Lab Hacks for Standardized Skills Assessment

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Learning Objectives

- List the most common mistakes made by veterinary technology students when learning laboratory assays
- Create a uniform laboratory evaluation for all students
- Use rubrics to provide constructive feedback to improve student success

- Make Veterinary Pathology Technicians!
- Our Goal: Everyone learn one new thing to try with their lab

Common Challenges of New Learners

- QC - running QC over and over again without troubleshooting why it might be failing
- UA - fat droplets as RBCs, Brownian motion, too cold (crystals)
- RBC - spherocytes, echinocytes vs acanthocytes, monolayer
- WBC - basophils vs lymph/monos, staying in monolayer
- Fecal floats - too much or too little feces

Preparing the Skills Assessment

- Which duties are most important to clinics? Where are voids?
  - Heartworm, tick-borne, other PoC tests
  - Running analyzers
  - Manual differentials
  - Urinalysis with sediment exam
  - Fecal floatation
  - Quality control/troubleshooting
  - Preparing/shipping to labs

Preparing the Skills Assessment

- Standardized vs. Organic
  - Standardized assessments will give each candidate equal comparisons, but they are more difficult to administer over multiple days
  - Organic assessments may give one student an unfair advantage, are easier to prepare, and the most difficult to grade

Preparing the Skills Assessment

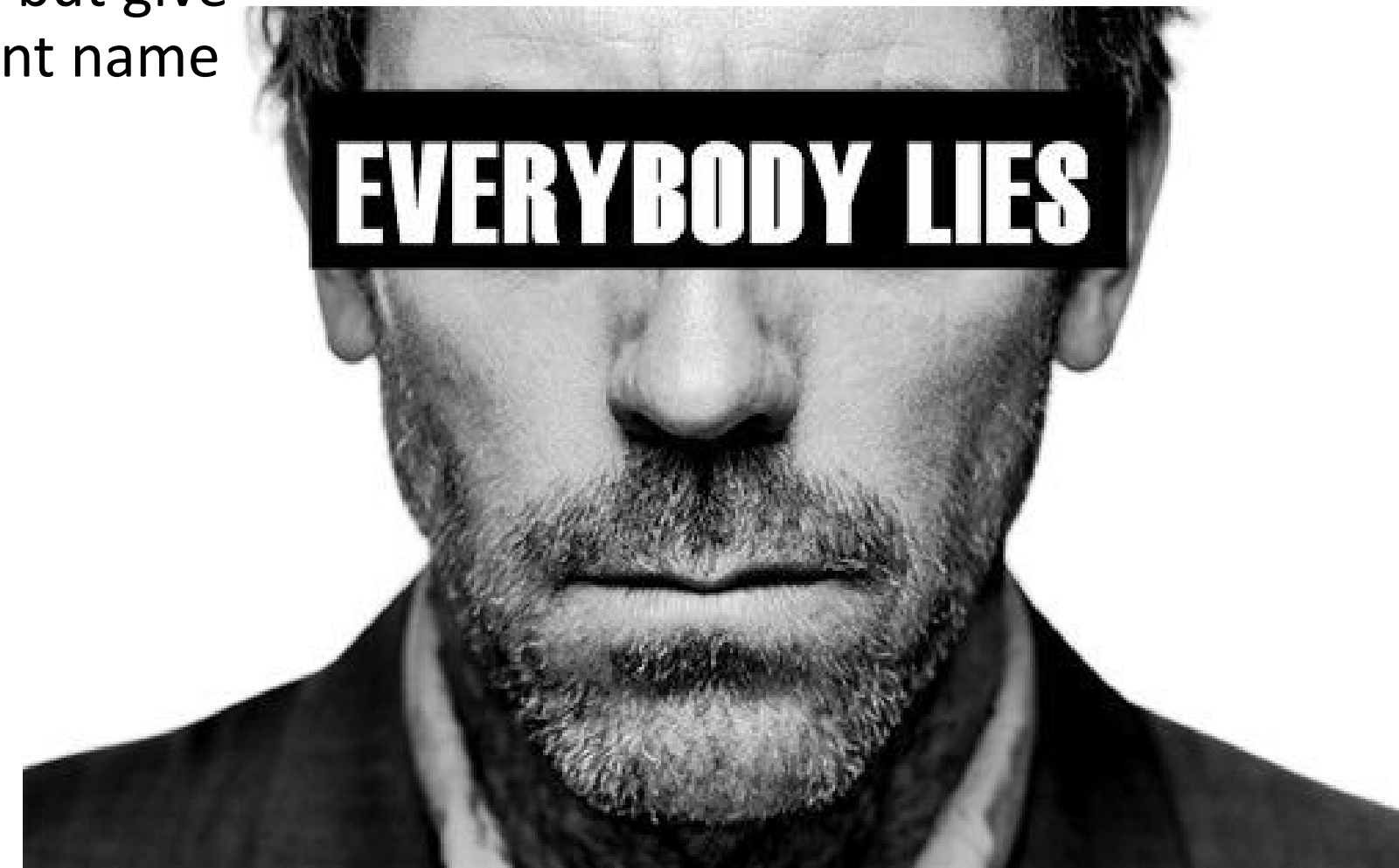
- Stability
  - EDTA-blood will become crenated
  - Parasite ova will hatch/Giardia will decompose
  - Urine crystals will dissipate

Preparing the Skills Assessment

- Abnormal vs. Within Normal Limits
  - Specimens lacking abnormal findings are just as important as positive samples
    - Negative fecal – air bubbles confused as ova
    - Plant material in fecals
    - Negative urinalysis - Brownian Motion confused as bacteria; oil droplets confused as erythrocytes
    - Negative serology
  - Easy to obtain a fresh sample

Lie

- Use the same specimen but give each container a different name or case number



Standardized Assessment

Skill: **CBC**

- Prior to Assessment: give students an SOP/flow chart
  - Complete a manual differential/manual platelet estimate/slide evaluation with every case

  Vs
  - Complete a manual differential/slide evaluation when anemia, leukopenia, leukocytosis, neutrophilia, lymphocytosis, thrombocytopenic, etc.

