm
brachy-	short
brady-	slow
cac-	bad, ill
calc-	stone
capit-	head
carcin-	cancer
cardi-	heart
caud-	tail
cephal-	head
chol-	bile
chro-	color
cis-	cut, kill
corp-	body
cyan-	blue
de-	down, from
derm-	skin
di-	two
dipl-	double
dis-	apart, away from
duct-	lead, conduct
dur-	hard
dys-	bad, improper
e-	out, from
ect-	outside, without
-ectomize	to subject to excision
-ectomy	excision of organ or part
ede-	swell
end(o)-	inside
enter(o)-	intestine
epi-	upon, after, in addition
erythro-	red
ex-	out of
extra-	outside of, beyond, in addition
fasci-	band
febr-	fever
-ferent	bear, carry
fiss-	split
for-	opening
gastr(o)-	stomach
gloss-	tongue
gran-	grain, particle
grav-	heavy
hem(at)-	blood
hemi-	half
hepat(o)-	liver
hetero-	the other
hist(o)-, hist(io)-	web, tissue

Element	Definition
hydro-	water
hyper-	above, beyond, extreme
hypo-	under, below
-ia	state or condition
idi-	peculiar, separate, distinct
infra-	beneath
inter-	among, between
intra-	inside
-ion	process
-itis	denoting inflammation
junct-	yoke, join
labi-	lip
later-	side
leuk-	white
lip-	fat
-logy	science of
lute-	yellow
ly-	loose, dissolve
macr-	large, long
mal-	bad, abnormal
medi-	middle
mega-	great, large
melan-	black
mes-	middle
micr(o)-	small
mon(o)-	one, single
morph(o)-	form, shape
multi-	many, much
my(o)-	muscle
narc-	numbness, stupor
ne(o)-	new, young
necr(o)-	corpse, dead
nephr(o)-	kidney
neur(o)-	nerve
ob-	against, toward, in front of
oc-	against
-odyn-	pain
-oid	resembling
olig-	few, small
-oma	tumor
oo-	egg
or-	mouth
orchi-	testicles

Element	Definition
orth-	straight, right, normal
oss-	bone
ot(o)-	ear
par-	give birth to, bear
para-	beside, beyond
path-	that which undergoes sickness
pen-	need, lack
per-	through
peri-	around
phil-	have an affinity for
phleb(o)-	vein
phob-	fear, dread
pne-	breathing
pod-	foot
poly-	much, many
post-	after, behind in time or place
pre-	before in time or place
pro-	before in time or place
pseudo-	false
py(o)-	pus
re-	back
ren(o)-	kidney
retro-	backwards
-rrhage	excessive flow
-rrhea	flow or discharge
sanguin-	blood
sarc-	flesh
-sect	cut
-sis	state or condition
-stalsis	contraction
sub-	under, below
super-	above, addition, implying excess
supra-	above, upper, over
syn-	with, together
tac-	order, arrange
tachy-	swift, rapid
tens-	stretch
tetra-	four
therm-	heat
thorac-	chest
thromb(o)-	lump, clot

Element	Definition
tom(y)-	cut
tox-	poison
tract-	draw, drag
tri-	three
uni-	one

Element	Definition
ur(o)-	urine, urinary organs or tract
vas-	vessel
vit-	life
zyg(o)-	union, join

See worksheets, pp. 73-75.

2 Hematology

COLLECTION OF BLOOD

Finger puncture

MATERIALS:

Cotton balls
70% alcohol
Sterile blood lancet

PROCEDURE:

1. With cotton moistened with 70% alcohol, cleanse pad of finger.
2. With a piece of dry cotton, thoroughly dry pad of finger.
3. Pick up a sterile blood lancet and remove wrapper.
4. With right hand, firmly grasp sterile lancet.
5. With left hand, firmly grasp patient's middle finger.
6. With a quick drop and a quick rise of lancet, make *deep* stab on pad of finger.
7. Take a piece of dry cotton and wipe away first drop.
8. Form a large rounded drop of blood at site of puncture.
9. Perform tests desired.
10. Place a piece of cotton on puncture until bleeding stops.

