PART III

VTPA 346.3
2015-2016
LABORATORY MANUAL

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Student Slide Boxes

January 2016

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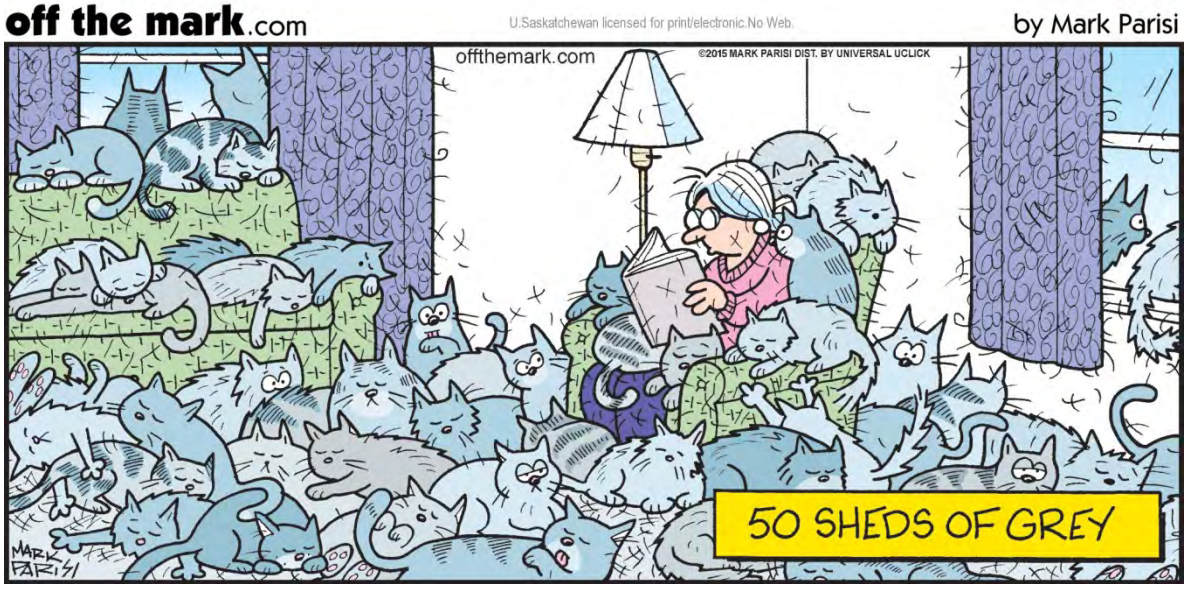
* Not all slide boxes contain these slides.



Cartoon copyrighted by Mark Parisi, printed with permission.

LAB 1: COMPLETE BLOOD COUNT (CBC)

Procedures: (Using the EDTA anticoagulated whole blood provided.)
A. Determine the total leukocyte number using the Bioanalytic Leuko-tic
B. Measure the packed cell volume (PCV) and calculate RBC indices using RBC count and hemoglobin values provided
C. Measure the total protein using the refractometer
D. Practice making blood smears
E. Stain your best smear

The final component of a CBC (for subsequent labs):
F. Blood smear evaluation (for subsequent labs)
- ***. Scanning the smear at low magnification***
- ***. Estimating leukocyte numbers***
- ***. Estimating platelet numbers***
- ***. Evaluating RBC morphology***
- ***. Leukocyte differential count***

Procedures:

A. Determine the total leukocyte (WBC) count using the Bioanalytic Leuko-tic:

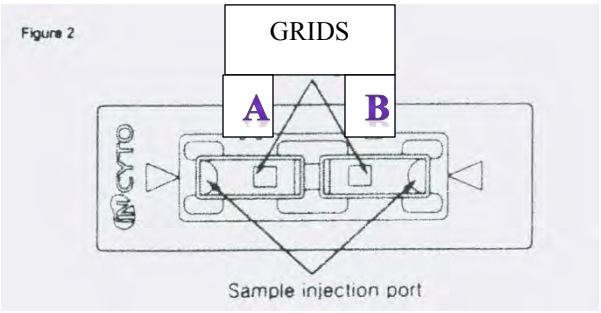
1. Follow procedure for sample dilution given in the manufacturer's instructions (pg 8-9) or use the "Speedy Version", page 7.

2. Charge the C-Chip disposable hemocytometer as follows:

a. Touch the end of a ¾ filled microhematocrit tube to the bottom of the crescent shaped opening (sample injection port in Figure 2 below). Allow fluid to fill counting area. Avoid overflow by not expelling a large drop prior to filling. Overflow is indicated by liquid in the center between counting areas.

b. Fill **both** the A and B sides of the hemocytometer.

Figure 2



Note: grids are visible on the hemocytometer, with the naked eye. This will help you position them on your microscope. (10x objective)

3. Set the hemocytometer aside for **1- 3** minutes to allow the cells to "settle" before counting.

4. **Lower the substage condenser** to optimize viewing of grids and WBCs. Use the 10x objective and find the grid. Grids are located near the "inside ends" of the filled areas and are visible with the naked eye.

5. Count the total number of WBCs in the 4 large corner squares of **both grids** (see diagram page 7)**,** calculate the average of the two grids and use this figure for step #6 below; target agreement between the 2 grids is 10%.
Average # of WBCs in 4 large corner squares calculated as follows:
[Side A (total, 4 large corner squares) + side B (total, 4 large corner squares)] ÷ 2

6. Calculate the total WBC count as follows:

Average # of WBCs in 4 large corner squares X 50 = # WBCs/µl
For SI units: ÷ 1000 = # of WBCs x 10^9/L
Example: If the average number of cells counted is 100, the total count is:
100 X 50 = 5000/ µl*
5000 divided by 1000 = 5 x 10^9 /L
(*5000/ µl = 5 x 10^6/ml = 5 x 10^9 /L)
***Note that the figure of 50 that is used in the initial formula is derived from the following: dilution factor (1:20) x 10/4 (ie. Volume factor/ 4 large corner squares counted)**

Leuko-tic: requires dilution of blood (to lyse the RBCs), hemocytometer, and a microscope.

Advantages: Inexpensive; can be used in clinic; can count nucleated cells in any fluid (CSF, thoracic and abdominal fluid, synovial fluid).

Disadvantages: Potential dilution and counting errors mean that the error rate using this method is approximately 20%. Time-consuming.

Automated counter: most instruments work on the electrical impedance principle or laser separation.

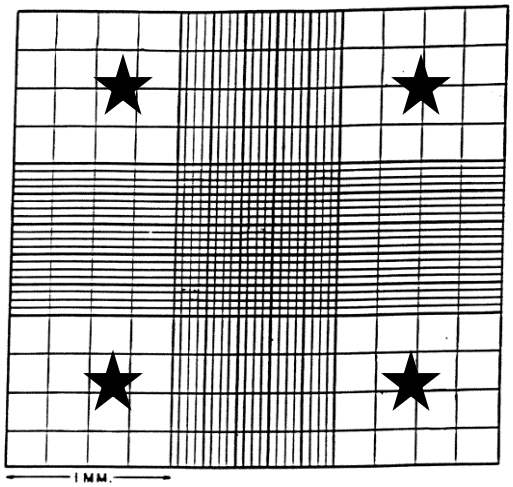
Advantages: Can count nucleated cells in any fluid, fast and accurate. Error rate ~5%.

Disadvantages: Expensive; errors may occur due to nucleated erythrocytes, clumping of cells, rupture of fragile cells and interference by platelet clumps and other particles in blood.

"Speedy Version"

WBC Count – Leuko-tic System

1) Using Capillary holder, fill a 20 uL pipette end to end with whole blood.
2) Using a Kleenex, wipe off outside-adhering blood without changing sample volume.
3) Drop filled capillary into diluent vial.
4) Quickly invert many times to mix (capillary tube remains in vial).
5) Wait a minimum of 30 sec. for RBC lysis.
6) Using a microhematocrit tube (not the capillary tube in the diluent), fill sides A & B of C-Chip disposable hemocytometer.
7) Allow to sit 1 – 3 minutes (allows cells to settle).
8) Lower condenser. Find grid (x 10 objective).
9) Count WBCs in 4 large corner squares of each side. If cells are overlapping lines, count either left & bottom or right & top).
10) Average the totals of the two sides.
11) See page 6, #6 for calculation.



Hemocytometer Grid: each hemocytometer has 2 grids (A&B); each grid has 9 large squares; count WBCs in 4 large corner squares (as marked with ★), on both grids, and average the 2 grids. This figure represents one grid.
Some cells will overlap the bordering grid lines. Count these cells either on the left & bottom **or** the right & top.

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Leuko-TIC®

1 : 20 • blue

REF

Single tests for quick, uncomplex, clean and precise counting of WBC.

Cont.

4013-0006/-0007/-0008 (EDMA 13 01 09 90 00) 100pcs

Product information for quantitative visual microscopic counting of white blood cells (WBC). Leuko-TIC »blue« corresponds improved Türk's solution and occurs without distracting background of RBC membranes.

Principle

Microscopic counting of white blood cells (WBC) in the counting chamber after lysis of the Red Blood Cells (RBC) and fixation of the WBC nucleus. The WBC nucleuses appear distinctly in front of a clear background. Gentian violet stain the WBC nucleus lightly violet-blue.

Leuko-TIC's for WBC counting allow quick, uncomplex, clean, and precise method of operation. The vial contains LeukoCount solution. Sample volume amounts 20µl blood (dilution 1 : 20).

Reagent

Leuko-TIC's are ready for use and have a shelf life at room temperature (+15...+25 °C) until to the imprinted expiry date. Remove vials only for use. Store vials on a dark place (closed box) and upright in the package.

Don't use if reagent is not clear, blue and free of particles.

Risks and Safety

Please maintain the necessary precautions for use laboratory reagents. Applications should occur only from expertly personal. Wear protective clothing and disposable gloves while handling.

Content / main components

004013-0006	1x 100pcs	Leuko-TIC 1:20 blue (Single tests) 380µl of acetate buffer pH = 3.0 isotonic to WBC, gentian violet 0,1g/l, detergent (Octylphenolethoxylate < 0,3%) as stabilizer. Reagent is azide free and contains no toxic and environment polluting mercury compounds (Thimerosal etc.).
004013-0007	1x 100pcs 1x 100pcs 1x 100pcs	Leuko-TIC 1:20 blue (Single tests) End-to-end volume capillaries 20µl Chamber filling capillaries.

Replacement pack

TIC-CP20	1x 1x 100pcs 1x 100pcs	TIC 20µL Capillary pack, contents: End-to-end volume capillaries 20µl Chamber filling capillaries.

Do not use other capillaries which are not declared for this test kit.

Additional required materials

099920-0001 Capillary holder.

Counting chamber (Neubauer improved, Neubauer); Microscope for medical laboratory purpose.

Sample

Fresh capillary blood process up at once. K_2- or K_3-EDTA blood, sterile and well closed can be used up to 24h when stored at +2...+8 °C. Don't freeze!

Out of the Leuko-TIC WBCs can be counted up to 48h.

Procedure

With included capillary pipettes (004013-0007):

Fill up one of the 20µl end-to-end volume capillaries free of bubbles from end to end with blood. For this procedure it is recommend to use a capillary holder (see ordering Information). Don't use the first resigning capillary blood drop. Remove outside adhesive blood with a fluffless tissue - don't change the blood volume. Give the filled volume capillary into the opened vial and shake very well until all blood is removed from inside the capillary. Wait for a minimum of 30 seconds for lysis RBCs is complete. Don't remove the capillary out of the vial.

Shake the vial once more before filling the counting chamber. Fill the filling capillary about ½ length and close on the upper end with the finger. Adduct in a small angle to the cover slip and refill the counting chamber.

Without capillary pipettes (004013-0006):

Instead of the 20µl end-to-end volume capillaries and the chamber filling capillaries use an automatic pipette 20µl (only usable for EDTA-blood). This mode of operation is only recommended for laboratory experts!

Examination / calculation

Microscopic counting with phasing-contrast or transmitting light (lowered condensing lens) at 100× enlargement.

Counting chamber Neubauer / Neubauer "improved".

Count the WBCs of the 4 big corner squares of each 1mm² surface, consisting from 4 × 4 squares. If you use the Neubauer „improved" counting chamber, count cells by the middle line.

Sum out of the 4 big corner squares × 50 = WBCs/µl blood

Capability characteristics

In comparison to (in the meantime obsolete) method with Türk's solution and diluting by means of blood mixing pipettes Leuko-TIC ® can be valued clearly advantageously.

Range / Limitation

Strongly incremented or degraded cell values can complicate a correct cell counting. In these cases a suitable diluting should be chosen and this is to be considered in the calculation.

Precision Leuko-TIC®

In the series n = 25	mean value [10³/µl]	SD [10³/µl]	CV [%]
Specimen 1	6.75	0.424	6.27
Specimen 2	9.91	0.618	6.23

Precision Türk's Solution and blood mixing pipette

In the series n = 25	mean value [10³/µl]	SD [10³/µl]	CV [%]
Specimen 1	6.88	0.794	11.54
Specimen 2	10.1	1.073	10.62

We hold an efficiency comparison to automated cell counters for not right, because these are strongly dependent on correct calibration.

Information

For in-vitro diagnostic use.

Special product information for counting very low WBC values on request available.

Support / Infoservice

Methodical and technical support by email (support@bioanalytic.de) or by fax (German, English).

Waste management

Please see regulations by law of your country.

Literature

Legends for used graphic symbols and tags are according to the norm or available on our internet pages.

[1] DIN 58932

[2] Wintrobe, Clinical Hematology, S. 1795 (1974), Lea & Febiger Philadelphia.

[3] Rick, Klinische Chemie und Mikroskopie, 24 (1977), Springer Verlag Berlin.

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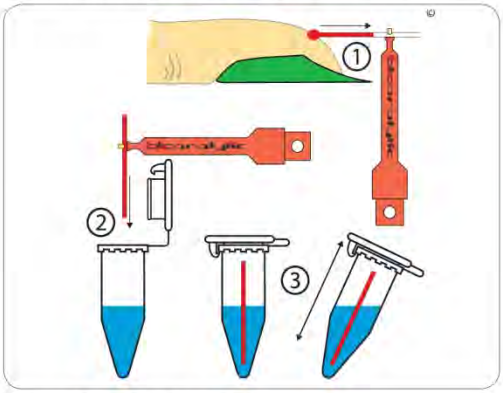
TIC's®

Ery-TIC® • Leuko-TIC® • Thrombo-TIC®
Reti-TIC®

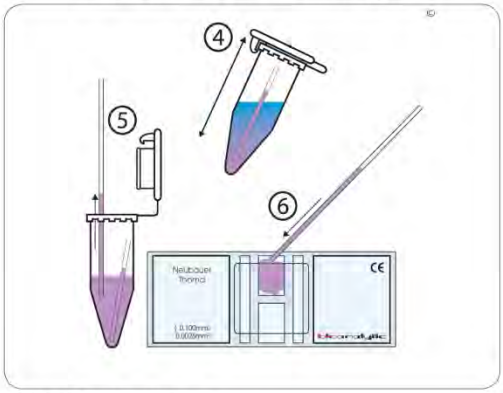
(EDMA 13 01 09 90 00)

Save working time ... work profitable and safe ...



1. Fill end-to-end capillary with blood.
2. Drop down capillary in the vial.
3. Close vial and mix blood out.

... with the „-TIC's" of bioanalytic !



4. Mix before chamber filling.
5. Fill chamber-fill-capillary (capillary attraction).
6. Fill counting chamber and count the cells.

Safe
Completely single use tests for simple, quick, safe and highly precise microscopic blood cell counting. They are packed in practical rack-boxes made from CFC free polystyrene.

Simple
Prepared high-quality reagents of manufacturer bioanalytic make possible a simple and safely handling.
There is no circumstantial handling of expensive equipment (graduated pipettes, vials, vibrators). There is also no expensive pipette cleaning and drying and no silicone coating for prevention of platelet-glass-aggregation (to low values).
The microscopic cell counting of cells will be made as known.

Inexpensively
Save high costs of pipettes, cleaning agents, working time and expiration of reagents by contamination risk.

High precision
The dilution is in agreement with the recommendations of the norm DIN 58932 and the WHO (World Health Organization). They can be exactly reproduced.
Fresh prepared samples can be counted still after 48 h (RBC, WBC) respectively 12 h (PLT)

Hygienic
bioanalytic „-TIC's" are in accordance with the rules and ordinances for do not pipette with the mouth. There is no contact with blood or blood reagent mixture at usage as recommend.

Information
WHO
Already in the year 1988, the WHO (World Health Organisation) has declared the use of Thoma type pipettes to **obsolete** [1] - therefore classified as not usable and deprecated, because they are **very inaccurate** [3] and **cracking easily** (risk of infection).
Instead of this it is recommend to use separate pipettes for blood admixing to ready for use reagent vials.
These recommendations are in accordance to the bioanalytic single tests Ery-TIC®, Leuko-TIC® and Thrombo-TIC®, which started in the market **by bioanalytic 10 years sooner**.