  - Always run a manual PCV/TS when:
    - Dogs: HCT <35% or >60% and TP is normal/low
    - Cats: HCT <30% or >60% and TP is normal/low

  - Always include a reticulocyte count/estimate when:
    - Dogs: HCT <35%
    - Cats: HCT <30%

Standardized Assessment

Skill: **CBC**

- Use fresh blood for each candidate
  - Gently centrifuge blood from a healthy patient, separate, then remix
  - Or add other patient's plasma or serum to accomplish this

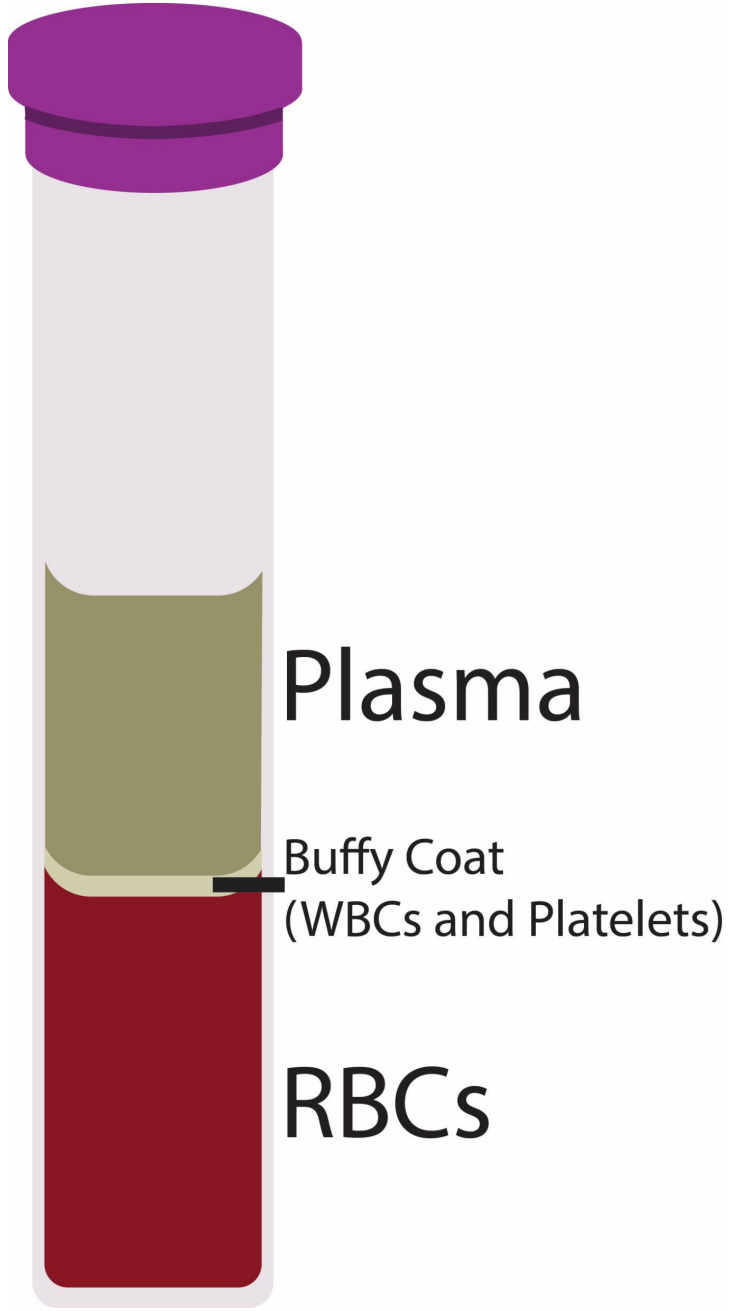
Spin, Separate, and Mix

- Overall Process