Venipuncture

MATERIALS:

Cotton balls
70% alcohol
Tourniquet
Needle (20 gauge)
Vacutainer tube and holder

PROCEDURE:

1. Assemble Vacutainer shell and needle. (The diameter of a needle is given by its gauge number. The smaller the number, the greater the diameter.)
2. Apply tourniquet above bend in elbow.
3. Select vein.
4. Moisten a piece of cotton with 70% alcohol and thoroughly rub cotton on vein you have selected.
5. Select a proposed point of entry into vein. Now place left thumb about

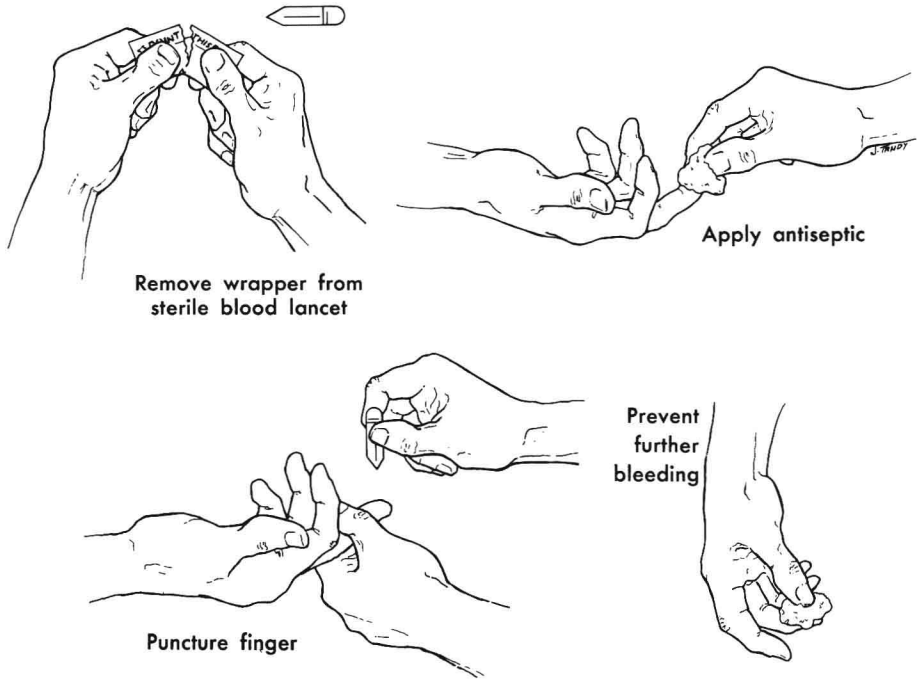

Fig. 2-1. Finger puncture technique.

2.5 cm (1 in) below this proposed point of entry. Press down firmly with thumb and pull skin toward yourself.

6. Point needle in exactly the same direction as vein is running.
7. Hold Vacutainer at a 15° angle and needle bevel up.
8. Push needle firmly and deliberately into vein.
9. Withdraw blood.
10. Release tourniquet.
11. Pick up a piece of cotton and gently hold it on puncture.
12. Withdraw needle.
13. When needle is out of arm, press cotton on puncture.

ESTIMATION OF HEMOGLOBIN

Hemoglobin is a conjugated protein present in the red blood cells. It is responsible for the red color of blood. The prosthetic (nonprotein) compound combined with protein (globin) to form hemoglobin is called *heme.* Heme is an organic compound containing iron in chemical combination (iron porphyrin). This iron has a valence of +2 (ferrous iron).

It is the function of hemoglobin to combine loosely with oxygen in the lungs and to take it to the tissues, where a part of this oxygen is released. Hemoglobin combined with oxygen is called *oxyhemoglobin.* Oxyhemoglobin shows three absorption bands when scanned in a spectrophotometer (absorption at a wavelength of 578, 542, and 415 nm).

Methods for the determination of hemoglobin concentration of whole blood might be divided into two groups: primary and secondary methods. The primary methods are, for all practical purposes, too tedious and time consuming to