Ordering Information
Capillary holder (for multiple use) is not included and it is recommended to order it separately at your first order. With the capillary holder you can handle the capillaries more simple and safely and minimize the blood contact risk.

Products / Ordering numbers
Products, ordering numbers and further information you find on the internet under www.bioanalytic.de.

Shortcuts
Ery = RBC (red blood cells; erythrocytes); Leuko = WBC (white blood cells; leukocytes); Thrombo = PLT (platelets; thrombocytes); Reti = reticulocytes.

Support
If there are any questions, don't hesitate to contact us by phone (German), eMail or fax (German, English).

Literature + Information:
[1] Recommended methods for the visual determination of white cell and platelet counts, WHO-report WHO/LAB/88.3, WHO, CH-1211 Genf 27. Publication (Germany): Laboratoriumsmedizin 13, Nr. 2: BDL 17 (1989).
[2] Laboratoriumsmedizin 13, Nr. 5: BDL 54 (1989).
[3] The inaccuracies applies not only to the volume of the Thoma type pipettes, but also to the application proceeding - therefore it is also valid for calibrated or certified (CE indicated) pipettes.

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(1/1) product information TIC's overview information + WHO 2008-08-25 (en) 004010-PR01

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Thrombo-TIC®

1 : 100

Single Tests for Quick, Simple, Clean and Precise Counting of Platelets (PLT).

Product information for quantitative visual microscopic counting of platelets (PLTs).

Principle

Microscopic counting of platelets (PLTs) after lysis of red blood cells (RBCs) and disaggregation of platelets (separation and shape change). The platelets appear nearly round, frequently have one or more dendritic processes, colourless, with darker rim and have a size of about 30 % of red blood cells (RBCs).

Thrombo-TICs® for platelet counting allow quick, uncomplicated, clean and precise method of operation. The vial contains ThromboCount® solution. Sample volume amounts to 10 µl blood (dilution 1 : 100).

Reagent

Thrombo-TICs® are ready for use and have a shelf life at room temperature (+15 ... +25 °C) up to the imprinted expiry date.

Remove vials only for use. Store vials on a dark place (closed box) and upnght in the package.

Don't use if reagent is not clear, colorless and free of particles or if there are any crystallizations.

Risks and Safety

Please maintain the necessary precautions for use of laboratory reagents and body fluids. Applications should be performed by expert personnel only. Wear protective clothing and disposable gloves while handling. Use a capillary holder for volume capillaries.

For further safety information please see the corresponding Safety Data Sheet (SDS). Download www.sds-id.com/100038-8 or via this QR-Code

Content / main components

004015-0006	1× 100 pcs	Thrombo-TIC® 1:100 (Single tests) *
		990 µl of 1 % oxalate buffer pH = 6.0.
004015-0007	1× 100 pcs	Thrombo-TIC® 1:100 (Single tests)
	1× 100 pcs	End-to-end volume capillaries 10 µl
	1× 100 pcs	Chamber filling capillaries.

Replacement pack

TIC-CP10	1×	TIC 10 µl Capillary pack, contents
	1× 100 pcs	End-to-end volume capillaries 10 µl
	1× 100 pcs	Chamber filling capillaries.

Do not use other capillaries which are not declared for this TIC test kit.

Additionally required materials

099920-0001	Capillary holder.*
CC-NEUI	Counting chamber (Neubauer improved)*
	Microscope for medical laboratory purpose
	Humidity chamber (moisturized filter paper in Petri Dish)

* Available at Bioanalytic GmbH

Sample

Process fresh capillary blood immediately after collection.

K_2- or K_3-EDTA blood, sterile and well closed can be used up to 24 hrs [5] when stored at +4 ... +8 °C. Don't freeze!

With Thrombo-TIC diluted samples count within 6 hrs. Resuspend the cells before.

For sample collection, storage and labeling follow the standards of technology and related instructions.

Range of reference

	[10^3/ µl]
	100 ... 440 [5]

Detailled referenced ranges in respect to age see literature [5].

Procedure

Use the reagent at room temperature of 18 ... 25 °C.

With capillary pipettes:

Fill one 10 µl end-to-end volume capillary free of bubbles from end to end with blood. For this procedure it is recommend to use a capillary holder (see ordering information). Discard the first drop of capillary blood. Remove remaining blood from the outside of the capillary with lint free tissue without drawing blood out of the capillary. Give the filled volume capillary into the opened vial, close and shake very well until all blood has been removed out of the capillary. Wait for a minimum of 5 minutes for cell lysis is complete. Don't remove the capillary from the vial.

Shake the vial once more before filling the counting chamber. Fill the filling capillary about ¼ ... ½ length and close the upper end with the finger. Adduct in a small angle to the cover slip and refill the counting chamber.

For sedimentation of the blood cells incubate the counting chamber in a humidity chamber for 10 ... 20 minutes.

With automatic pipette:

This mode of operation is recommended for laboratory experts only!

Instead of the 10 µl end-to-end volume capillaries and the chamber filling capillaries use an automatic pipette 10 µl (only in case of EDTA-blood). Rinse pipette tip sufficiently. Shake the vial once more before filling the counting chamber.

Examination / Calculation

Microscopic counting with phase-contrast optics or with transmitting light at 400× magnification.

Counting chamber Neubauer ("improved"):

Count 25 group squares (each of them contains 16 smallest squares). This is the complete middle field of 1 mm². In the border squares count cells up to the middle line.

Sum × 1'000 = platelets (PLT) / µl blood

Thrombocytopenia

For thrombocytopenia the following options are recommended:

a) Count the complete Neubauer grid (= 9 mm²). This is 9× the normal count, so calculating factor changes.
Calculating factor = 1000 / 9 = 111.

Change the dilution. Take instead of 10 µl for normal a bigger blood volume with automatic pipette. Rinse pipette tip multiple with Thrombo-TIC solution.

b) Recommended dilution for values < 80000:
20 µl blood (Dilution 990 + 20 = 1010. 20 : 1010 = 1 : 50.5).
Calculating factor = 505.

c) Recommended dilution for values < 40000:
50 µl blood (Dilution 990 + 50 = 1040. 50 : 1040 = 1 : 20.8).
Calculating factor = 208.

Thrombocytosis & PRP

a) For thrombocytosis with results of > ~400 × 10^3/µl it is possible to count only 5 of the 25 group squares or 4 lines.
Calculating factor = 5000.

b) With results > ~1000 × 10^3/µl, as for the count of platelets from platelet rich plasma (PRP), a higher dilution is required (e.g. 1 : 250 or 1 : 550). Please download/request special instructions.

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Capability characteristics

Thrombo-TIC® is evidently advantageously in comparison to the method with solution diluting by means of blood mixing pipette (meanwhile obsolete).

Range / Limitation

Strongly increased or decreased cell values can complicate a correct cell counting. In these cases a suitable dilution should be chosen, which has to be considered in the calculation.

Precision Thrombo-TIC®

Intraday n = 25	mean value [10^3/ µl]	SD [10^3/ µl]	CV [%]
Specimen 1	218	12.2	5.58
Specimen 2	394	20.9	5.30

Precision Thrombo-Solution and blood mixing pipette

Intraday n = 25	mean value [10^3/ µl]	SD [10^3/ µl]	CV [%]
Specimen 1	202	21.3	10.6
Specimen 2	367	37.8	10.3

Information

For in-vitro diagnostic use.

Classifications

EU: EDMA: 13 01 09 90 00; IVD Class A
AU: Class I; IVD
CA: HC: Class I; exempt; for in-vitro diagnostic use
US: FDA: JCG; Class I; exempt; for in-vitro diagnostic use.

Support/Infoservice

Methodical and technical support by eMail (support@bioanalytic.de) or by fax (German, English).

Check the actuality of this product information periodically on our website.

Waste Management

Please refer to the legal regulations.

Used and rejected solutions must be disposed of according to local regulations. Decontaminated packaging may be treated like household waste or recycled, unless otherwise specified.

Literature and Footnotes

Legends for used graphic symbols and tags are according to the norm or available on our internet pages.

[1] DIN 58932
[2] Wintrobe, Clinical Hematology, S. 1795 (1974), Lea & Febiger Philadelphia.
[3] Rick, Klinische Chemie und Mikroskopie, 24 (1977), Springer Verlag Berlin.
[4] WHO-Bericht Lab/88.3
[5] Thomas L., Labor und Diagnose, (1992) 4. Aufl., Die Medizinische Verlagsgesellschaft Marburg

*1 Alternatively we recomend Thrombo-TIC® fix-20 for dilution of 1 : 20. Therefore only 1/5 of the squares have to be counted, however the value of counts is identical.

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● biomedical analysis technology
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Disposable hemocytometer - C-chip

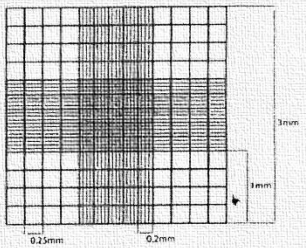
CYTO C-Chip™
Disposable Haemocytometer

INTENDED USE

InCyto C-Chip™ disposable haemocytometer is a disposable plastic haemocytometer for manual counting of cells.

INTRODUCTION

InCyto C-Chip™ disposable haemocytometer is made of optical transparent thermoplastic polymethyl methacrylate with 2 counting chambers on each slide. The grid is exactly the same as the Neubauer improved which consists of 9 large squares, each square measuring 1x1 mm, giving a total area of 3x3 mm counting area. The depth of the counting chamber is 0.1mm. Each of the 9 squares has a total volume of 0.1 mm^3.



Neubauer improved grid pattern

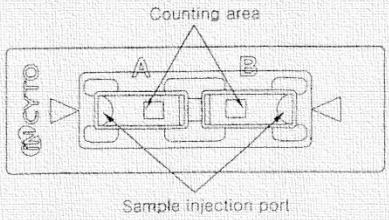
The disposable haemocytometer is of one piece construction and cover slip is not required. It is supplied in a sealed foil pouch to protect from dust.

STORAGE AND STABILITY

Store at any temperature below 40°C.

WARNING AND PRECAUTIONS

1. For single use only. Do not re-use.
2. Human specimens are potentially infectious: dispose used disposable haemocytometers according to established laboratory procedures.
3. All plastic material can attract dust by static electricity: store slide in the sealed pouch until use.
4. Carefully load sample into counting chamber to prevent introduction of air bubbles.
5. Over-filled chamber should not be used.



General cell counting

1. Mix the sample thoroughly just before pipetting.
2. Pipet 10 to 15 µl of sample into an unused counting chamber on the slide.
3. Allow it to fill the chamber by capillary action. Do not flood the chamber.
4. Allow the cells to settle for 1 minute before counting.

Erythrocyte counting (1:200 dilution)

1. Mix blood sample thoroughly.
2. Dilute blood using accepted laboratory method.
3. Pipet 10 to15 µl of sample into an unused counting chamber on the slide.
4. Allow it to fill the chamber by capillary action. Do not flood the chamber.
5. Count erythrocytes in 5 small squares under a microscope.

Leukocyte counting (1:20 dilution)

1. Mix blood sample thoroughly.
2. Dilute blood and lyse red cells using accepted laboratory method.
3. Pipet 10 to 15 µl of sample into an unused counting chamber on the slide.
4. Allow it to fill the chamber by capillary action. Do not flood the chamber.
5. Count leukocytes in 4 large corner squares under a microscope.

Mammalian cell counting

1. Treat cell sample with trypsin-EDTA.
2. Carefully remove the supernatant with a pipette without disturbing the pellet.
3. Add an appropriate amount of growth media or PBS to dilute to a final concentration of approximately $5x10^3$ cells/ml to 5×10^6 cells/ml.
4. Thoroughly resuspend the cell pellet with a pipette.
5. Check visually if there are any cell clumps or agglomerates left.
6. Pipet 10 to 15 µl of sample into an unused counting chamber on the slide.
7. Allow it to fill the chamber by capillary action. Do not flood the chamber.
8. Count cells under a microscope.

FEATURES

- Precise fixed depth counting chamber with superior accuracy and reproducibility
- No need to place cover glass slip. No broken or misplaced cover slip.
- Reduce the risk of exposure to potentially infectious material.
- Increases productivity by eliminating cleaning and work interruptions.
- Very light and unbreakable, compared with glass.
- Optical transparent material, optical character comparable to glass.

Innovatek Medical Inc
Vancouver, Canada

Version B: June 2011
PN-1226-B

ORDERING INFORMATION:

Disposable Hemocytometers:

VWR - Phone 800-668-6348

www.vwr.com

Order # 82030-468 (box of 50) - $259.12

Must set up an account to place an order

Protocol Hema 3 (formerly "Diff Quick"), **Leuko-TIC, Thromb-TIC & Capillary Holders:**

Fisher Scientific - Phone 800-234-7437

www.fishersci.ca

Protocol Hema 3:

Hema 3 Stain Kit: 500 ml of each solution -- # 22122911 - $244.33

OR

Fixative -- # 23122929 - $307.61 / 3.78L
Solution 1 (pink) -- # 23122937 - $471.14 / 3.78L
Solution 2 (purple) -- # 23122952 - $471.24 / 3.78L

Leuko-TIC: # 361022139 - $283.95 / box of 100

Thrombo-TIC: # 361022140 - $281.26 / box of 100

Capillary Holder: # 361022142 - $44.23 each

B. Measure the PCV and calculate RBC indices:

1. Packed cell volume (PCV) determination:

Principle: The microhematocrit (capillary or PCV) tube method produces good packing of cells in all animal species. It is rapid and uses a small volume of blood. The portion of the capillary tube which contains red cells is measured relative to the total plasma volume in the tube.

Method:

1. Well-mixed EDTA anticoagulated blood is drawn into the PCV tube by capillary action. Fill **two** tubes approximately 3/4 full. Avoid filling to blue line as prohibits visualizing top of plasma layer after centrifugation.

2. Seal one end by pressing the tubes into the "Critoseal" (plastic sealant).

3. Place the tubes across from each other in the centrifuge with the **sealed ends to the outside against** the rubber liner. **Place the cover** and set the timer for 4 minutes. A speed of 8000g. is preset, and the centrifuge will automatically shut off after 4 minutes.

4. Reading the PCV with the Hematocrit Reading Chart: (see diagram pg 15)

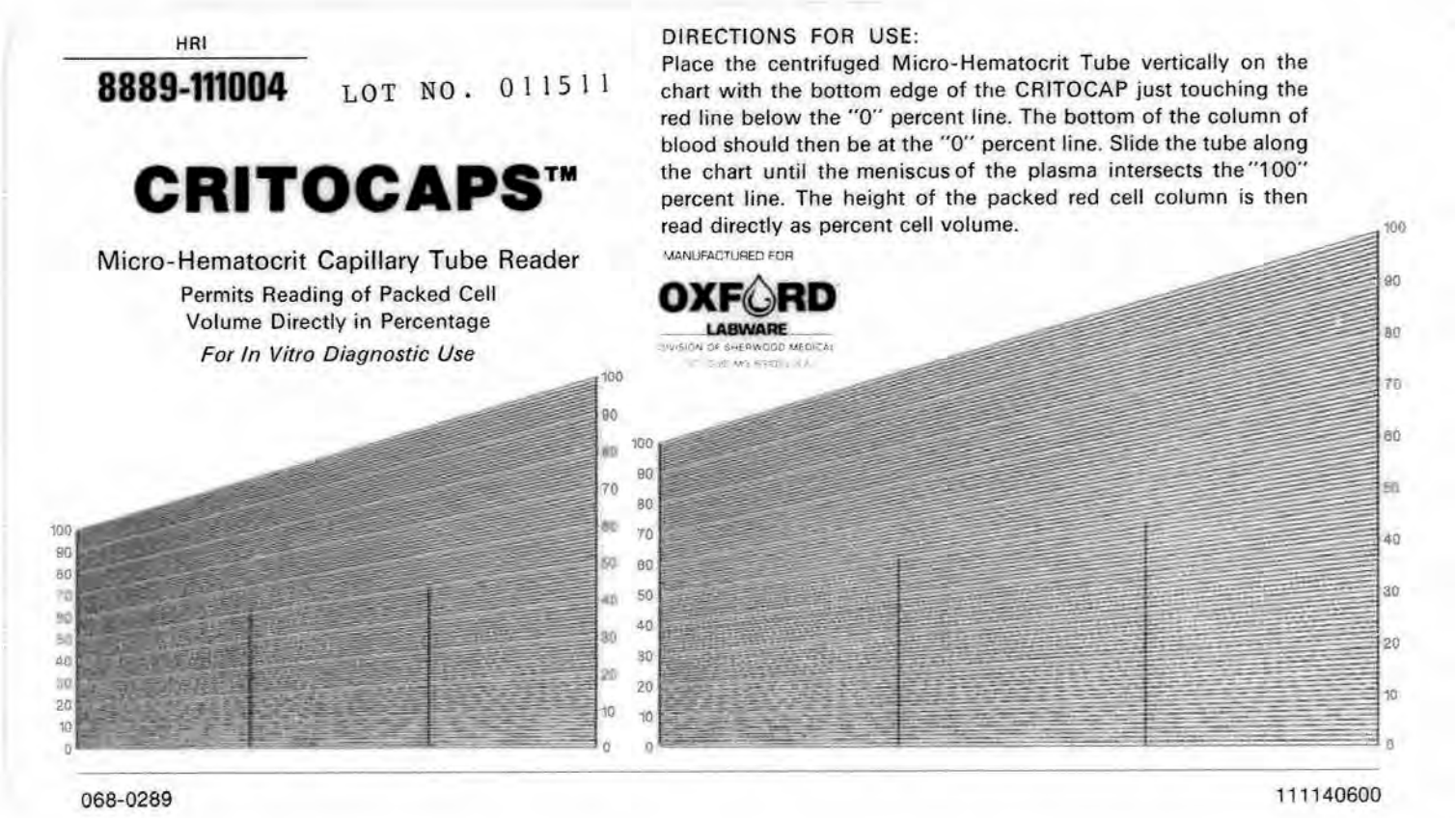
   a) Place the bottom of the red cell layer on the 0 line.
   b) Slide the tube until the top of the plasma falls on the 100 line. Make sure that the tube is vertical.
   c) The PCV result is that point where the top of the RBC layer intercepts a line; leukocytes and platelets (buffy coat) are not included in the measurement. The result is in %, but can be converted to L/L by dividing by 100.
   d) Plasma appearance should be noted i.e. hemolysis (red), lipemia (opaque white), yellow (carotenes or icterus). Check for an appreciable increase in the height of the buffy coat (the layer of WBCs and platelets above the RBCs).