- Separate in any combination:
  - Red cells
  - Buffy coat
  - Serum

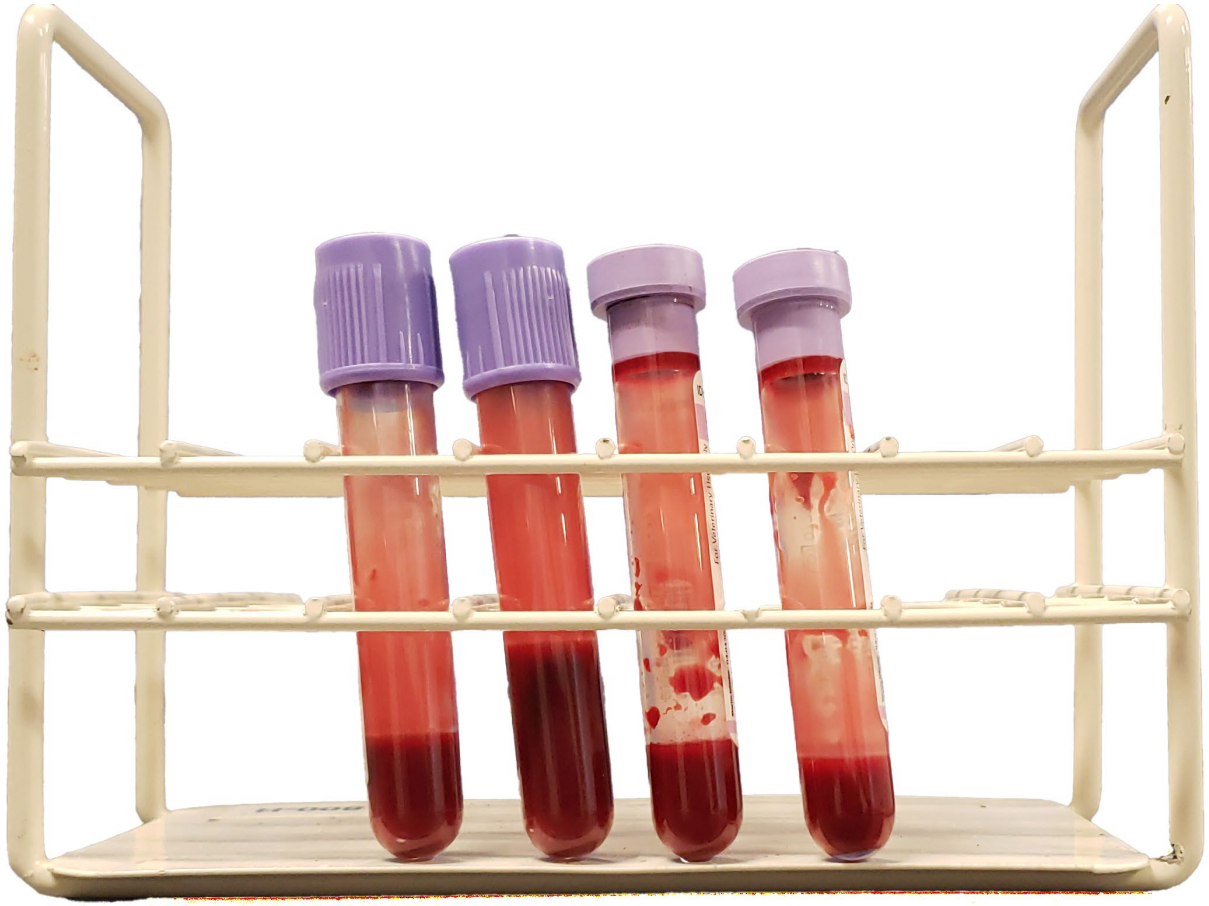
Spin, Separate, and Mix Tips

Tip #1: Remembering the components in each layer is key to separating and mixing tubes



Spin, Separate, and Mix Tips

Tip #2: Combine multiple healthy patients into a non-additive tube for centrifugation



Don’t use another EDTA-tube

- It will crenate the RBCs

Spin, Separate, and Mix Tips

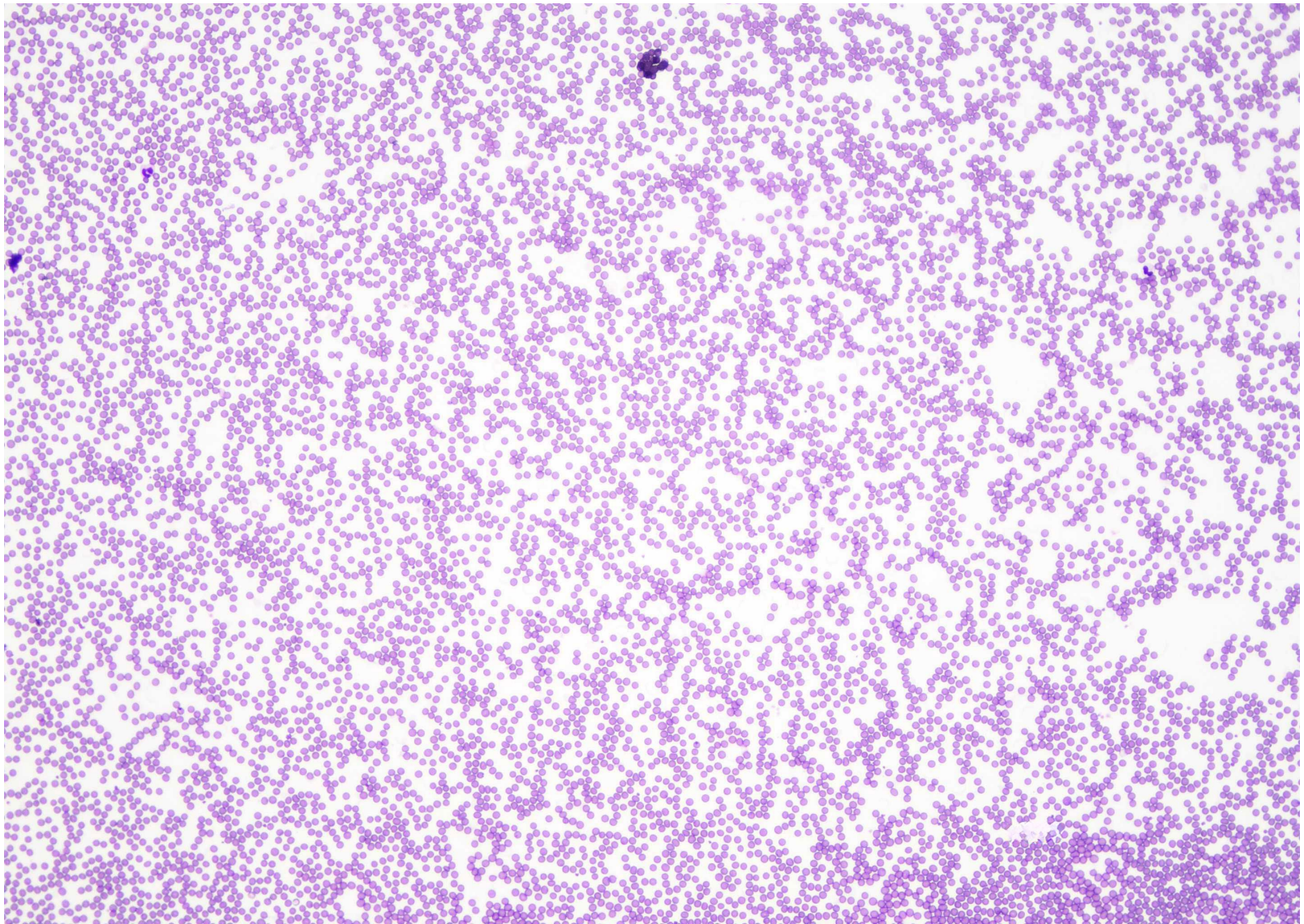
Tip #3: “Recipes” – use 2-3 tubes worth of blood and rearrange to form:

Abnormality to Mimic	Red Cells	Buffy Coat	Plasma
Anemia (Non-Regenerative)	1	2-3	2-3
Polycythemia	1.5-2	1	1
Leukopenia and Thrombocytopenia	2	0	2
Leukocytosis	1	3 or more	1
Anemia, Leukopenia, and Thrombocytopenia	1	1	2-3 (may add serum too)

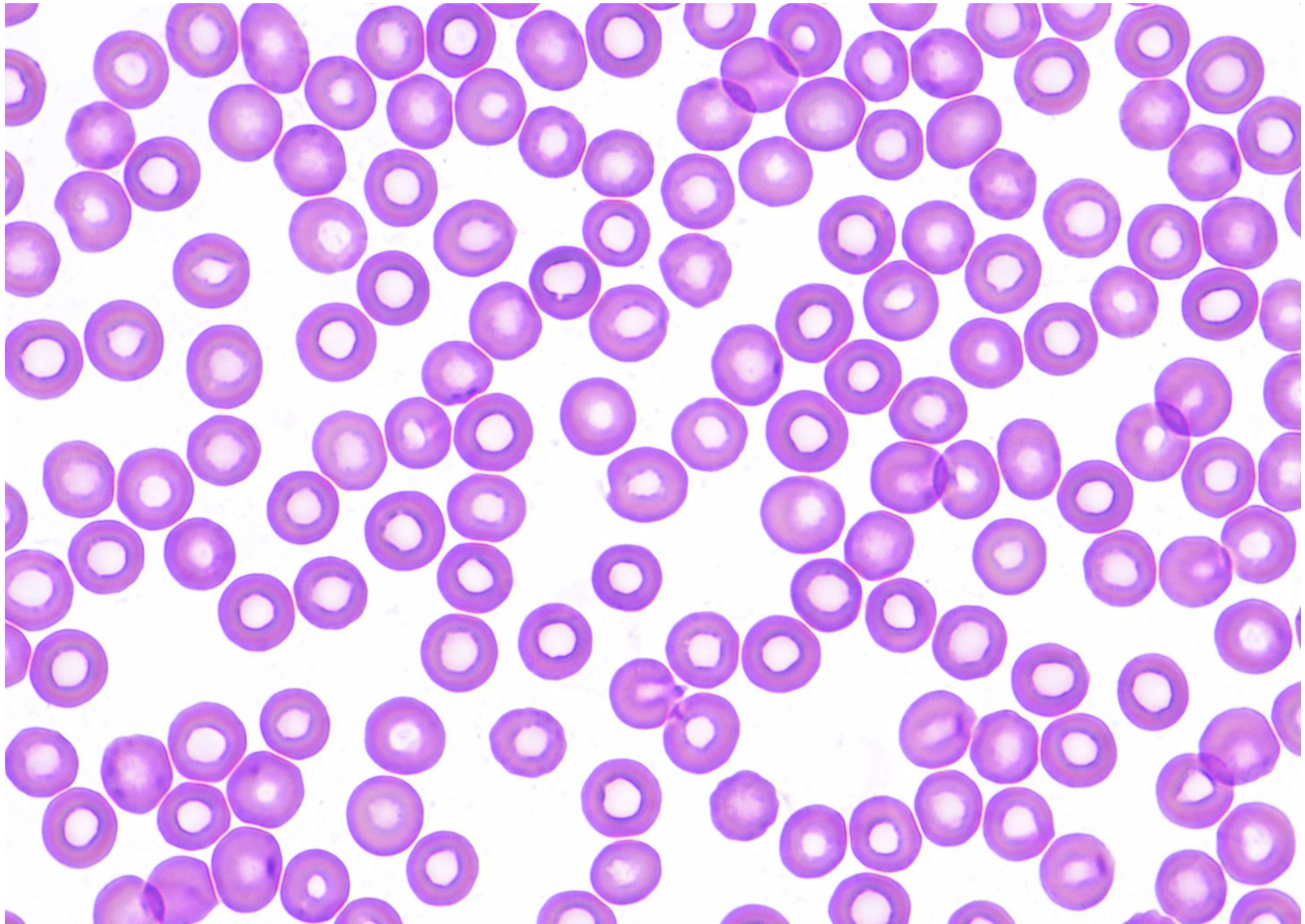
Spin, Separate, and Mix Example

- Goal: Anemia and Leukopenia (Thrombocytopenia by default)
- Materials: 2 EDTA-tubes
- Combined: 1 RBC + 1 Buffy Coat + 2 Plasma (+1 drop water)