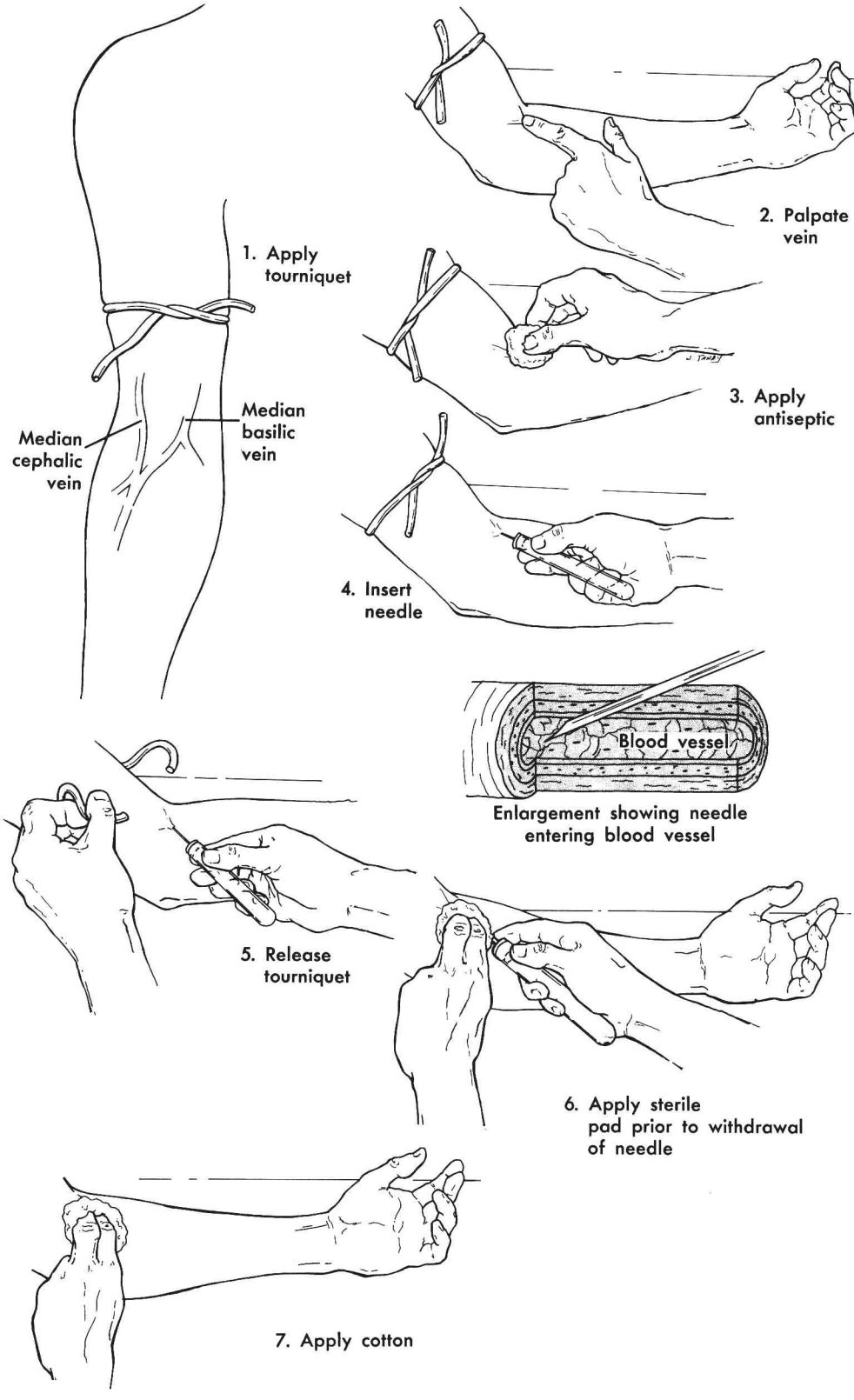

Fig. 2-2. Venipuncture technique.

be used as a routine method for hemoglobin analysis, but they have their value in that they can be used for the standardization of routine procedures (secondary methods). The properties of hemoglobin that serve in primary methods are essentially two: (1) the oxygen-combining property of hemoglobin and (2) the iron content of the hemoglobin molecule. Most of the secondary methods are based on spectral characteristics of hemoglobin or its derivatives.

Each gram of oxyhemoglobin is capable of combining with 1.34 volumes percent (vol%) of oxygen. Therefore if the oxygen capacity of blood is divided by 1.34, the quotient gives the number of grams of hemoglobin per 100 ml of blood. In fully oxygenated blood from a normal person there will be about 20.9 vol% of molecular oxygen. Therefore an average value of 15.6 of hemoglobin is present.

Each 100 g hemoglobin contains 335 mg iron. Therefore if the iron contained in 100 ml of blood is determined, and this value is divided by 3.35, the quotient equals the grams of hemoglobin per 100 ml of blood.

The blood oxygen capacity measures functional hemoglobin only and is inaccurate in that 2% to 12% of adult hemoglobin may be of an inactive form (unable to take up oxygen), which cannot be regenerated, and therefore it would not be measured by this method.