Causes of error:

- *in vitro* hemolysis resulting in falsely low PCV
- too little blood added to a standard volume of EDTA resulting in dilution
- use of uncalibrated microcentrifuge (i.e. too fast or too slow) can also affect PCV

NOTE: The terms **hematocrit (Hct)** and **packed cell volume (PCV)** are often interchanged. Automated instruments calculate the Hct using the total RBC count and MCV **(Hct = MCV x total RBC count**). In the calculation of the Hct the instrument is capable of error, most commonly in cases of RBC agglutination. The manual PCV can be used to check the calculated Hct.

Hematocrit Reading Chart:



"Cartoon copyrighted by Mark Parisi, printed with permission."

PCV = % divided by 100 = L/L

Plasma color = (visual assessment of hemolysis, lipemia or yellow)

Buffy coat = (visual assessment of the height, do not measure. The height of the buffy coat is proportional to the total number of leukocytes)

2. Calculation of **RBC indices**: You will be provided with the RBC count and the hemoglobin concentration (HgB) of the blood sample; use the PCV determined above for the following calculations.

Mean cell volume (MCV) - Volume of total red cells (PCV) divided by RBC count. It is an index of mean volume. Reference interval for MCV is rather wide.

$$\textbf{MCV} = \frac{\textbf{PCV (L/L)}}{\textbf{RBC count (\# of RBC x } 10^{12}\textbf{/L)}} = \textbf{fL, where 1 fL} = 10^{-15}\ \textbf{L}$$

(e.g. when PCV is 30% (0.30 L/L) and RBC count is 4.45 x 10^{12}/L:

MCV = 0.30 L/L ÷ 4.45 x 10^{12}/L = .0674 x 10^{-12}L
= 67.4 x 10^{-15}L
= 67.4 fL)

Mean corpuscular hemoglobin concentration (MCHC) - the proportion of the solid RBC which is comprised of hemoglobin.

Hemoglobin concentration is divided by PCV or Hct.

$$\textbf{MCHC} = \frac{\textbf{HgB (g/L)}}{\textbf{PCV (L/L)}} = \textbf{g/L}$$

Mean corpuscular hemoglobin (MCH) - hemoglobin concentration divided by the absolute RBC count. It is affected by anything that affects the absolute RBC count.

$$\textbf{MCH} = \frac{\textbf{HgB (g/L)}}{\textbf{RBC count (\# of RBC x } 10^{12}\textbf{/L)}} = \textbf{pg where 1 pg} = 10^{-12}\ \textbf{g}$$

C. Total protein determination:

You will be using either:
1. **blue-boxed** refractometer which requires plasma from 2 PCV tubes to fill. Read the protein directly using the left hand scale.
   or
2. **denim-cased** refractometer which may require 2 tubes of plasma. Read from **right hand scale** and use conversion chart on page 11.

***Clean the glass platen with tap water and kimwipes first.**

Principle: The Goldberg refractometer is based on the principle that a beam of light is bent as it passes through a dense medium. The greater the concentration of a solution, the greater the refraction.

Method: The protein determination is done after the PCV has been read:

1. Break the capillary tube at the plasma/cell junction.

2. Let the plasma flow onto the chamber by capillary action, or by tapping the **unbroken** end of the tube to the platen. (Plasma from 2 tubes may be required to fill the platen)

3. Direct the instrument at a light source, and hold the top shield firmly. Bring the scale into focus by rotation of the eyepiece and obtain reading.

4. Wipe the fluid off the refractometer platen with a soft, non-abrasive tissue.

   Note: The presence of gross lipemia or hemolysis in the plasma, or an improperly cleaned or underfilled chamber will result in a "fuzzy" intercept line, and you will be unable to read the protein.

5. Use the conversion chart on the next page to obtain protein reading, only if using **denim-cased** refractometer. Readings are taken directly from the instrument with **blue-boxed** refractometer.

Report results in g/L.

Conversion chart for total protein concentration using the TS meter in the **denim** case. When taking your reading from the refractometer, remember to use the **right hand scale** viewed in the instrument. Convert the N reading to protein concentration in g/L

N	Protein concentration	
	100 ml	g/L
1.3382	1.4	14
1.3384	1.5	15
1.3385	1.6	16
1.3387	1.7	17
1.3389	1.8	18
1.3391	1.9	19
1.3393	2.0	20
1.3395	2.1	21
1.3397	2.2	22
1.3389	2.3	23
1.3401	2.4	24
1.3403	2.5	25
1.3405	2.6	26
1.3407	2.7	27
1.3408	2.8	28
1.3410	2.9	29
1.3412	3.0	30
1.3414	3.1	31
1.3416	3.1	31
1.3418	3.2	32
1.3420	3.3	33
1.3422	3.4	34
1.3424	3.5	35
1.3426	3.6	36
1.3428	3.7	37
1.3429	3.8	38
1.3431	3.9	39
1.3433	4.0	40
1.3435	4.1	41
1.3437	4.2	42
1.3439	4.3	43
1.3441	4.4	44
1.3443	4.5	45
1.3445	4.6	46
1.3447	4.7	47
1.3449	4.8	48
1.3451	4.9	49
1.3452	5.0	50
1.3454	5.1	51
1.3456	5.2	52
1.3458	5.3	53
1.3460	5.4	54
1.3462	5.5	55
1.3464	5.6	56
1.3466	5.7	57

N	Protein conc.	
	g/100ml	g/l
1.3468	5.8	58
1.3470	5.9	59
1.3472	6.0	60
1.3473	6.1	61
1.3475	6.2	62
1.3477	6.3	63
1.3479	6.4	64
1.3481	6.5	65
1.3483	6.6	66
1.3485	6.7	67
1.3487	6.8	68
1.3489	6.9	69
1.3491	7.0	70
1.3493	7.1	71
1.3495	7.2	72
1.3496	7.3	73
1.3498	7.4	74
1.3500	7.5	75
1.3502	7.6	76
1.3504	7.7	77
1.3506	7.8	78
1.3508	7.9	79
1.3510	8.0	80
1.3512	8.1	81
1.3514	8.2	82
1.3516	8.3	83
1.3517	8.4	84
1.3519	8.5	85
1.3521	8.6	86
1.3523	8.7	87
1.3525	8.8	88
1.3527	8.9	89
1.3529	9.0	90
1.3531	9.1	91
1.3533	9.2	92
1.3535	9.3	93
1.3537	9.4	94
1.3539	9.5	95
1.3540	9.6	96
1.3542	9.7	97
1.3544	9.8	98
1.3546	9.9	99
1.3548	10.0	100
1.3550	10.1	101
1.3552	10.2	102

D. Make blood smears:

1. Use clean, dry slides. The spreader should have a bevelled edge. (Note: all of the slides used in these labs are bevelled.)

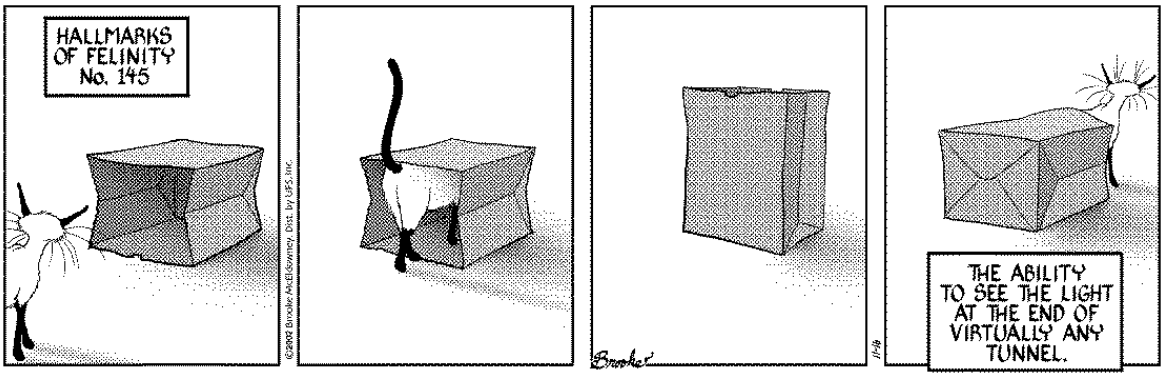
2. Using a PCV tube, place a medium-sized drop of blood **near the frosted end** of the slide.
Hold the spreader at a **45°** angle (adjust angle if smear is too thick or too thin). Anchor slide opposite blood drop with index finger. (If right-handed, anchor the end of slide with left index finger and vice versa.)
Pull the spreader in to the drop of blood; once the blood reaches both ends of the spreader, **push** the spreader to the end of the slide (**away** from the frosted end) in a swift flowing motion. Stop at index finger. Do not apply pressure or lift off.
One clean spreader can be used to make 4 smears (turn spreader over between smears and use both ends). Make several smears until you are happy with your technique and the result.

E. Stain your best smear:

Hema Protocol 3 (Romanowsky Stain) Procedure:

1. Dip slide 5 times for 1 sec each in Hema 3 Fixative Solution. Allow excess to drain.
2. Dip slide 3 – 5 times for 1 sec each in Hema 3 Solution I. Allow excess to drain.
3. Dip slide 3 – 5 times for 1 sec each in Hema 3 Solution II. Allow excess to drain.
4. Rinse slide with deionized water.
5. Air dry.
6. Write your name in pencil on the frosted end. Place your slide in the metal trays on the lab counter. Slides will be coverslipped and returned in the next lab.

NOTE: Prolonged or repeated exposure to stains 1 and 2 may be required with thick film preparations (e.g. bone marrow and lymph node aspirates).



9 CHICKWEED LAND, Brooke McEldowney. Used by permission of UNIVERSAL UCLICK. All rights reserved.

LAB 2: SMEAR EVALUATION: WBC estimate and 100 cell differential; platelet estimate; RBC morphology; assessment for anemia

*****COMMIT THE COMPONENTS OF SMEAR EVALUATION TO MEMORY!*****
(including formulas for WBC and platelet estimates)

Smear Evaluation:

A. Scan on low power for:

- on 10x scan the smear noting staining characteristics of cells and background, distribution of cells, whether there are clumps of cells and/or platelets particularly at the feather edge, "intactness" of cells, etc. (Note: there will always be an increase in leukocytes and disintegrated cells at the feather edge. Also, RBC and leukocyte morphology becomes distorted at the extreme feather edge.)

B. Estimate leukocyte numbers:

- on 10x **(for virtual microscopy, use 7.5x, instead of 10x)**, count the leukocytes in at least 3 fields in the **monolayer** (this is the region where the red cells form a uniform sheet, with minimal overlapping of adjacent cells and no large empty spaces between cells)

- divide the total number of leukocytes counted by the number of fields to obtain the average number per field

- divide the average number of leukocytes counted by 4 in order to approximate the number of leukocytes x 10^9/L

- **Note: this provides an approximation only; most important is to gain an appreciation whether the WBC count is low, normal, or high. Under some circumstances it may be difficult to use this formula (e.g. if WBC numbers are extremely high or low, or if WBCs are unevenly distributed). With very high WBCs, count ½ or ¼ of field and calculate accordingly.**

C. Leukocyte differential (100 cells): (see Lab 3)

D. Estimate platelet numbers:

- on oil immersion (100x) **(for virtual microscopy, use 75x)**, count the number of platelets in at least 5 fields of the monolayer and calculate the average for 1 field

- multiply the average for 1 field by 20 x 10^9/L. If platelets are clumped (especially at the feather edge), the formula will underestimate the platelet count. Do the estimate, but

comment if there is clumping. If platelets are clumped and plentiful, numbers are assumed to be adequate.

- less than 3 - 4 per 100x field (60 - 80 x 10^9/L) represents a significant thrombocytopenia

(Note: Pick up the smear you made last week. You may add this blood smear to your slide box and examine it when you wish. This is a very busy lab and there is insufficient time to evaluate your own smear at this time.)

E. Evaluate RBC morphology (x 100 oil immersion):

Background:
RBC morphology includes: size, shape, colour, and inclusions.
The RBC morphology terms, pictures, and significance are provided on pages 6 and 7 of Veterinary Clinical Pathology: An Introduction; colour pictures begin on page 54.

RBC morphology is quantified as follows: (***students are not expected to quantify RBC morphology, but you are expected to recognize and report morphology***) Numbers are per 200 RBCs; there are about 200 RBCs/100X field in the monolayer in non-anemic samples.
slight (<5) 1+ (6-10) 2+ (11-25) 3+ (>25)

Exceptions:
The "slight" terminology is applied only to acanthocytes, eccentrocytes, ghost cells, keratocytes, schizocytes, and spherocytes.
Agglutination and hypochromasia are reported only as "present".
RBC inclusions (basophilic stippling, nuclear remnants, Heinz bodies, and organisms) are reported as few, moderate, or many.
Polychromasia is reported as follows:

1+ (5-15/200 RBCs) (=2.5-7.5% reticulocytes)	2+ (16-30) (=8-15%)	3+ (>30) (= >15%)

A reticulocyte count is done on any sample with a low hematocrit or PCV or sample with $\geq$ 1+ polychromasia. (Equine samples are the exception since regeneration is rarely manifested in peripheral blood.)

Rouleaux is reported as follows:

	1+	**2+**	**3+**
free cells	majority	at least half	almost none
# of chains	some	many	most
cells/chain	max 2-3	usually <5	usually >5

Method:

- **find an appropriate area on 40x (e.g. the monolayer - don't go into thick area and don't go into the feather edge!)**
- on oil immersion (100x), examine erythrocytes for variations in size, shape, and color, and for the presence of inclusions
- note: your assessment on 10x would have included observation for the presence of rouleaux, agglutination, an expanded monolayer (suggesting the presence of anemia), or a shortened monolayer (suggesting erythrocytosis or hemoconcentration). **An expanded or shortened monolayer only has significance in properly made smears.**

Do WBC estimates, platelet estimates and assess RBC morphology in slides # 1 and 3 below (species normals). These are good comparisons for the abnormal smears to follow.

1. Normal canine peripheral blood: (D1232422)

SIZE - Canine erythrocytes are the largest of the species you will be studying. There is slight variation in size (anisocytosis). In most normal dogs anisocytosis is minimal.
SHAPE - Normal canine erythrocytes are round. Short projections around the cell (crenation/echinocytes) may be due to prolonged exposure to anticoagulants or an artifact due to improper drying of the blood film.
COLOR - There are rare pale purple cells (polychromatophilic erythrocytes). Canine erythrocytes normally exhibit central pallor.
PLATELETS - These are normal in number but enlarged, and platelet clumps may be present in this smear. Be aware that they may resemble inclusions when superimposed on erythrocytes.

***NOTE: RDW (Red Cell Distribution Width):** The RDW is an electronic measure of the variation in red cell size (ie. anisocytosis). It is the standard deviation (SD) of erythrocyte volumes expressed as a percent of the mean.

$$\text{RDW} = \frac{\text{SD}}{\text{MEAN}} \times 100$$

The utility of the RDW has not been extensively evaluated in veterinary medicine. It is expected to be increased in cases where the degree of anisocytosis (as estimated on the stained blood film) is increased.