Spin, Separate, and Mix Example



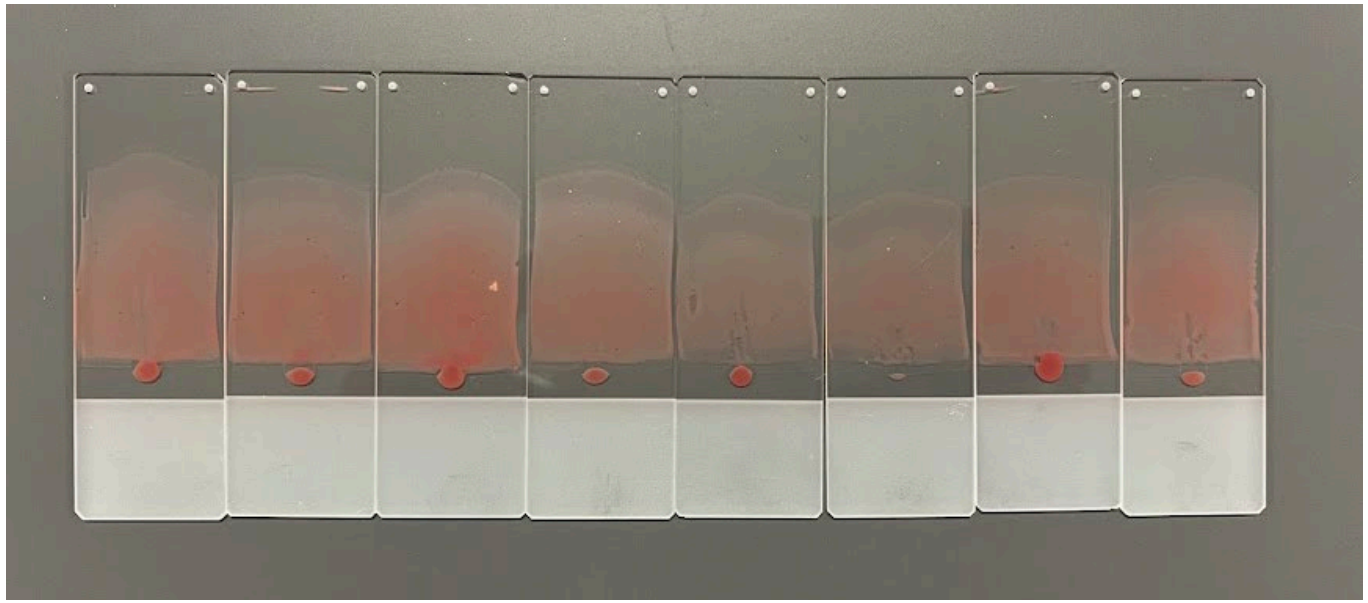
Spin, Separate, and Mix Example

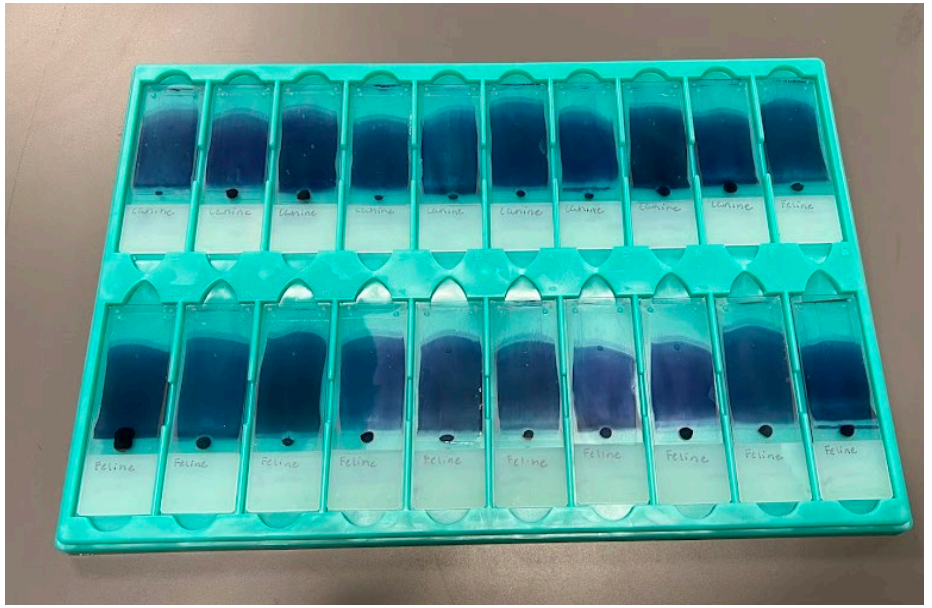


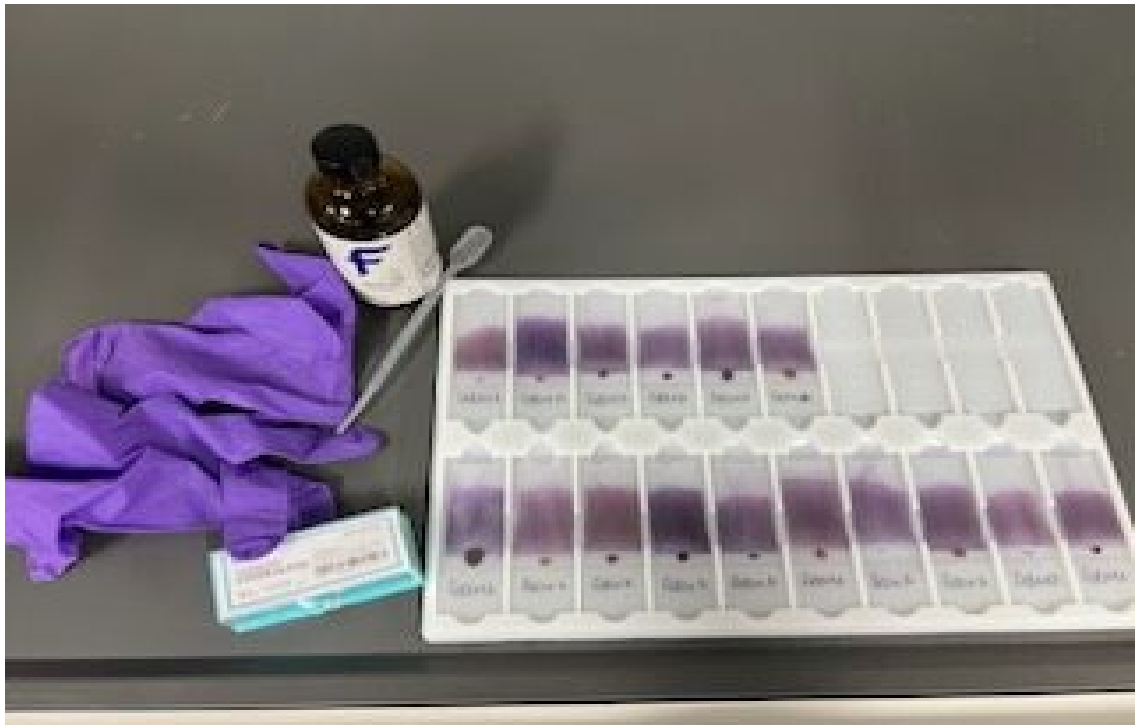