Total-blood-iron measurement for all practical purposes may be regarded as being bound to hemoglobin, the serum iron level being relatively small. Total-blood-iron analysis is considered the best method for the primary standardization of routine hemoglobin analysis.

Cyanmethemoglobin method

PRINCIPLE: In the cyanmethemoglobin technique the blood specimen is diluted with Drabkin's reagent. The potassium ferricyanide converts hemoglobin iron from the ferrous state to the ferric state to form methemoglobin, which then combines with potassium cyanide to produce the stable pigment cyanmethemoglobin. The absorbance of the cyanmethemoglobin is then read at 540 nm.

MATERIALS:

1. Drabkin's reagent:
   1.0 g sodium bicarbonate
   0.05 g potassium cyanide
   0.20 g potassium ferricyanide
   Distilled water to 1 liter
   (This solution should be kept in brown bottle not longer than 1 month. The solution is clear and pale yellow. Discard if it appears turbid.)
2. 5 ml transfer pipette
   20λ (0.02 ml) pipette
   Cuvettes
   Spectrophotometer

PROCEDURE:

1. Measure 5.0 ml Drabkin's reagent into cuvette.
2. Draw blood into a hemoglobin pipette until it is slightly above the 0.02 ml

mark. Wipe excess from outside of pipette and adjust exactly to 0.02 ml mark by touching tip of pipette to finger.

3. Blow blood into diluent and rinse pipette at least three times with diluent. Cover cuvette with parafilm and mix contents by inverting several times.
4. Let stand 5 min.
5. Adjust spectrophotometer to zero absorbance with cuvette filled with Drabkin's reagent at a wavelength of 540 nm.
6. Place cuvette containing blood sample in spectrophotometer. Read and record the reading.

CALCULATIONS: Transfer this reading to the standard curve and obtain the hemoglobin concentration in grams per deciliter of blood.

NORMAL VALUES:

Male:	15 to 19 g/dl (at 4400 ft)
	14 to 18 g/dl (at sea level)
Female:	13 to 17 g/dl (at 4400 ft)
	12 to 16 g/dl (at sea level)

HEMATOCRIT

PRINCIPLE: The hematocrit is a test to determine the ratio of cells to fluid in blood. This test is generally considered more accurate than the red cell count.

MATERIALS: Capillary tubes (1 mm bore and approximately 75 mm in length): a blue-tipped tube does not contain any anticoagulant and is used when whole blood has already been treated with an anticoagulant. A red-tipped tube contains heparin and is used with capillary blood.

PROCEDURE:

1. Fill a capillary tube 2/3 to 3/4 full of blood. If using capillary blood, tilt tube back and forth to allow heparin to mix with blood and thus prevent coagulation.
2. Seal end of capillary tube with clay.
3. Centrifuge tube in a microhematocrit centrifuge at 12,000 rpm for 5 min.

CALCULATIONS: The volume of packed cells is expressed as a percentage of the total length of the column of blood. A special hematocrit reader is available for this measurement.

NORMAL VALUES:

Male:	45 to 51 (at 4400 ft)
	40 to 47 (at sea level)
Female:	40 to 49 (at 4400 ft)
	37 to 47 (at sea level)

WHITE BLOOD CELL COUNT

PRINCIPLE: The diluting fluid hemolyses all nonnucleated red cells but does not alter leukocytes, thus facilitating enumeration of the white blood cells.

MATERIALS:

3% glacial acetic acid
White blood cell pipette
Hemacytometer counting chamber

PROCEDURE:

1. Draw blood slightly above 0.5 mark of white cell pipette. Wipe off outside of pipette and adjust blood exactly to 0.5 mark by touching pipette tip to finger.
2. Draw 3% acetic acid diluent to 11 mark.
3. Shake pipette (3 min by hand).
4. Expel and discard first 4 drops from pipette.
5. Place clean coverslip on counting chamber. Allow counting chamber area under coverslip to be completely filled with mixture.
6. Under low power, count number of leukocytes in each of the four large corner squares.

CALCULATIONS:

Dilution 1:20

Volume counts 4 per 10 cu mm

Number of cells counted × 10/4 × 20 = number of cells per cubic millimeter

NORMAL VALUES: 5000 to 10,000 per cu mm. Values above 11,000 are usually considered as representing leukocytosis, whereas those below 4000 indicate leukopenia.