2. Normal feline peripheral blood: (PDS1533045)

SIZE - Feline erythrocytes are smaller than those in dogs. They normally exhibit some anisocytosis. Some rouleaux is evident - cells are in a "stack of coins" formation.
SHAPE - These cells are normally round. Echinocytes I are prominent in this smear.
COLOR - Feline erythrocytes exhibit little or no central pallor. There is slight polychromasia.

INCLUSIONS - A rare erythrocyte contains a deep purple, non-refractile, round inclusion which is a nuclear remnant.
PLATELETS - Note that feline platelets exhibit considerable variation in size. Giant platelets are common in this smear. Platelet clumping is common on feline blood films. Adequate numbers of platelets are present.

3. Normal bovine peripheral blood: (D0901964)

SIZE - RBCs are similar size to feline RBCs.
SHAPE - Normal
COLOR - there is no polychromasia; there is evidence of central pallor.
PLATELETS - platelets are within the reference interval, and there are a few large platelets.

4. Normal equine peripheral blood: (D0213119)

SIZE - Intense rouleaux is common in horses. There is 1+ anisocytosis evident on this slide.
SHAPE - Note the varying degrees of echinocyte formation.
COLOR - Equine erythrocytes have little or no central pallor. Horses do not release reticulocytes even when erythropoiesis is intense, therefore polychromasia is rarely observed.
PLATELETS - Equine platelets may not stain as darkly as those of other species and may be difficult to see. There should be five or more platelets per field at 100x oil magnification.

Examine a New Methylene Blue Slide:

Slide #12 (D1220167) is stained with New Methylene Blue which stains reticulocytes and Heinz Bodies. Reticulocytes per 1000 erythrocytes are counted and expressed as a percentage. In order to be counted as a reticulocyte, the precipitated RNA must be in clumps or strands (aggregated); cells with individual blue dots (punctate), particularly common in feline blood, are not included in the count. The large blue less distinct cells are leukocytes. Refractile material is artifact.
For this lab: Examine the slide and recognize the difference between aggregate and punctate reticulocytes. Do not count reticulocytes for this lab.

FOR REFERENCE ONLY:

***New Methylene Blue (NMB) – for reticulocytes and Heinz Bodies**:*

1. Add equal volumes of NMB and whole blood (e.g. 2 drops each), anticoagulated by EDTA, in a 10 mm x 75 mm disposable test tube.
2. Incubate for 15 minutes at room temperature or 10 minutes at 37°C.
3. Prepare two smears, as you would a blood smear; solution is dilute, so "perfect" smears are not required. Increasing the spreader angle may be helpful.
4. Air dry.

Evaluate the erythrocyte morphology in slides #9, 20, 21, and 23 and answer the following questions. Also examine #24 which is a New Methylene Blue (reticulocyte) stain of #23. Refer to CBC results in Appendix I.

Slide #9: (D1104952)

With a PCV of 18%, is this dog anemic? What is the reference interval for PCV/Hct in dogs?
Is there evidence of RBC regeneration? What criteria can you use to evaluate regeneration when examining a smear? What criteria can you use to assess regeneration when you have all the CBC data to evaluate?
What 3 RBC morphologic findings suggest a mechanism for the anemia in this case?
Why is a PCV provided rather than Hct, in this case?

Slide #20: (D0832489)

With a Hct of 0.18 L/L, is this dog anemic?
Is there evidence of RBC regeneration?
What 3 RBC morphologic findings suggest a mechanism for the anemia?
What information provided on the CBC would support this mechanism?
Platelet numbers are increased – why?

Slide #21: (D0701735)

This cow has a Hct of 0.171 L/L; what is the reference interval for Hct for cattle?
Is there evidence of regeneration? If so, what are the criteria you can use from smear evaluation?
What are possible etiologies for the anemia?

Slide #23: (D0914274)

This dog has a Hct of 0.190 L/L.
Is there evidence of RBC regeneration?
Are there RBC morphologic finding(s) that suggest a mechanism?
What are possible etiologies for the anemia?

Slide #24: (D0914274)

This is a New Methylene Blue-stained smear of the same case as #23.
What is the purpose of this stain?
What do we count when we evaluate this smear?
What does this information correlate to on the Wright-Giemsa-stained smear?

LAB 3: LEUKOCYTE DIFFERENTIAL and MORPHOLOGY I

A. Performing a leukocyte differential:

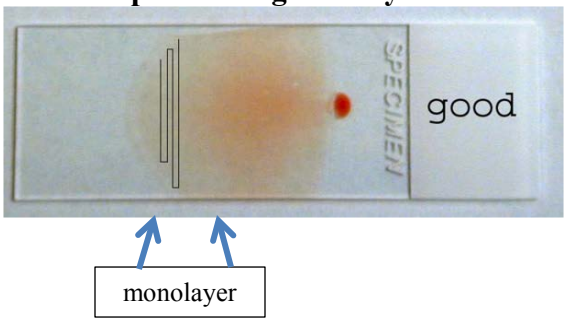
1. Microscope condenser should be raised and diaphragm open to maximize light. After examining the smear on 10x **(7.5x with virtual microscopy)** to obtain a general evaluation of WBC numbers, platelet clumps, presence of large parasites or abnormal cells, etc., the remainder of the leukocyte examination is done using 100x (oil) **(75x with virtual microscopy)**. The differential should be performed in the **monolayer** of the blood film (starting approx. 1 field on 10x from midpoint of feather edge). Leukocytes cannot be identified in the thick part of the smear as they round up and become very darkly stained.
2. 100 leukocytes should be counted when doing the differential.
3. The **absolute number** for each leukocyte type can be calculated by multiplying the percentage by the total WBC count. For example, if the total WBC count is 10 x 10^9/L and 66/100 (66%) are neutrophils, then the absolute neutrophil count is 66/100 x (10 x 10^9/L) = 6.6 x 10^9/L.
4. The following Leukocyte Differential Worksheet is useful for keeping track of the cell types identified and total numbers counted:

Leukocyte Differential Worksheets: (free apps also available for diffs on some e-devices)
Each large square is made up of 100 small squares. Each small square represents one cell. When you have filled the large square you have complete a 100 cell leukocyte differential count. The number of small squares occupied by a specific letter (**N=neut; B=band; L=lymph; M=mono; E=eos; Baso=baso**) is equal to the percentage of that cell type. Nucleated RBCs are counted in addition to the 100 WBCs and reported as number per 100 WBCs.

N	N	N	L	L	E	M	M	N	N
N	N	N	N	N	M	M	N	L	N
M	M	N	E	L	L	E	N	E	L
N	N	N	N	L	L	L	N	M	E
N	N	N	N	N	E	L	N	L	N
L	L	L	L	E	M	L	N	N	E
N	N	N	M	N	L	L	L	N	M
L	N	N	N	N	N	N	B	N	E
L	L	N	L	N	N	N	B	N	N
E	M	M	N	N	N	N	N	N	N

% (from counting squares)		Absolute count (if total WBC=10x10^9/L)	
Neuts:	53 %	Neuts:	5.3 x 10^9/L
Bands:	2 %	Bands:	0.2 x 10^9/L
Lymphs:	23 %	Lymphs:	2.3 x 10^9/L
Monos:	12 %	Monos:	1.2 x 10^9/L
Eos:	10 %	Eos:	1.0 x 10^9/L
Basos:	%	Basos:	
Other:	%	Other:	
nRBCs:	0/100 WBCs		

Pattern for performing leukocyte differential:



Example of a good smear

***Use the formula below if nRBCs are > 5 per 100 leukocytes counted in the differential. No need to memorize this formula as it will be provided. (See slide #9 which contains many nRBCs)**

Corrected white blood cell counts (cWBC) - automated counters may and manual counts always include nucleated erythrocytes (metarubricytes and rubricytes) in the total white blood cell count. It is necessary to calculate a corrected white blood cell count that excludes the nucleated erythrocytes (nRBCs) so that we can more correctly grade a patient's WBC count as low, normal or high. When nRBCs are counted as part of the leukocyte differential count the following formula is used:

***Corrected WBC count = (WBC count x 100) ÷ (100 + nRBCs)**

Example: If nRBCs are 15 per 100 WBC and the WBC count from the automated counter is 30 x 10^9/L, the corrected WBC count is:

(30 x 10^9/L x 100) ÷ (100 + 15) = 3000 x 10^9/L ÷ 115 = 26.1 x 10^9/L

Leukocyte Changes:

Terminology for increased leukocyte numbers in peripheral blood: leukocytosis due to one or more of: neutrophilia, monocytosis, lymphocytosis, eosinophilia, basophilia.

Terminology for decreased leukocyte numbers in peripheral blood: leukopenia due to one or more of: neutropenia, lymphopenia, monocytopenia.

Example: if the total count is high and it is due to increased neutrophils without bands, this change would be described as a moderate to marked leukocytosis (depending on how high) characterized by a mature neutrophilia.

Determine possible reasons for deviations from normal and the clinical relevance of these changes.

MORPHOLOGY OF LEUKOCYTES IN THE PERIPHERAL CIRCULATION

1. **Segmented Neutrophil** (mature neutrophils)
The most common leukocyte in the peripheral blood of dogs, cats, and horses. The equivalent cell type in certain species (e.g. birds, rabbits, reptiles) is the heterophil.

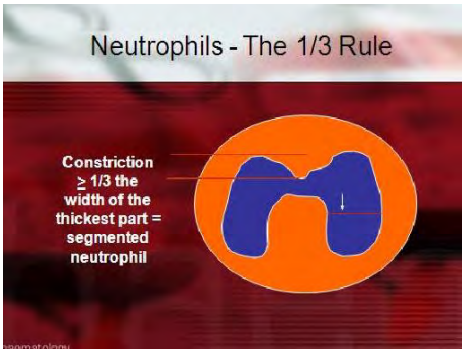
2. **Band Neutrophil**
It is very important to distinguish band from segmented neutrophils as increased bands indicate release of immature granulocytes, usually in response to a severe inflammatory process. All of the following features should be considered.

Band versus segmented neutrophils:

1) **nuclear shape** - nuclear borders are parallel to each other for the majority of the band nucleus.

2) **chromatin pattern** - segmented neutrophils have a more clumped chromatin compared to bands; clumped chromatin indicates maturity.

3) **nuclear constriction** - the "1/3" rule applies - if a constriction within the nucleus is > 1/3 the width of the thickest part of the nucleus, the cell is classified as segmented; if the constriction is < 1/3 the width of the thickest part of the nucleus, it is a band. **If in doubt, err on the side of maturity (call it a seg).**



4) **cell size** - bands tends to be larger cells than segs.

5) **cytoplasm color** - bands may have bluer cytoplasm.

Toxic change of cells in the neutrophil series:
Toxic change is a sign of immaturity of the granulocyte cytoplasm. Toxic change occurs when there is accelerated movement of cells through the maturation pool in the bone marrow. Cells are moved more rapidly through the maturation pool when peripheral demand for granulocytes is increased. Toxic change refers to cytoplasmic features only and includes retention of primary granules; increased blue staining due to retention of ribosomes; foamy vacuolation; blue granulation from deposits of rough endoplasmic reticulum (if in a large dark blue deposit, called Döhle body).

Grading toxic change:
Categories are: **sl** (slight), **1+** (mild), **2+** (moderate) and **3+** (marked). **You are not expected to grade toxic change, but you should determine if it is present.**

Left shifts:

Usually a healthy animal releases mature neutrophils and very low numbers of band neutrophils. However, an increased demand for neutrophils in the tissues which exceeds the storage pool in the bone marrow will result in the release of increased numbers of immature neutrophils (usually

bands, but sometimes metamyelocytes and myelocytes). Despite the release of immature neutrophils, usually there is a neutrophilia with higher numbers of segmented neutrophils than immature forms. Sometimes, the demand for neutrophils is so great and so acute, that the marrow cannot compensate quickly enough and the total neutrophil count is within the reference limits or lower and immature forms approach or surpass mature forms in number **(degenerative left shift)**. The prognosis is worse in this situation. However, it is important to appreciate that changes can occur quickly in the peripheral blood and monitoring the CBC over time can be a valuable tool in determining response to treatment and prognosis.
Adult cattle have a lower reserve of mature neutrophils in the bone marrow than most other species. Therefore, cattle may develop a degenerative left shift more readily and the prognosis is not necessarily as poor as in other species. The leukocyte responses of young calves (up to 3 - 4 months) are more similar to those of dogs than to adult cattle.

3. **Metamyelocytic Neutrophils**

- Metamyelocytes are not found in the peripheral blood of healthy animals.

4. **Lymphocytes**

- Lymphocytes are the major leukocyte of bovine blood (they outnumber neutrophils in normal ruminants) and the second most numerous white cell in dogs, cats and horses.
- both small and large lymphocytes may be seen normally.
- intensely blue staining lymphocytes may be reactive/immunologically stimulated.

5. **Monocytes**

- Monocytes are the largest cell in the peripheral blood but they can vary in size (9-22 μ).
- nuclei are highly variable - from multilobulated to kidney-shaped.
- the cytoplasm is a characteristic blue-grey colour and sometimes vacuolated (this helps distinguish them from other leukocytes; “pseudopod”-like projections are sometimes seen.
- monocytes are generally larger than granulocytes.
- occasionally it is difficult to differentiate large lymphocytes from monocytes.

6. **Basophils**

- Basophils are uncommon in the peripheral circulation of all species and granularity is variable - the granules of basophils are water soluble and so with staining and washing, may be leached.
- the nucleus resembles that of a neutrophil.
- cytoplasmic granulation varies with the species (e.g. granules are scant in dogs, pale orange-lavender in cats, blue-black and numerous in cows, and irregular in size and number in horses).

7. **Eosinophils**

- Eosinophils are usually quite distinctive due to their orange-pink granules.
- eosinophil nuclei are band-formed to bilobed.
- cytoplasmic granulation varies with the species (eg. highly variable size and number in the dog, large and bright orange-red in the horse, numerous and small in cattle and cats).
- there are some intraspecies differences e.g. vacuoles rather than granules are seen in Greyhound eosinophils.

8. **Platelets**

a) Dog - generally small pale blue and contain a few purple granules.

b) Cat - very pleomorphic - giant forms are common; the shape varies from round to elongated to cigar-shaped.

c) Horse - indistinct, pale staining with irregular barely discernible membranes and a few indistinct granules.

d) Cow - variable in size, light blue cytoplasm and reddish purple granules.

In each smear first find classic examples of each cell type and study the details of the staining of nucleus and cytoplasm.

B. Do full smear evaluations on the following 2 smears (see pp 20-22 for the components of full smear evaluation, if you have not already committed these to memory):

#2 & #4: compare your results with those provided in Appendix I

2. Normal Feline Peripheral Blood: (PDS1533045)

4. Normal Equine Peripheral Blood: (D0213119)

LAB 4: LEUKOCYTE DIFFERENTIAL AND MORPHOLOGY II:

A. Do <u>full smear evaluations</u> (see pp 20-22 if you have not yet committed this to memory) on slides #35 & #36. Examine slides 41 and 47 and identify the eosinophils and basophils:

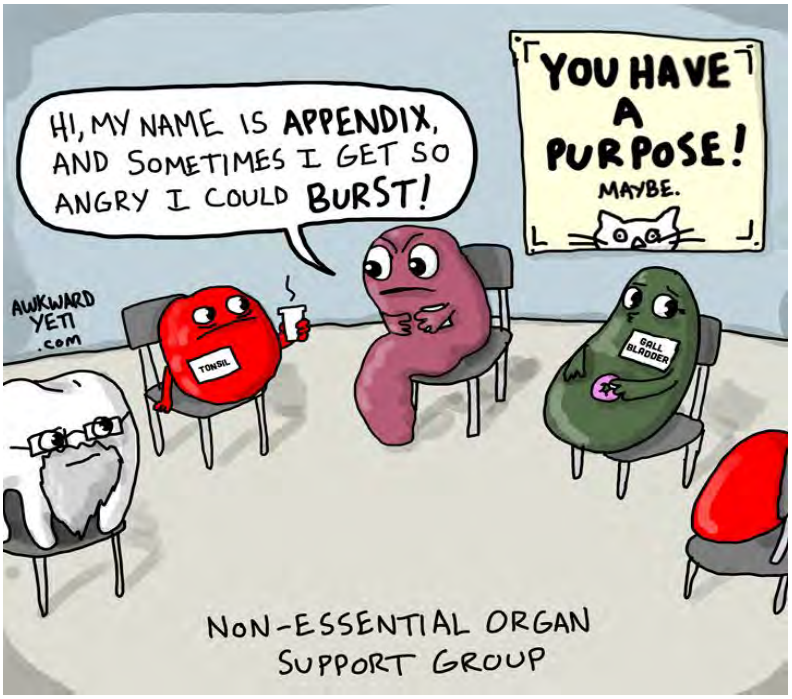
Compare your results with those provided in the Appendix.