Standardized Assessment

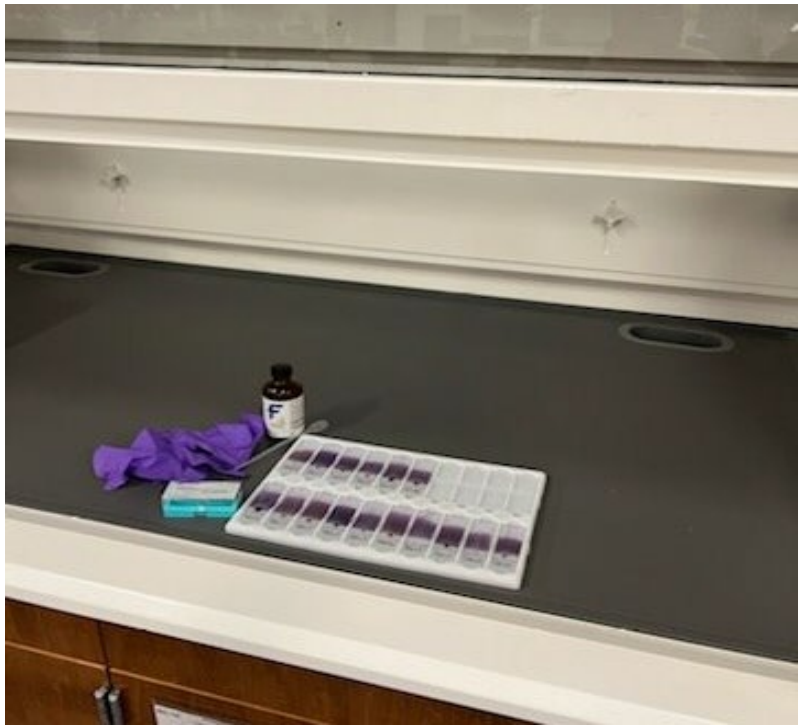
Skill: **CBC**

- Lysis buffers
- Saving unique blood films to be used for assessments
  - Don't forget the CBC!