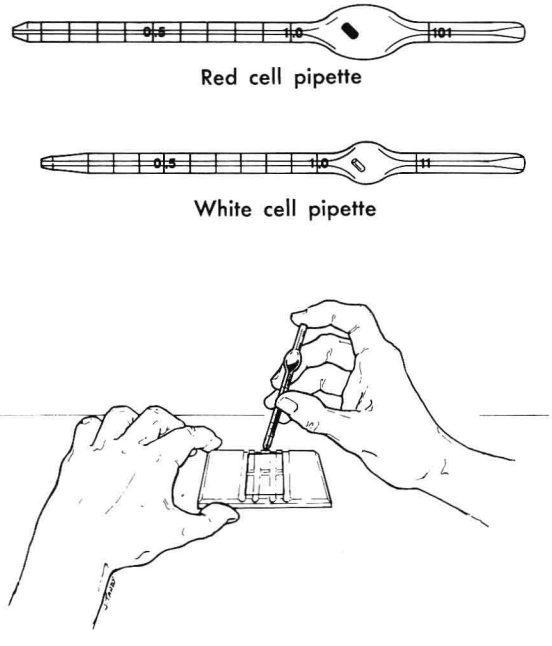

Fig. 2-3. Method for charging hemacytometer counting chamber.

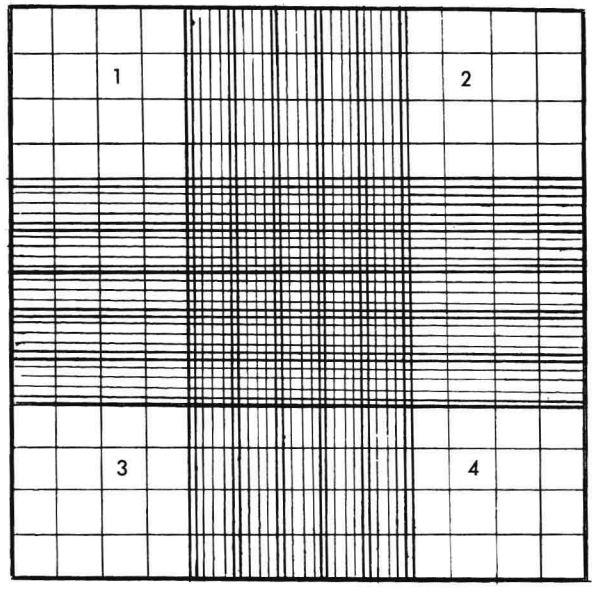

Fig. 2-4. The hemacytometer.

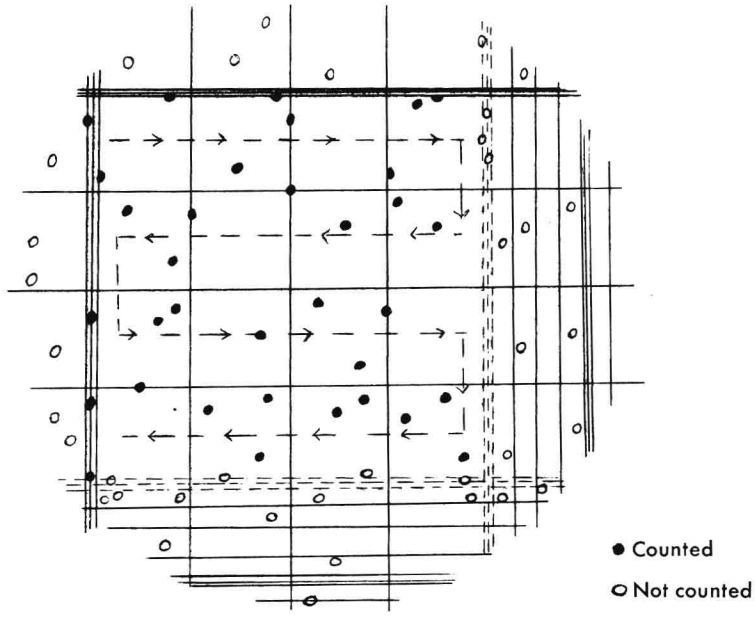

Fig. 2-5. Diagram of white cell count. A white cell is counted only once by counting those within the medium-sized square and those touching any line at the left and top, but not counting those at any line at the right and bottom of the medium-sized square. All cells touching the triple lines shown as broken lines will be excluded.