35. Equine left shift: (D0316574): Essence, 6 year old Arabian mare. Colic of 12 hours duration. Just foaled and had rectal prolapse after foaling.
<u>Describe</u> your leukogram findings.
<u>Interpret</u> these findings.
What would you find if you examined the bone marrow in this horse (with respect to the granulocytic series)?

36. Canine left shift: (D0921040): Chico, 11 year old Mc Pomeranian dog. Ruptured gall bladder; septic abdomen. Post-surgery for gall bladder removal.
<u>Describe</u> your leukogram findings.
<u>Interpret</u> these findings.
What would the bone marrow findings be?
Why is this dog anemic?

41. Feline basophilia: (D0108882): 15 year old Fs DSH cat. Vomiting 2 x daily for past 6 weeks, otherwise active and healthy.

47. Canine eosinophilia and basophilia: (D0220063): Grizz, 11 year old Mc Rottweiler dog. Presurgical bloodwork. What are potential causes of eosinophilia and basophilia in dogs?



Used with permission, Nick Seluk (The Awkward Yeti)

LAB 5: PARASITES AND LEUKEMIAS:

A. Examine slides # 8, and 13 which contain erythrocyte parasites and note results below:

Slide #8 - *Mycoplasma haemofelis* or *haemominutum* (feline): (D9927065) Greyby, 6 year old F DSH cat. C-section 24 hours ago to remove retained fetus.

- the morphology of the organism can be variable and includes basophilic rings, chains, or tiny dots or rods; it is important to differentiate stain precipitate or refractile artefacts from organisms.
- typically there is a good regenerative response unless *M. haemofelis* or *M. haemominutum* is associated with another disease process resulting in immunosuppression.
- a lack of regeneration could exist in the very early stages of infection (1-3 days) or could be due to immunosuppression and/or concurrent disease; repeating the CBC would be important in such a case.
- compare the CBC findings between slides 8 and 13.

Slide #13 - *Mycoplasma haemofelis* or *haemominutum* (feline): (D9814320) Old Yeller, 17 year old Mc DSH cat. Inappetence and weight loss.

B. Do <u>full smear evaluations</u> (you know what this means now, right?) on slides #14 and #84:

Slide #14: *Anaplasma phagocytophilum* (canine): (D0717365) Shadow, 10 year old Mc Nova Scotia Retriever x dog. Acute onset of lethargy, anorexia, and fever.
- note basophilic morulae within the cytoplasm of some of the neutrophils; scan the smear on 40X to find the organism more quickly.

Slide #84: *Dirofilaria immitis* (canine): (D9807490) Brownie, 1 year old Fs Terrier x dog. "Rescued" from beach in the Bahamas and brought to Saskatoon. Has diarrhea and enlarged spleen.
- note microfilariae (315 μm x 6 μm) on low magnification (feather edge and sides of smear).

C. Examine slides # 65, 66, and 68 which are leukemias of different cell types. Make sure you note the important features reported below and on the CBC results (Appendix I).

65. Basophilic Leukemia - feline: (D9929377) This 10 year old M(c) cat had been losing weight for about 3 weeks. (Note: these neoplastic basophils have distinct dark blue-purple granules compared to basophils in normal cats, which have indistinct orange-lavender granulation.) Compare to slide #41 - normal feline basophils.

66. Lymphocytic Leukemia - canine: (D0025145) This 7 year old M(c) Labrador Retriever dog had been anorexic for 5 days and had increased lymphocytes noted within blood vessels on skin biopsies.

68. Mast Cell Leukemia - feline: (D0910363) This 17 year old F(s) DMH cat had a decreased appetite and was losing weight and vomiting.
What is a potential mechanism for the vomiting?

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LAB 6: UNKNOWN BLOOD SMEARS: (Practice)

A. You will be provided with 2 peripheral blood smears and accompanying histories. Do **full smear evaluations** (you know the drill!) as you have done in previous labs. The CBC results will be discussed at the end of the lab period.

[See Blood Smear Evaluation Guide for Quizzes, pp. 22 and 23 (last 2 pages) of Appendix I]

Low Power:

- evaluation on 10x to determine smear quality (e.g. staining properties, distribution and intactness of cells, presence of platelet clumps, background appearance).

- determine if there is rouleaux, agglutination, increased/decreased WBCs, an increased or decreased monolayer suggesting anemia or hemoconcentration, respectively.

- estimate the total leukocyte count (on 10x with light microscopy; 7.5X with virtual microscopy).

High Power:

- on 100x, do a platelet estimate (75X with virtual microscopy), evaluate RBC morphology, and do a leukocyte differential count.

Describe your findings:
Interpret your findings:

LAB 7: UNKNOWN BLOOD SMEARS: (20 Marks)

A. You will be provided with 2 peripheral blood smears and accompanying histories. Evaluate these smears as you have previously; describe your findings; interpret your findings. These will be handed in and comprise 20% of your final grade.

LAB 8: URINALYSIS AND CASES:

The focus of this lab is the methodology and interpretation of a complete urinalysis.

1. Complete the urinalysis as described below for the sample case (history and signalment are provided on the next page).

2. Examine the **Demonstration slides –** different urine sediments are set up at the front of the lab.

*****Note that for this lab the urine sample has already been centrifuged and the supernatant poured off so that you will have 2 tubes.** *In veterinary practice you would describe color/clarity and perform the Urine Dipstik on <u>unspun urine</u>. Then centrifuge the urine for 5 minutes at 1500 rpm. Pour off the supernatant. The supernatant is used to read the refractometer & the sediment is used for microscopic examination. Please see detailed instructions for routine urinalysis that should be followed in a veterinary practice setting.*

Urine A = urine supernatant – use this tube for the Chemstrip 10A and specific gravity.
Urine B = urine sediment – prepare the microscopic slide from this sample.
In this lab you work in **PAIRS**. There are 20 stations set up throughout the lab.

There are only 10 refractometers. They are sitting by center sink. Use the U.G. Scale to read the Specific Gravity (will be the far left or the far right depending on which refractometer you are using). Return them as soon as you are finished.

All garbage (urine strips, plastic pipettes, Kleenex, gloves etc) **MUST** be put in supplied waste container found on the counter by the center sink (do not put anything in regular garbage cans). There is a red sharps container for the slides.

LAB INSTRUCTIONS

Urine A – Supernatant*

1. Describe Color & Clarity
2. Perform Urine Dipstik
    a. Dip the strip in the urine for not more than 1 second.
    b. Wait 60 seconds and read all results.
        i. The part of the strip with no reagent pads goes next to the lid of the urine container.
        ii. For blood there are 2 squares on the urine strip container that correspond to the 1 reagent square on the strip.
        iii. The very last reagent square on the strip is used for blanking automatic strip readers so do not read it.
        iv. Report the following results:
        pH, Protein, Glucose, Ketones, Bilirubin, Blood
3. Read Urine Specific Gravity using a refractometer.

Urine B – Sediment*

1. Make a microscope slide
    a. Place a drop of urine in 2 spots on a slide.
    b. To one drop of urine add a drop of sedistain.
    c. Mix urine and sedistain with the corner of a coverslip.
    d. Coverslip both drops.
    e. Examine stained & unstained sediment on low power (x10) and on high dry (40x).
    f. Use unstained sediment for quantifying urine sediment.

Urinalysis: **Sample Case**

History: A 7-year-old male Spaniel dog was presented because it had lost considerable weight over the past month or so. The dog seemed to be urinating more often. A cystocentesis sample was submitted for urinalysis.

Results: Please fill in your results in table below.

Physical	
Colour/Clarity	
Specific Gravity	
REAGENT STRIP	
pH	
Protein	
Glucose	
Ketones	
Bilirubin	
Blood	

SEDIMENT	
WBC/hpf	
RBC/hpf	
Epithelial cells	
Crystals	
Casts	
Bacteria	
Fat	
Other	

Interpretation:

Diagnosis or Differential Diagnoses:

Case assignment:
Students will be provided with urinalysis data from 2 cases (see handout). Please interpret the data and answer all associated questions. We will return to the classroom during the last 20 minutes to discuss these cases.

Chemstrip® 10 A

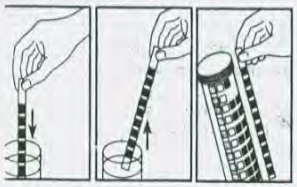
(EN) **Ten-patch test strip for the semiquantitative determination of specific gravity, pH, leukocytes, nitrite, protein, glucose, ketone bodies, urobilinogen, bilirubin, and blood in urine.**
For evaluation by reflectance photometry with Urisys® 1100, Urilux® S, Urisys® 1800, Urichem® 1000, Miditron® Junior and Miditron® Junior II urine analyzers.

or professional use

OR IN VITRO DIAGNOSTIC USE

ıdditionally required materials:
Urinalysis analyzer
Control-Test M calibration strip, Cat. No. 11379194-263
Controls as indicated below
General laboratory equipment

ıstructions for use:
. For instructions on evaluation of the **Chemstrip 10 A** test strip with the analyzers please read the operator's manual for the respective instrument. Use fresh urine that has not been centrifuged. Thoroughly mix the urine sample. The sample should be at room temperature when the test is performed and should not have been standing for more than two hours.
. Take a test strip out of the container. Close the container again with the original desiccant stopper immediately after removal of the strip. This is important as otherwise the test areas may become discolored due to moisture and incorrect results may be obtained.
Briefly (about 1 second) dip the test strip into the urine making sure that all test areas are moistened.
When withdrawing the test strip, wipe the edge against the rim of the vessel to remove excess urine.
For use with Urisys 1100 and Urilux S: Briefly (not more than 1 second) dab the long edge and then the back of the test strip on an absorbent surface (e.g. paper towel).
Insert the test strip in the instrument as directed in the **operator's manual** for the analyzers. If the test is to be read visually, wait 60 seconds (60–120 seconds for the leukocyte test area) and then compare the reaction colors of the test areas with the colors on the label. Compare the 10th (blood) test area with both color scales as separate color scales are given for erythrocytes and hemoglobin.



Any color changes appearing only along the edges of the test areas, or developing after more than 2 minutes, do not have any diagnostic significance.

Evaluation of results: After the test strip has been accepted by the analyzer, it is read by means of reflectance photometry. The results are then printed out on the report form in terms of "neg.", "pos." or as a concentration value. Like the results obtained by visual color comparison, the values appearing on the print-out corresponds to specific concentration ranges.

However, as a result of the differing spectral sensitivities of the human eye and the optical systems of the analyzers, it is not always possible to obtain precise agreement between the values obtained by visual reading and those obtained in the instrument.

Test principles and notes on individual test areas

Specific gravity: The test detects the ion concentration of the urine. In the presence of cations, protons are released by a complexing agent and produce a color change in the indicator bromthymol blue from blue via blue-green to yellow. On visual reading, 0.005 should be added to the result if the urine has a pH of 7 or more. The instruments automatically carry out this correction. In the presence of small amounts of protein (100–500 mg/dL) or ketoacidosis the specific gravity measurements tend to be elevated. An increase in the specific gravity due to glucose concentrations >1000 mg/dL (>56 mmol/L) is not indicated by the test.

pH: The pH values found most frequently in fresh urine from healthy individuals lie between 5 and 6. The test is specific for the detection of hydronium ions, the pH being the negative common logarithm of the hydronium ion concentration. The test pad contains the indicators methyl red, phenolphthalein and bromthymol blue.

Leukocytes: The reaction detects the presence of esterases that occur in granulocytes. These enzymes cleave an indoxyl ester, and the indoxyl so liberated reacts with a diazonium salt to produce a violet dye. Both **intact** and **lysed** leukocytes are detected. On visual reading, reaction colors that cannot be unequivocally classed as negative or approx. 10-25 WBCs/µL urine after 60 seconds can generally be more easily assessed after 120 seconds. The reaction is not affected by bacteria, trichomonads or erythrocytes present in the urine. Formaldehyde (stabilizer) and medication with antibiotics containing imipenem, meropenem or clavulanic acid may cause false-positive reactions. If the urine specimen is strongly colored (for example due to the presence of bilirubin or nitrofurantoin) the reaction color may be masked. Urinary protein excretion in excess of 500 mg/dL and urinary glucose excretion in excess of 2 g/dL may diminish the intensity of the reaction color, as can medication with antibiotics containing cephalexin or gentamicin if administered in high daily doses.

Nitrite: The most common organisms causing urinary tract infections, E. coli and most urinary pathogens, convert dietary nitrate to nitrite, which produces a pink coloration of the test area. The reaction thus indirectly detects the presence of nitrite-forming organisms in the urine. Even a slight pink coloration is an indication of significant bacteriuria. Prolonged urinary retention in the bladder (4–8 hours; ideally over night) is essential for a valid result. Administration of antibiotics or other chemotherapeutics should be discontinued 3 days before the test.
The test is based on the principle of Griess' test and is specific for nitrite.

Protein: The test is based on the principle of the protein error of pH indicators and is particularly sensitive to albumin. Quinine, quinidine, chloroquine and tolbutamide do not affect the test, nor does a high pH (up to pH 9). False positive results may be obtained after infusion of polyvinylpyrrolidone (blood substitute), or if the urine specimen collection vessel contains residues of disinfectants based on quaternary ammonium compounds or chlorhexidine.

Glucose: The determination of glucose is based on the specific glucose-oxidase/peroxidase reaction.
This test is independent of the pH and specific gravity of the urine and is not affected by the presence of ketone bodies. The **effect of ascorbic acid** (vitamin C) has been **largely eliminated** so that at glucose concentrations of 100 mg/dL (5.5 mmol/L) and above even high ascorbic acid concentrations are not likely to give false-negative results.

Ketone bodies: This test is based on the principle of Legal's test and is more sensitive to acetoacetic acid than to acetone.
Phenylketones and phthalein compounds produce red colors on the test area. These are, however, quite different from the violet colors produced by ketone bodies. Captopril, mesna (2-mercaptoethanesulfonic acid sodium salt) and other substances containing sulfhydryl groups may produce false-positive results.

Urobilinogen: A stable diazonium salt reacts almost immediately with urobilinogen to give a red azo dye. No discoloration of the test area or colors lighter than that shown for 1 mg/dL (17 µmol/L) constitute a normal finding.
The test is specific for urobilinogen and is not susceptible to the interfering factors known to affect Ehrlich's test. Larger amounts of bilirubin produce momentary yellow coloration of the test area which may turn green to blue after about 60 seconds.

Bilirubin: The test for bilirubin is based on the coupling of bilirubin with a diazonium salt to give an azo dye. Even the slightest pink coloration constitutes a positive, i.e. pathologic, result. Other urinary constituents produce a more or less intense yellow discoloration.

Blood: Hemoglobin and myoglobin catalyze the oxidation of the indicator by an organic hydroperoxide contained in the test paper.
The values appearing on the print-out refer to **intact erythrocytes.**
The value range of 5–10 RBCs/µL also applies to hemoglobin from 5–10 RBCs/µL.
Separate color scales for erythrocytes and hemoglobin are given on the label of the test strip container. Individual to closely packed green dots on the yellow test area are indicative of intact erythrocytes. Hemoglobin, hemolyzed erythrocytes and myoglobin are indicated by a uniform green coloration of the test area.
At concentrations of about 5–50 RBCs/µL, significant hemolysis (such as may occur on prolonged standing of the urine) leads to values which are higher than the corresponding concentrations given for intact erythrocytes.

Note: In women the test for blood may be falsified from 3 days before to 3 days after a period. It is therefore advisable not to perform the test during this time. After physical activity, e.g. strenuous jogging, raised values for **erythrocytes** and **protein** may occur without being signs of disease.
Vitamin C (ascorbic acid) has virtually no effect on the test.

Compensation area: This white area, which is not impregnated with reagents, is used to compensate for an intensive intrinsic color of the urine which might affect the evaluation of the parameters leukocytes, nitrite, protein, glucose, ketone bodies, urobilinogen, and bilirubin.

Reactive components per cm²: **Specific gravity:** ethyleneglycoldiaminoethylethertetraacetic acid 182.8 µg; bromthymol blue 36 µg; **pH:** bromthymol blue 13.9 µg, methyl red 1.2 µg, phenolphthalein 8.6 µg; **Leukocytes:** indoxyl ester 15.5 µg, methoxy-morpholinobenzene diazonium salt 5.6 µg; **Nitrite:** hydroxytetrahydrobenzoquinoline 33.5 µg, sulfanilamide 29.1 µg; **Protein:** tetrachlorophenoltetrabromosulfophthalein 13.9 µg; **Glucose:** tetramethylbenzidine 103.5 µg, GOD 6 U, POD 35 U; **Ketones:** nitroprusside sodium 157.2 µg, glycine 4.2 mg; **Urobilinogen:** methoxybenzene diazonium salt 67.8 µg; **Bilirubin:** dichlorobenzene diazonium salt 16.7 µg; **Blood:** tetramethylbenzidine 52.8 µg, dimethyldihydroperoxyhexane 297.2 µg.