Feline





Coverslipping Slides

Supplies and Equipment

- Chemical hood
- Slides – stained, dry, and ready to go
- Cover glass – 1.5 thickness, rectangle shape (24 x 60mm)
- Disposable gloves, lab coat
- Paper towels and Kim wipes
- Swabs with wood end
- Slide tray
- Mounting medium
- Nail polish (optional)

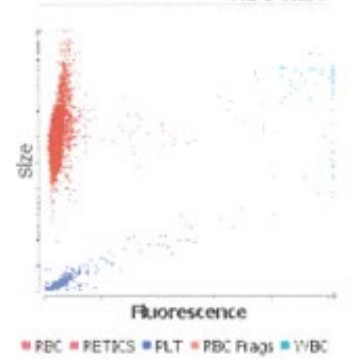
CBC Assessment

Patient Name: Bailey
Species: Canine
Breed: Mixed

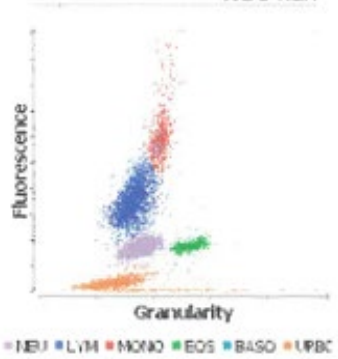
Weight:
Age: 3 Years
Doctor: Andrew Dunn

Test	Results	Reference Interval	LOW	NORMAL	HIGH
ProCyte Dx (April 4, 2024 9:44 AM)					
RBC	6.86 M/µL	5.65 - 8.87			
HCT	44.5 %	37.3 - 61.7			
HGB	16.6 g/dL	13.1 - 20.5			
MCV	64.9 fL	61.6 - 73.5			
MCH	24.2 pg	21.2 - 25.9			
MCHC	37.3 g/dL	32.0 - 37.9			
RDW	18.4 %	13.6 - 21.7			
%RETIC	0.3 %				
RETIC	22.6 K/µL	10.0 - 110.0			
RETIC-HGB	27.3 pg	22.3 - 29.6			
WBC	11.17 K/µL	5.05 - 16.76			
%NEU	61.1 %				
%LYM	28.3 %				
%MONO	4.8 %				
%EOS	5.7 %				
%BASO	0.1 %				
NEU	6.82 K/µL	2.95 - 11.64			
LYM	3.16 K/µL	1.05 - 5.10			
MONO	0.54 K/µL	0.16 - 1.12			
EOS	0.64 K/µL	0.06 - 1.23			
BASO	0.01 K/µL	0.00 - 0.10			
PLT	88 K/µL	148 - 484	LOW		
MPV	14.5 fL	8.7 - 13.2	HIGH		
PDW	15.1 fL	9.1 - 18.4			
PCT	0.13 %	0.14 - 0.46	LOW		

RBC Run



WBC Run



Clinical Pathology I Laboratory Skills Assessment

Perform microscopic exam of blood film: perform leukocyte differential–normal vs abnormal, evaluate erythrocyte morphology–normal vs abnormal*, calculate absolute values**

*Perform CBC to include: estimate platelet numbers**

Student: _____ Date: _____

Leukocyte differential: _____/12 pt
Did you perform an accurate count of WBCs?
Were cells identified correctly?

Absolute value calculations: _____/6 pt
Did you correctly calculate all required values?
Did you use the correct units?

Erythrocyte morphology: _____/9 pt
Were you able to identify variations in cell size?
Were you able to identify variations in cell color?
Did you correctly identify variations in cell shape?

Platelet estimation: _____/10 pt
Did you properly identify a platelet?
Were you aware of how to perform a platelet estimate?
Did you accurately calculate the estimated value?

Results submission: _____/5 pt
Did you accurately and completely fill out the Blood Film Evaluation form?

Microscope use: _____/8 pt
Did you use the microscope properly?
Was the microscope cleaned and stored correctly?

Final grade: _____/50 pt

Skills sign-off:

WBC Differential	Yes	Not yet
Erythrocyte morph.	Yes	Not yet
Calculations	Yes	Not yet
Platelet est.	Yes	Not yet

CBC Assessment Results

Collin College
VTHT 2323 – Veterinary Clinical Pathology I

Blood Film Evaluation Form

Student name: _____ Date & time of collection: _____

Patient identification: _____ Species: _____

Breed: _____ Age: _____ Sex: _____

Date & time of results: 4/10/24 2:54 pm

CELL TYPE	RELATIVE COUNT	ABSOLUTE COUNT	LOW/NORMAL/HIGH
Total Leukocyte Count	100 %	WBC=11.17 K/μL	Normal
Segmented Neutrophils	82 %	9,159 /μL	High
Band Neutrophils	0 %	0	Normal
Lymphocytes	11 %	1,228 /μL	Low
Eosinophils	0 %	0	Low
Monocytes	7 %	781 /μL	Normal
Basophils	0 %	0	Normal

Avg. # of platelets = 12 Platelet Estimation = 252,000 K/μL (low/normal/high)

Leukocyte morphology observations: No abnormal findings

Erythrocyte morphology observations: No abnormal findings

Other findings: No abnormal findings

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VTHT 2323 – Veterinary Clinical Pathology I