Please note: Diagnosis or therapy should never be based on one test result alone but should be established in the context of all other medical findings.
Not all effects of drugs or their metabolites upon the individual tests are known. In doubtful cases, it is therefore advisable to repeat the test after discontinuation of the medication.
Use only clean, well-rinsed vessels to collect urine. False-positive readings, particularly for glucose, protein and blood, can result from residues of detergent or strongly oxidizing disinfectants in the specimen collection vessel.
Do not add stabilizers to the urine. Do not expose urine specimens to sunlight as this induces oxidation of bilirubin and urobilinogen and hence leads to artificially low results for these two parameters. Drugs that turn red in an acid environment (e.g. phenazopyridine) may produce false-positive readings or reddish discolorations on the test areas for nitrite, protein, urobilinogen, and bilirubin. Large quantities of ascorbic acid (vitamin C) can lead to low or false-negative results for nitrite and bilirubin.

Calibration
Control-Test M calibration strips are used for the calibra tion of the photometer unit of the analyzer. For details se Operator's Manual of the analyzer.

Quality Control
For quality control, use commercially available urine cor trols, or other suitable control material. The control interva and limits must be adapted to the individual laborato requirements. Values obtained should fall within establishe limits. Each laboratory should establish correctice measur to be taken if values fall outside the limits.

Expected Values
See Appendix 1. Each laboratory should investigate th transferability of the expected values to its own patie population and if necessary determine its own referen range.

Result Values
See Appendix 1. For visual reading see color label on th test strip vial.

Performance Characteristics
See Appendix 2. The values specified for the analytic sensitivity are defined as the concentration of the analy which leads to a positive result in > 90% of the examin urines. For specific gravity and pH, analytical sensitivity not applicable (N. A.).
The method comparison data for Urisys 1100/Urilux S a Urichem 1000 (Miditron M) are based on the comparis with visual reading, the data for Urisys 1800 and Miditr Junior/Junior II are based on the comparison with Miditr M. The values for NEG and POS indicate the rate of concor ant negative or positive results.
For performance characteristics using visual reading the test strip see package insert of Chemstrip 10, Cat. N 11203479119.

Storage and shelf-life: Do not store the Chemstrip 10 Pack at temperatures below +2°C or above +30°C. In t original container the test strips are stable up to the da printed on the pack, even once the container has be opened.

Disposal: Please dispose of used test strips accordi to the safety regulations applicable at your facility.

The stopper of the test strip container is filled with a n toxic silicate-based desiccant. If inadvertently ingested should be flushed down with plenty of water.

Presentation: Packs of 100 test strips
(REF 11379208119).

For an explanation of the symbols used and a list references please refer to the end of this insert.

Last updated: 11/2004

Routine Urinalysis:

1. Use a freshly voided specimen (midstream), catheterized or cystocentesis specimen collected in a clean container. If analysis is to be delayed, refrigerate the sample and be sure to warm it to room temperature (minimum of 30 minutes) before proceeding with the urinalysis. Cooling may produce crystal formation. **Never freeze a urine sample if there is to be a microscopic examination** - freezing will rupture any cellular structures in the urine.

2. Gently **mix, do not shake,** the urine.

3. Note and record the volume of the urine sample. A 3 mL sample volume is ideal. Interpretation of the microscopic examination is based on a standard starting volume of 3 mL.

4. Note the color and turbidity (clarity) of the urine sample.

5. Dip a "Chemstrip 10A" strip (see preceding page for product information) into the well-mixed urine sample. Replace the lid on the test strips immediately. Read the results between **60 and 120 seconds** after dipping by comparing the colours to the test pad colours on the vial. Make sure to line up squares correctly (top part of strip where there are no squares is next to lid, very last square on strip is for the automated strip reader thus not read). Record the results on the urine requisition form. **Do not report the results of the leukocyte, nitrite, or urobilinogen test pads.**

   Note: In evaluating urinary blood pigment there are two separate scales - one for whole blood, one for hemoglobin. Scattered or compact dots on the test pad are indicative of intact erythrocytes. Hemoglobin, hemolyzed erythrocytes and myoglobin are indicated by a uniform green coloration on the test pad.

6. Centrifuge the urine sample for 5 minutes at 1500 rpm. Decant the supernatant quickly into a clean test tube (eg. quickly turn the tube upside down over the clean tube; don't worry, the sediment will remain in the upturned tube). **Determine the urine specific gravity (USG) on the supernatant** using a refractometer.

7. If the protein value from "Chemstrip 10A" strip is considered to be significantly increased then further quantification of urine protein may include one or more of the following tests:
   Determination of urine protein concentration using an automated chemistry analyser.
   Determination of a urine protein to creatinine ratio.

8. Do the microscopic evaluation of the urine sediment (**Note: Lower the condenser to increase visibility of the sediment constituents.)**

   a) Use 10 µL (1 drop) urine sediment for "unstained" and 10 µL (1 drop) urine sediment + 10 µL (1 drop) Sedistain for "stained" preparations. Mix stain and urine with the edge of the coverslip, then place a coverslip on both drops.

b) Scan on low (10x) or high dry (40x) power for crystals, bacteria, debris and fat. Quantify on high dry (40x) as **scant** (< 5 %); **mild** (5-25 %), **moderate** (25-75 %) or **abundant** (>75 %). **NB:** Percentages are based on area of the 40x field occupied by these components.

c) Scan on low (10x) power and numerically quantify casts.

d) Scan 15 - 20 fields on high (40x) power and average the counts for cellular elements. Report as a numerical range up to 100/HPF. If >100/HPF report as such or, if excessive, as "field obscured by RBCs, WBCs".

e) Report sperm only as present (do not quantify).

f) Use 100x to specify bacterial morphology (rod, cocci, chains) or aid in identification of anything obscure at lower magnifications.

Physical Examination of Urine:

1. **Measurement of urine volume**

- measurement is only valuable when all the urine formed over a known period is collected, ideally at least 24 hours. Seldom possible without using a metabolism cage.

Polyuria: > 50 - 70 ml/kg/day
Oliguria: < 7 ml/kg/day
Anuria : < 2 ml/kg/day

2. **Appearance of urine**

a) color - normal urine appears yellow due to the presence of urochromes (breakdown products of bile pigments)

Colorless to pale yellow	- dilute urine (low USG)
Yellow	- "normal"
Red/pink	- if red and cloudy, intact RBCs in urine **(hematuria)**
	- if bright red and clear, Hgb free in the urine **(hemoglobinuria)**

Red, brown and various shades in between may be seen with hematuria, hemoglobinuria or myoglobinuria. Additional testing may be necessary to further distinguish myoglobin.

Yellow/yellow-orange/amber	- concentrated urine
	- bilirubin
	- drugs

b) transparency - urine is normally transparent. Turbidity may be due to crystalluria, excessive numbers of cells, microorganisms, or natural secretions.

c) odour - natural odours are due to pheromones (especially obvious in male cats) and metabolites of food. Abnormal odours include ammonia, acetone and putrefaction.

3. **Specific gravity**

Dilute urine : USG $\leq$ 1.007
Isosthenuria : USG = 1.008 -1.012 (same as glomerular filtrate)

- there is no "normal" range for USG. To interpret USG need to know:
a) hydration status of the animal
b) plasma or serum concentrations of urea and creatinine
c) drugs or fluids that have been administered
d) water consumption

Urine specific gravity is measured with a refractometer or total solids (TS) meter, as for plasma protein in Lab 1.

Practice Tip: Be sure to check the **zero reading of the refractometer** regularly using distilled water (SG of 1.000). You can check the upper limit of the scale by making a 7.5% solution of NaCl (7.5 gm of NaCl in 100 ml of distilled water) and check the reading - it should read 1.035 +/- 0.003

Chemical Examination of Urine:

The level of chemical constituents of the urine and urinary pH can be most conveniently measured using commercial test strips.

1. Urinary pH
range from 4.5 to 8.5
- pH reflects diet - protein/meat diets result in acid urine; plant/cereal diets result in higher pH.
2. Urinary protein (SI units are g/L)
- commercial strip tests are more sensitive to albumin (mainly of renal origin) than to other proteins (globulin, hemoglobin, myoglobin, Bence-Jones protein and mucoprotein).
- always interpret urinary protein relative to urine SG - the same amount of protein would be more significant with a low SG than with a high SG; urine protein to creatinine ratio determination will allow for comparison regardless of differences in SG.

3. Urinary glucose
   - SI units are mmol/L, but we report as normal to 4+.
   - based on glucose oxidase/peroxidase reaction. False positive results can be obtained if there is contamination with hydrogen peroxide, hypochlorite or chlorine.
4. Urinary ketones
   - recorded as **trace**, **small**, **moderate** or **large** amounts (trace, 1+, 2+ or 3+).
   - strip reacts mainly with acetoacetic acid and only to a slight extent with acetone. The tablet test (Acetest) reacts with both. **Neither detects β-hydroxybutyric acid.**
5. Urinary bilirubin
   - detectable concentrations are usually recorded as **small**, **moderate** or **large** (1+, 2+, and 3+).

   Dogs: - when canine urine is within its usual specific gravity range (1.015 - 1.045) only 2+ or 3+ readings are significant.
   - renal threshold for bilirubin is low and renal tubular epithelium can conjugate bilirubin.

   Cats: - the renal threshold for bilirubin is 9 times higher than in the dog; therefore, any reading is significant.
6. Urinary blood pigment
   - commercial test strips may attempt to distinguish between blood/hematuria (intact RBCs in the urine) and hemoglobinuria/myoglobinuria (hemoglobin or myoglobin free in the urine).
   - when hematuria is present, individual RBCs lyse on the test area giving a speckled appearance. If there is free hemoglobin or myoglobin pigment present, the color change is expected to be uniform (solid color). However when intact RBCs are present in microscopic examination, you can see either a speckled or solid pattern; the solid pattern indicating lysis of RBCs in the urine.
   - the **trace** result on the strip corresponds to 5-15 $x10^6$ RBCs/L.

Practice Tips:
1. Make sure the reagent strips you are using are not outdated; **do not cut them in 2 to save $**.
2. Make sure you replace the lid as soon as you remove a reagent strip. Moisture will make the strips unreactive and lead to false negative results.
3. When dipping reagent strips into the urine, do not leave in any longer than it takes to get them wet. Leaving the reagent strips in too long will dilute out the chemicals in the test pads and result in false negative values.
4. Make sure the urine is at room temperature - lower temperatures will cause reactions to be lower or even negative.
5. Make sure the urine is well mixed.
6. Make sure to read all reactions between 60 and 120 seconds after dipping.

Miscellaneous Urinalysis Interference Information

For glucose determination:

On Chemstrip:

1) The reagent pad is specific for glucose; it will not interact with other sugars like fructose, galactose, etc.
2) Ascorbic acid, at high levels, can cause a false negative reaction.

For ketone determination:

The Chemstrip reagent pad will not react with β-hydroxybutyrate which is quantitatively the most important ketone body. If one is concerned with detecting very mild ketosis this could be a problem. In most dogs and cats with diabetic ketoacidosis and in cattle with ketosis this does not present a problem - the other ketone bodies (acetone and acetoacetate), which will react with the Chemstrip reagent pad, are present in high enough quantities.

Specific Gravity:

The urine specific gravity will be falsely increased with any substance which could increase the refractive index of the urine sample. Thus the determination of urine specific gravity should only be performed on the **supernatant**, not unspun urine. The crystals or cells suspended in the urine can increase the urine specific gravity.

Urine Sediment:

The urine sediment should be prepared for examination as soon as possible following collection in order to minimize *in vitro* changes. Refrigeration will help preserve the cells if delay is unavoidable.
Examination of unstained preparations is recommended for routine use. **The microscope condenser should be lowered to increase contrast**.

Normal urine sediment findings:

RBC: < 5/HPF
WBC: < 5/HPF (≤ 3/HPF in samples collected by cystocentesis)
Epithelial cells: Few
Casts: Few (hyaline 0-2/LPF; granular 0-1/LPF)
Crystals: Some
Other: Fat droplets, sperm

Cells in the urine sediment:

For routine purposes, cells are examined as unstained wet-mounts of urine sediment. Under some circumstances, air-dried smears and cytocentrifuge preparations are prepared and stained with hematologic stains.

RBCs and WBCs are quantified as "cells/HPF" (40x objective). Other cell types are usually subjectively listed as "scant, mild, moderate or abundant".

The morphology of **RBC in unstained urine sediments** depends largely on the concentration of the specimen and the time lapse following collection.

Fresh RBC tend to have a red or yellow color. Prolonged exposure results in a pale or colorless appearance as hemoglobin may be lost from the cells.

In fresh samples with SG of 1.010-1.020, RBC may retain the normal disc shape. In more concentrated urine (SG > 1.025) RBC tend to shrink and appear as small, crenated cells. In more dilute urine samples, they tend to swell. At SG < 1.008 and/or highly alkaline pH, RBC lysis is likely. Lysed RBC appear as very faint "ghosts" or may not be visible.

RBC up to 5/HPF are commonly accepted as normal. Increased RBC in urine (hematuria) can be due to hemorrhage, inflammation, necrosis, trauma or neoplasia somewhere along the urinary tract (or urogenital tract in voided specimens). The method of collection must be considered in interpreting hematuria and localizing the source. Catheterization, cystocentesis and manual expression of the bladder can induce hemorrhage.

WBC in unstained urine sediments typically appear as round, granular cells 1.5 - 2 times the diameter of RBC. The details of the nuclear shape are often difficult to discern, especially if the specimen is not fresh. WBC in urine are usually neutrophils. Staining of an air-dried smear or cytocentrifuge preparation with hematologic stains is useful for specific identification. Like RBC, WBC may lyse in very dilute or highly alkaline urine.

WBC up to 5/HPF are commonly accepted as normal. Greater numbers (pyuria) generally indicate inflammation somewhere along the urinary tract (or urogenital tract in voided specimens). Pyuria is often caused by urinary tract infection and bacteria may be seen on sediment preparations. Depending on clinical signs, pyuria may be an indication for urine culture despite the absence of identifiable bacteria. Non-septic causes of inflammation, such as uroliths and tumors, also must be considered.

Squamous epithelial cells are the largest cells which can be present in normal urine samples. They are thin, flat cells, usually with an angular or irregular outline and may have a small, round nucleus, however a nucleus is often not visible. They may be present as single cells or in variably sized clusters. Squamous cells are common in low numbers in voided specimens and generally represent contamination from the genital tract. Many dogs with squamous metaplasia of prostatic epithelium have extremely high numbers of squamous cells in their urine.

Transitional epithelial cells originate from the renal pelvis, ureters, urinary bladder and/or urethra. Their size and shape depends on the depth of origin in the mucosa. Most often they are round. They are generally smaller and smoother in outline than squamous cells but larger than WBC. They may develop refractile, fatty inclusions as they degenerate in older specimens. Usually transitional cells are few and appear as single cells or small clusters. Specimens collected by catheter sometimes contain large sheets of cells scraped off during the procedure. In inflammatory conditions causing hyperplasia of the urinary mucosa, large numbers/clusters may exfoliate. In such cases, differentiation from neoplastic transitional cells may be difficult.

Neoplastic cells may be seen in urine sediments of patients with tumors (e.g. transitional cell carcinomas) of the urinary tract.

Crystals: General

A variety of *in vivo* and *in vitro* factors can influence crystal formation. *In vivo* factors include: 1) the concentration of the crystallogenic substances, 2) the urine pH, 3) the solubility of crystallogenic substances and 4) the excretion of diagnostic and therapeutic agents.
In vitro factors include: 1) temperature (solubility decreases with temperature), 2) evaporation (increases solute concentration), and 3) urine pH (changes with standing and bacterial overgrowth).

Crystals: Common types

Listed here are some of the more commonly encountered types of crystals. All these can be seen in samples from "normal" animals. Conversely, under certain circumstances, many of the same crystals can be clinically significant.

1.) **Bilirubin crystals** tend to precipitate onto other formed elements in the urine. They are golden-brown fine needle-like crystals. Bilirubin crystals are seen most commonly in canine urine, especially in highly concentrated specimens. They are less common in the urine of other species. In dogs, they are often of no clinical significance - healthy dogs can have low, but detectable, bilirubin levels in urine. Bilirubin crystals or a +ve chemical reaction on the urine dipstick in feline, equine, or bovine urine should be investigated since an underlying cholestatic process is likely.

2.) **Struvite crystals (magnesium ammonium phosphate)** usually appear as colorless, 3-dimensional, prism-like crystals ("coffin lids"). Struvite crystals are the most common type in urine from dogs and cats. They are often seen in urine from clinically "normal" individuals. Though they can be found in urine of any pH, their formation is favoured in neutral to alkaline urine. Urinary tract infection with urease +ve bacteria can promote struvite crystalluria and urolithiasis by raising urine pH and increasing free ammonia.

3.) **Calcium oxalate dihydrate crystals** typically are seen as colorless squares or rectangles whose corners are connected by intersecting lines (resembling an envelope). They can occur in urine of any pH. The crystals vary greatly in size. In some cases, large numbers of tiny oxalates

may appear as amorphous crystals unless examined at high magnification. These crystals are often seen in normal urine from horses and cattle (derived from the high oxalate content of herbivore diets), but are less common in urine from normal dogs and cats. Calcium oxalate dihydrate crystals can also be seen in dogs and cats with uroliths composed of calcium oxalate, in cases of ethylene glycol intoxication, or sometimes in hypercalcemic patients. If observed in large numbers in the urine of a dog or cat with acute renal failure, ethylene glycol consumption should be considered.

4.) **Calcium carbonate crystals** usually appear as large yellow-brown or colorless spheroids with radial striations. They can also be seen as smaller crystals with round, ovoid or dumbbell shapes. These crystals are common in the urine of normal horses, rabbits, guinea pigs and goats. They are rarely observed in urine from dogs or cats.

5.) **Amorphous crystals** appear as aggregates of finely granular material without any defined shape.

Crystals: Less common types

The following urine crystals are not expected in normal individuals.

1.) **Calcium oxalate monohydrate crystals** vary in size and may have a spindle, oval or dumbbell shape. Most commonly they appear as flat, elongated, six-sided crystals ("fence-pickets"). This form of calcium oxalate crystals is usually associated with **ethylene glycol toxicity.**

2.) **Ammonium urate (also called ammonium biurate) crystals** generally appear as brown or yellow-brown spherical bodies with irregular protrusions ("thorn-apples"). Their formation is favoured in neutral to alkaline pH.
These crystals may be seen in conditions in which there is decreased conversion of ammonia to urea, are fairly common in dogs and cats with congenital or acquired portal vascular anomalies, with or without concomitant ammonium urate uroliths; they may also be seen in patients with liver failure. They can be seen in the urine from normal Dalmatian dogs or English bulldogs. They are rarely, if ever, seen in urine from normal cats or dogs of other breeds.

3.) **Cystine crystals** are flat colorless plates with a characteristic hexagonal shape with equal or unequal sides. They often aggregate in layers. Their formation is favoured in acidic urine. Cystine crystalluria or urolithiasis indicates defective renal tubular reabsorption of certain amino acids including cystine. Sex-linked inheritance is suspected since male dogs are almost exclusively affected. Renal function otherwise appears to be normal and aside from a tendency to form uroliths, the defect is without serious consequence.

4.) **Tyrosine crystals** are usually seen as brownish, fine needles. These can be associated with severe liver disease in humans, but they are very rarely seen in domestic animals.

5.) Many **drugs** excreted in the urine have the potential to form crystals. Most common among these are the sulfa drugs. Different appearances are possible for the same drug and may relate to variation in drug concentration, urine pH and other factors.

Infectious Agents:

Infectious agents of various classes can be observed in urine sediments. In most cases, their significance can be properly assessed only in light of the clinical signs, method of collection, post-collection interval and other findings in the urinalysis.
Bacteria can be identified in unstained urine sediments when present in sufficient numbers. Rod-shaped bacteria and chains of cocci are often readily identifiable. Voided samples may be contaminated with bacteria from the urethra. Bacteriuria of clinical significance is usually accompanied by pyuria. However, diabetic animals and those with hyperadrenocorticism may have bacteriuria in the absence of a leukocyte response.
Yeast, fungi, or parasite ova could be seen in urine samples.

Urinary Casts (see figure on next page):

Casts are cylindrical structures composed of mucoprotein from the tubular epithelium and other structures (cells, etc) which are present at the time of formation.

Hyaline casts are formed in the absence of cells in the tubular lumen and their presence suggests renal protein loss. They have a smooth texture and reduced illumination is essential to visualize.

Cellular casts indicate degeneration and necrosis of tubular epithelial cells, often due to hypoxia or anoxia.

WBC casts indicate renal inflammation; however differentiation from epithelial cells is usually difficult as cells undergo aging changes.

Granular casts have a textured appearance which ranges from fine to coarse in character. They usually form as a stage in the degeneration of cellular casts.

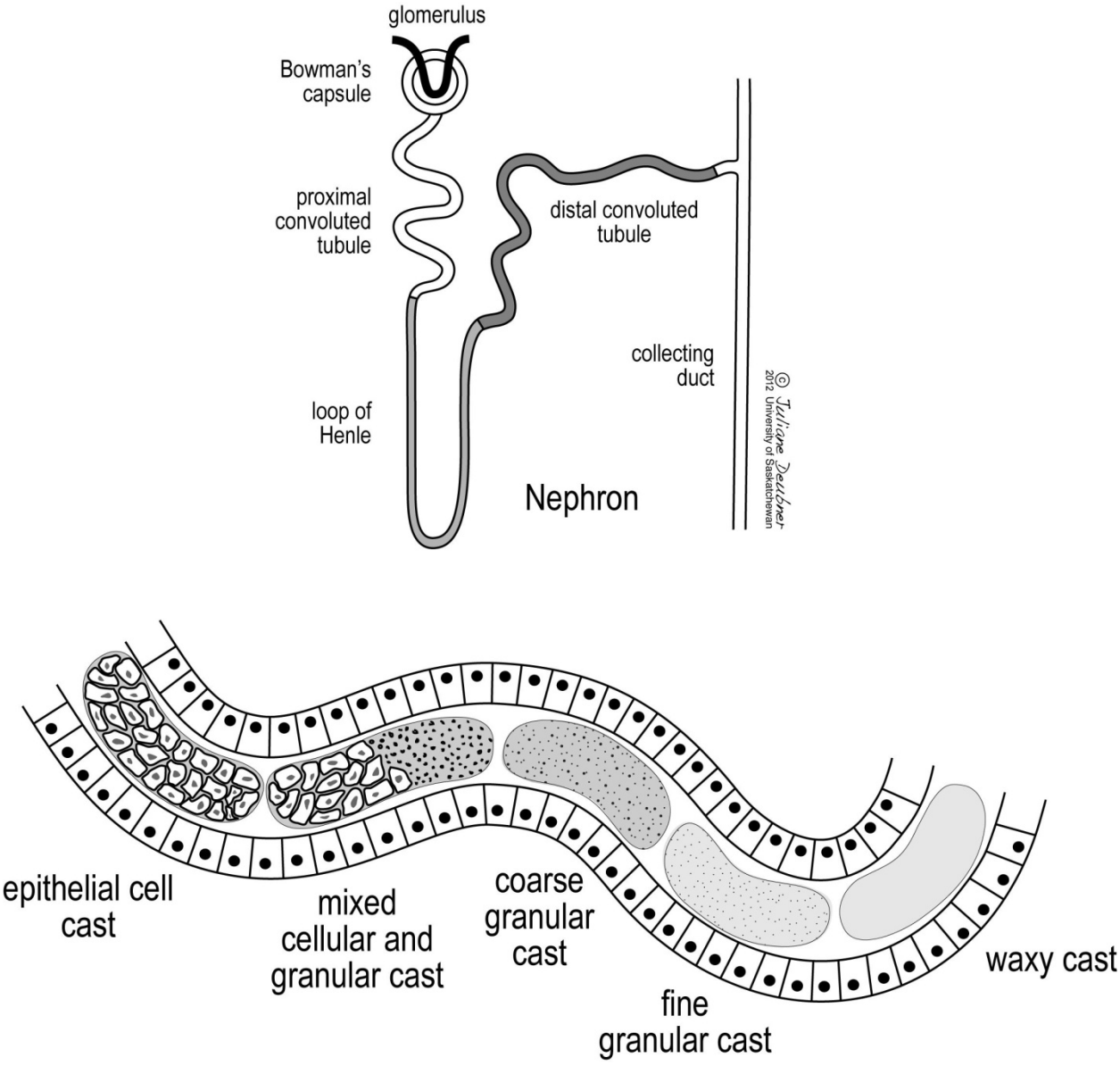
Fatty casts are identified by the presence of refractile lipid droplets. The background matrix of the cast may be hyaline or granular in nature. Often, they are seen in urine in which free lipid droplets are present as well. Fatty casts are most commonly seen in cat urine.

Waxy casts have a smooth consistency but are more refractile and therefore easier to see compared to hyaline casts. They commonly have squared off ends, as if brittle and easily broken. The significance of waxy casts is the same as described for cellular and granular casts.

Contaminants:

Extraneous contaminating materials of many types can make their way into urine specimens, especially those collected by free flow. Common contaminants include: mold spores, pollen, bacteria, fecal material, plant material, and starch granules (from surgical gloves).

Cast formation
Illustration showing transition between epithelial cell, coarse, fine granular, and waxy casts formed in the loop of Henle, distal tubules and collecting ducts (shaded area of nephron).



LAB 9: CYTOLOGY:

This laboratory will consist of 2 parts:

1.) **<u>Microscopic slide review:</u> 10:30-11:45 am, Room 2115**

a. Instructor will utilize virtual microscopy scanned slides: We will learn how to examine a cytology slide, how to differentiate inflammation from neoplasia, how to classify different types of neoplasms, features of malignancy and will evaluate a few common cytological samples. A description of this material is provided in your laboratory manual. The following slides will be demonstrated:

i. **D0822182.** 5 year old male Golden Retriever X dog. Lost 15 kg over the last 3 months. Vomiting usually after exercise and drinking water. Now in respiratory distress. We received 2.5 ml cloudy salmon colored pleural fluid that contained white flocculent material (therefore nucleated cell count (NCC) not available). Total protein 43 g/L.

ii. **D0610182.** 17 year old MC cat. Off food for 2-3 days. Owner cannot see urine or stool on litter. Abdomen is distended. Cat has had a heart murmur for 1 year. Received 1 ml cloudy abdominal fluid containing flocculent material. NCC not available. TP 41 g/L.

iii. **D1128942.** 6 year old MC German shepherd X dog. Large 2.8 cm x 3.0 cm mass on left medial forelimb. FNA smears received.

iv. **D0822455.** 12 year old MC Terrier X dog. 1 cm firm subcutaneous lump on ventral chest, has been growing. FNA smears received.

v. **D1127471.** 2 year old male, Boxer dog. Mass on RH limb, cutaneous, firm, round, raised, 6 mm, mobile and ulcerated. Owner noticed it 3 weeks ago, dog has been licking at it. FNA smears received.

vi. **D0901958.** 16 year old FS German Shepherd X dog. Losing weight (3 kg in last 8 months), still eating, increased drinking, sleeping a lot. Increased size of submandibular and prescapular lymph nodes. We received FNAs from 4 of these nodes. We will examine smear from left prescapular lymph node.

vii. **D1130275.** 5 year old MC Golden Retriever dog. The dog has a large pink ulcerated tumor arising from the RH 4th digit that was previously diagnosed by biopsy as an anaplastic tumor. Now the right popliteal lymph node is mildly enlarged. We received a FNA smear from this lymph node.

viii. **D0900540.** 17 month old male Border Collie X dog. Glaucoma, uveitis, chest mass, depression, fever and lymphadenopathy. We will examine a FNA smear from the Left submandibular lymph node.

b. Students will examine 3 of these slides using their own computers (see histories and signalment above):

i. D0822182
ii. D0610182 – look at clumps at periphery of smear
iii. D0901958 -

2.) <u>Cytology wet lab:</u> 11:45-12:20 am.

The following procedures will be demonstrated in **room 2115**, after which the students will proceed to **room 1364 (Physiology laboratory)** where they will find a station (students will work in pairs) in order to practice these techniques.

a. **<u>Fine needle aspirate (FNA) and non-aspiration techniques</u>** will be demonstrated using oranges and students will be shown how to make cytology smears using a <u>slide over slide method.</u>

b. **<u>Impression smear making</u>** will be demonstrated using orange segments.

c. **<u>Processing fluid samples</u>**: During the demonstration students will be shown how to make a direct smear, line preparation, and how to use a swab to capture any particulate/mucoid material. In a clinic setting, a fluid sample may be concentrated by centrifugation and direct smears may be made from the sediment. At each station, students will find a fluid sample for cytology. Please make a direct smear (blood smear technique) and line preparation as described during the demonstration.

****REMINDER: Do not put <u>any</u> waste in the floor garbage cans, other than hand-drying paper towel. Waste and sharp containers will be provided.**

A. Fluid Cytology Preparation and Analysis (for future reference)

- After collection of the sample, place 3-5 ml (depending on tube and sample size) of the fluid into an EDTA (purple top) tube (important in order to prevent clotting) and some fluid in a sterile red top tube (for potential culture).

- Make a few direct smears (blood smear technique and a line smear – see diagram below), allow them to air dry. It is important to make fresh smears as soon as possible following collection of the fluid to prevent cellular disintegration.

- Perform a physical assessment of the fluid- eg. turbidity, color. A turbid fluid may indicate the presence of increased cells or lipid (eg. chylous effusion with chylothorax), bacteria, fibrin, or GI ingesta. Red or maroon color may indicate RBCs or free hemoglobin. You can compare PCV of fluid to that of peripheral blood to determine if it is similar to fresh blood. If the fluid is centrifuged and the red color is only seen in the sediment, then this finding indicates the presence of intact RBCs rather than hemoglobin (entire sample remains red or maroon color).

- Perform a nucleated cell count (NCC) using a hemocytometer (as for a white blood cell count on a CBC) on fluid from the EDTA sample. If NCC is very high, this may be very labor intensive and you may report your findings as TNTC (too numerous to count).

- If cellularity of the fluid is low, a concentrated sample can be prepared by centrifugation of the fluid (5 minutes at 1500 rpm) and then direct smears can be made from the sediment (always remember to indicate on your label that this is a sediment slide).

- Perform a total protein measurement using a refractometer (see CBC instructions). Total protein measurement should be done on the supernantant of a centrifuged sample if the fluid is cloudy. The sample can be centrifuged in a capillary tube. If the amount of fluid is not adequate in the EDTA tube, this can interfere causing a false increase in the total protein value.

- Send unstained slides to a diagnostic laboratory OR if you are staining the slide in your clinic to evaluate, then use Protocol Hema 3 (same stain used for a CBC). Just remember that Protocol Hema 3 may not stain or may weakly stain mast cell granules, therefore you cannot rely on it if you are seeing round cells.

- Microscopic examination of all slides.

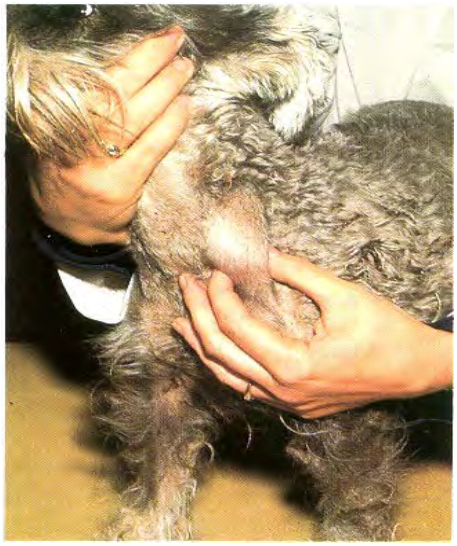
- Classification of body cavity fluids (eg. thoracic, abdominal) as tranudate, modified transudate or exudate. A transudate is a fluid that has a nucleated cell count $\leq 1.5 \times 10^9$ /L and a total protein value of ≤ 25 g/L. An exudate is a fluid that has a nucleated cell count $>7.0 \times 10^9$ /L and a total protein value of > 30 g/L. A modified transudate is a transudate that is modified by either increased cells and/or increased protein, therefore if the values for NCC and total protein do not fall into the classification for transudate or an exudate, then the fluid is classified as a modified transudate.

- Interpretation of all findings.

B. <u>Fine needle aspiration (FNA)</u>

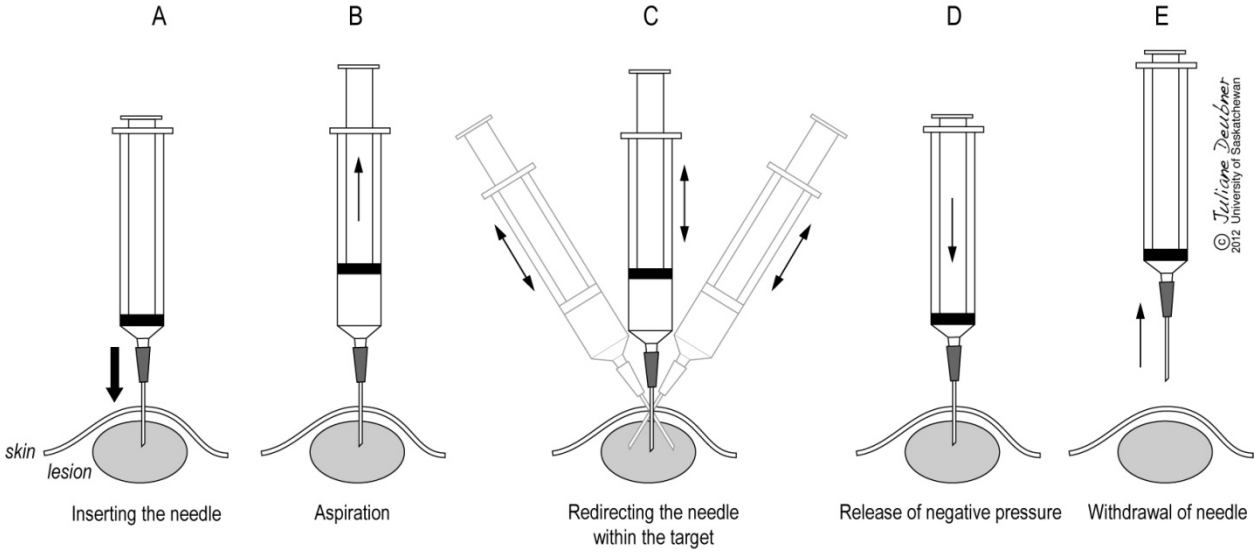
- A needle can be inserted into almost any tissue in order to obtain cells. Be sure the aspirate is deep into the mass or lymph node in order to obtain a representative sample. The needle should be no larger than a 22 gauge even for large animal species as a larger needle will only lead to blood contamination of your sample. Use a 22-25 gauge needle attached to a 3 ml or 10-12 ml syringe.

- Make direct smears using either slide over slide (see diagram below) or blood smear method. Allow smears to air dry. The smears may be submitted unstained to a diagnostic laboratory (a good habit to develop is to stain one of the smears with Protocol Hema 3 stain to be sure that your smears contain cells for evaluation).



Left hand of handler demonstrating immobilization of an enlarged prescapular lymph node in preparation for FNA.
Compendium Small Animal 9(2), 1987, 104-112, Fig 22

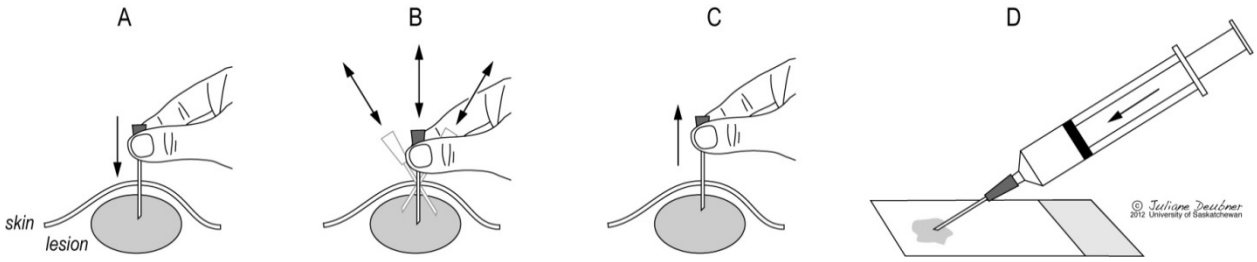
Fine needle aspiration (FNA)



A. Insert the needle into the mass.
B. Aspirate with as much suction as possible.
C. Re-directing the needle within the mass 2-4 times.
D. Release the negative pressure on the syringe.
E. Withdrawal of the needle attached to syringe from the mass without suction.

Disconnect the needle from the syringe, fill the syringe with air and reconnect to the needle. Express material onto a slide (near frosted end).

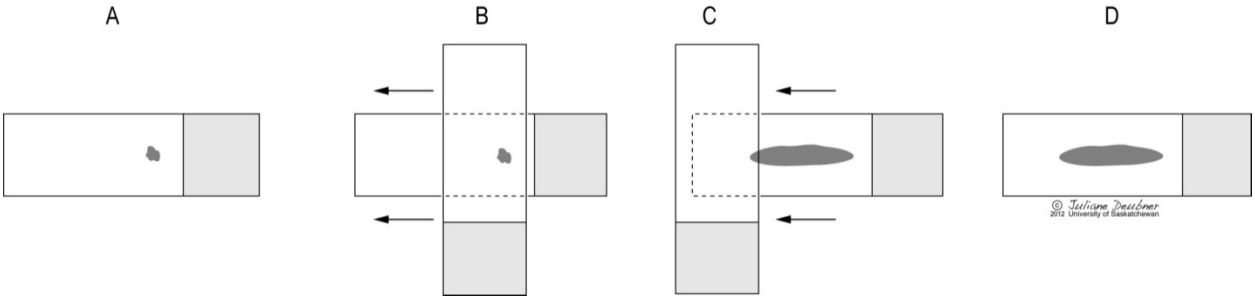
C. Fine needle capillary sampling (nonaspiration method)



A. Use needle only (22 g) and insert into target tissue.
B. Move needle back and forth within the target (3-6 times) varying the angle each time.
C. Withdraw the needle from the target tissue.
D. Attach syringe containing air to needle and express material onto slide(s).

D. Making smears

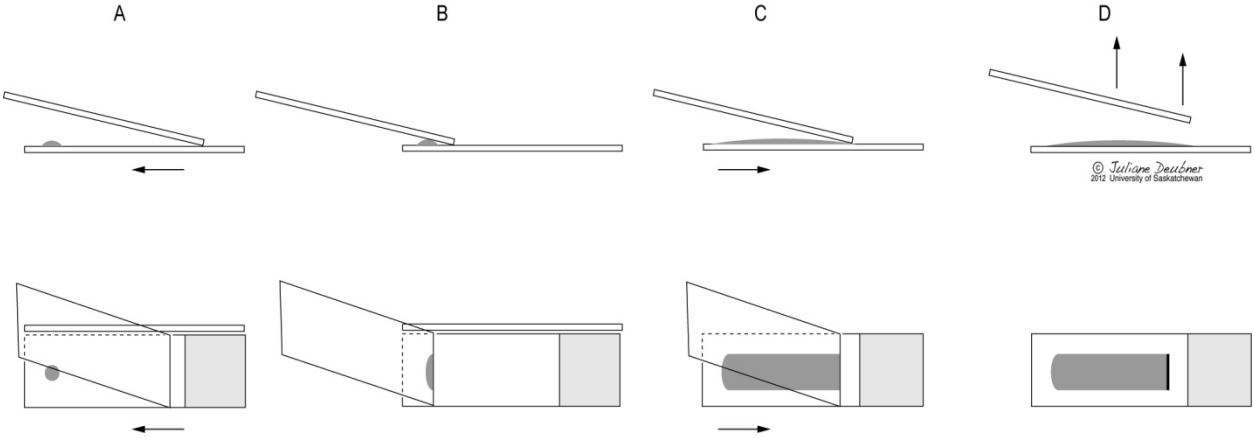
Slide over slide method



A. Place a small drop of fluid on slide.
B. Second slide is placed on top of the first slide at right angle.
C. Top slide is pulled across spreading the drop of fluid.
D. Final smear is complete.

Line smear method

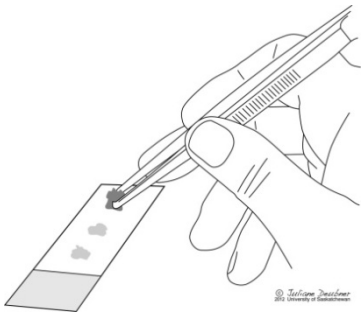
Make the smear but instead of creating a feather edge as for a blood smear, stop short and lift slide up. Good for low cellularity fluids.



A. Spreader slide is held at an angle and drawn into the drop of fluid.
B. Spreader slide is held until the fluid has spread.
C. Spreader slide is pushed in the opposite direction.
D. Spreader slide is lifted straight up creating a line of concentrated cells near the end of the smear.

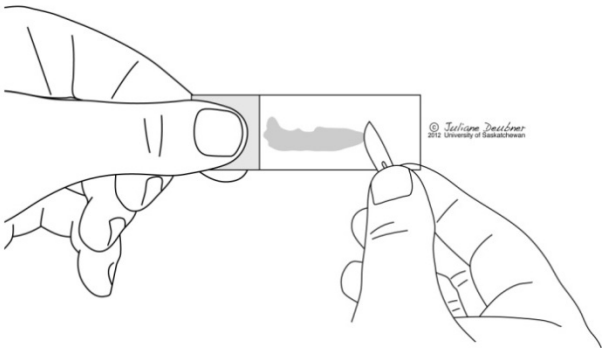
Impression smears

The cut surface of the tissue is first blotted on a paper towel to remove excess blood, then gently touched multiple times to the surface of a clean glass slide.



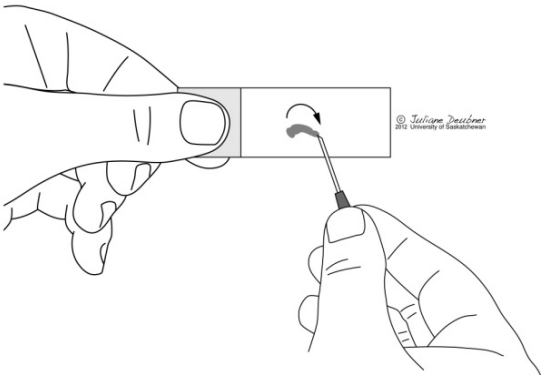
Tissue scraping

If the tissue does not adequately exfoliate, a scalpel blade may be used to scrape the surface of the tissue. The material on the edge of the blade is then dragged along the surface of a slide.



Tissue rolling

Small pieces of tissue that cannot be easily grasped with forceps for making an impression smear can be gently rolled on a slide using a 25 gauge needle.



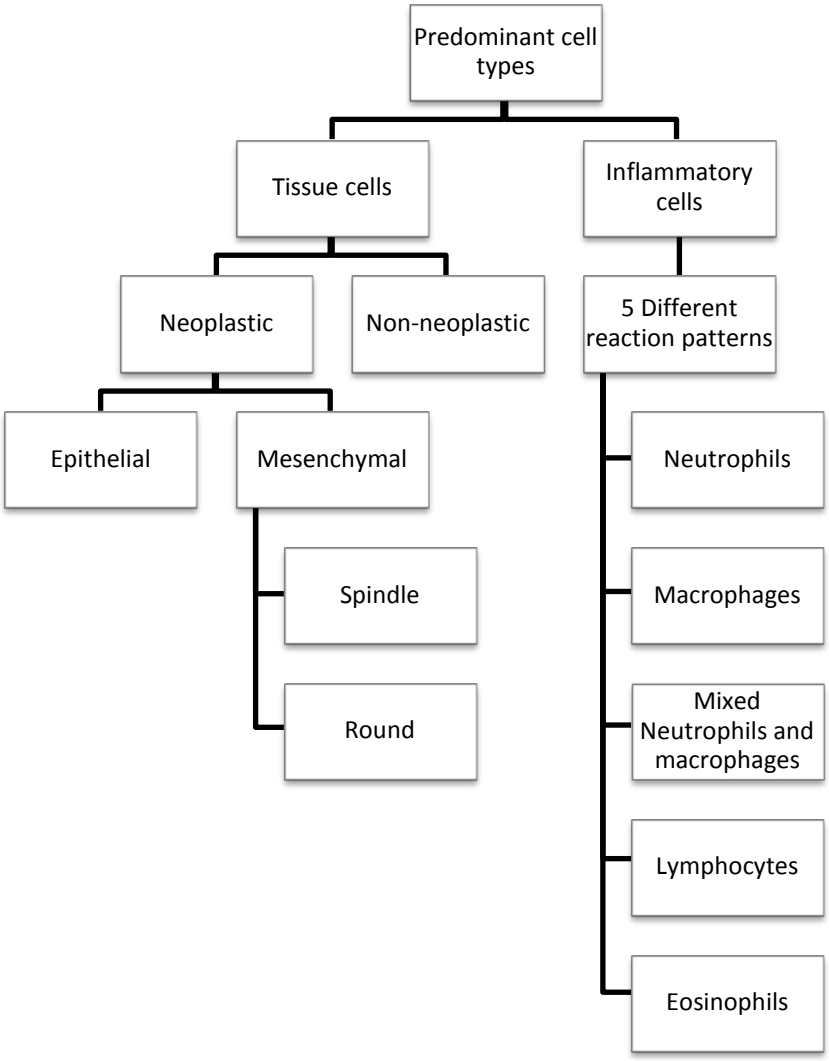
MICROSCOPIC EXAMINATION OF CYTOLOGY SAMPLES:

1. **Assess the following features at low power (x10-x20), describe and quantify (few, moderate or many) where appropriate:**
    a. Examine slide for clumps of cells especially at the periphery of the smear.
    b. Examine the slide for large organisms (e.g. fungal, or clumps of bacteria).
    c. Evaluate the degree of cellularity (high, moderate, low) – refers only to nucleated cell numbers (do not include erythrocytes or platelets in this assessment). Cellularity can only be assessed on direct smears, FNA or impression smears (not on concentrated preparations such as cytospins) or washes (e.g. transtracheal wash).
    d. Examine and describe the amount, color and distinguishing features of the background (e.g. pink staining material, mucus, disintegrating cells, granules, debris).
    e. Numbers of erythrocytes can be indicated (few, moderate, many). Note: Do not spend time trying to determine red cell morphologic abnormalities on cytology preparations.
    f. Comment on the presence and numbers of disintegrated cells since these may affect the ability to properly evaluate the slide. Disintegrated cells are those that have been traumatized during slide preparation.

2. **See cytology flow chart (next page) to assist with cell types and perform this assessment and description at higher powers (x40, x100).**
    a. If tissue cells are present, proceed to the cytology flow chart (left side). If the cells are determined to be neoplastic (population of abnormal tissue cells), determine if epithelial (cells in clumps) or mesenchymal (spindle or round cells) – see page 153 of your textbook for diagrams of the 3 basic tumor categories. Determine if the cells are malignant or benign (see page 151 of your textbook for a description of features of malignancy). In general, malignant cells reveal a higher degree of variability (e.g. anisocytosis, pleomorphism) than their benign counterparts.
    b. If only inflammatory cells are present, then proceed to the cytology flow chart (right side). List the types of inflammatory cells that are present in descending order of frequency. Determination of the predominant type of inflammatory cell will aid in determination of the disease process. Determine if neutrophils are non-degenerate or degenerate. Look carefully within inflammatory cells for etiologic agents (bacteria, fungi). Look within macrophages for evidence of erythrophagocytosis and/ or the presence of hemosiderin.

3. **Interpretation:** include a description of the type of inflammation present (eg. septic neutrophilic inflammation) and provide a specific diagnosis if a specific etiologic agent is found (e.g. blastomycosis) or in the case of a neoplastic process, the category of neoplasm (e.g. epithelial or mesenchymal (spindle or round) and whether it is benign or malignant. Possible differential diagnoses for the neoplasm based on cytological findings and history should also be provided.

CYTOLOGY FLOW CHART



LYMPH NODES

The cytologic appearance of a normal lymph node or lymphoid tissue (e.g. spleen) is heterogeneous (consists of predominantly small mature lymphocytes, with fewer medium lymphocytes and even fewer large lymphocytes). A neoplastic process involving lymphocytes (lymphosarcoma) reveals an increase in homogeneity of the normal lymphocyte population (usually > 50% large lymphocytes).

Another process that we commonly recognize is hyperplastic/reactive lymph nodes (secondary to antigenic stimulation). In these cases, we often see increased numbers of plasma cells. Other processes that we may detect in lymph nodes include lymphadenitis, or metastatic neoplasia.

The microscopic evaluation of lymph nodes should follow the same basic process as described above (#1). The lymphocyte population should then be assessed as either heterogenous (different sizes of lymphocytes) or homogeneous (lymphocytes appear similar in size). Next the percentage of the different sizes of lymphocytes should be estimated and the number of plasma cells evaluated to allow the examiner to categorize the lymphocyte population as normal, hyperplastic/reactive or lymphosarcoma (see chart below).

	Normal	**Reactive/ Hyperplastic**	**Lymphosarcoma**
Cell types	Heterogeneous	Heterogeneous	Homogeneous
Small Lymphocytes	75-95%	70-80%	Few
Medium Lymphocytes	5-15%	Few to moderate	Few
Large Lymphocytes	5%	Few to moderate	>50%**
Plasma cells	Few	Increased	Few

In order to determine the size of a lymphocyte, the nucleus should be compared to the size of an erythrocyte:

small lymphocyte 1-1.5 x RBC
medium lymphocyte 2-2.5 x RBC
large lymphocyte $\geq$ 3 x RBC

****Note that the % of immature (large) lymphocytes and not malignant criteria (as with other neoplasms) is important in making a diagnosis of lymphosarcoma.**