Blood Film Evaluation Form

Student name: _____ Date & time of collection: 4/10/2024 2:00pm

Patient identification: Poogie Species: Canine

Breed: Poodle Mix Age: 14y Sex: F/S

Date & time of results: 4/10/2024

CELL TYPE	RELATIVE COUNT	ABSOLUTE COUNT	LOW/NORMAL/HIGH
Total Leukocyte Count	100 %	11.17 K/μL	normal
Segmented Neutrophils	61 %	6.81 K/μL	normal
Band Neutrophils	0 %	0.0 K/μL	normal
Lymphocytes	27 %	3.01 K/μL	normal
Eosinophils	6 %	0.67 K/μL	normal
Monocytes	4 %	0.44 K/μL	normal
Basophils	2 %	0.22 K/μL	High

Avg. # of platelets = 17 Platelet Estimation = 340,000 /μL (low/normal/high)

Leukocyte morphology observations: All leukocyte morphology looked normal. No signs of toxicity, parasites, or activation. Some leukocytes appeared damaged/squished

Erythrocyte morphology observations: Few polychromatophilic erythrocytes

Other findings: Few Microplatelets

#	n=100
0	0-4
1	0-6
2	0-8
3	0-9
4	1-10
5	1-12
6	2-13
7	2-14
8	3-16
9	4-17
10	4-18
15	8-24
20	12-30
25	16-35
30	21-40
35	25-46
40	30-51
45	35-56
50	39-61
55	44-65
60	49-70
65	54-75
70	60-79
75	65-84
80	70-88
85	76-92
90	82-96
91	83-96
92	84-97
93	86-98
94	87-98
95	88-99
96	90-99
97	91-100
98	92-100
99	94-100
100	96-100

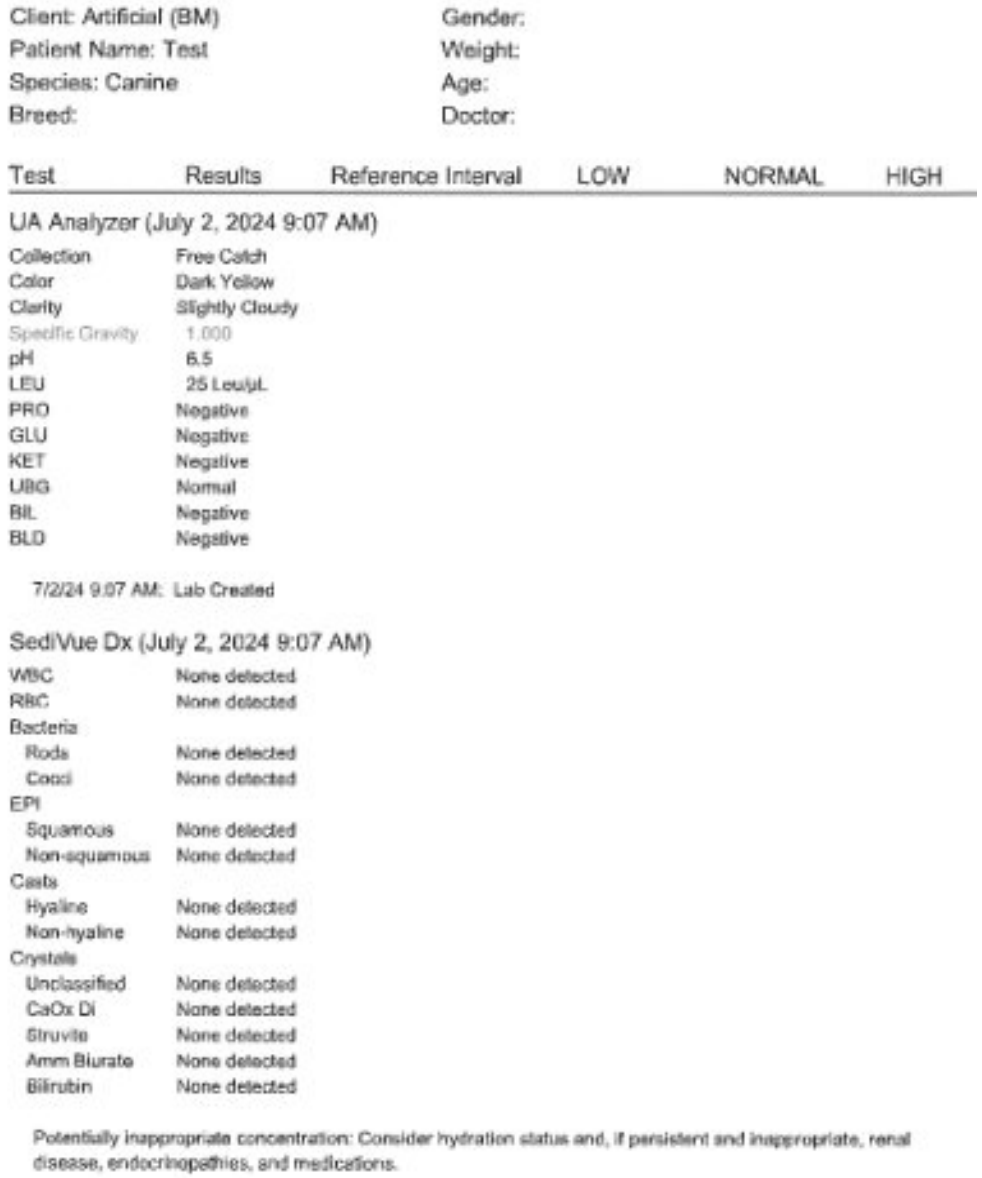
Standardized Assessment

Skill: **Urinalysis**

- Free-catch from an employee dog or shelter animals
- Artificial urine (also great for events with children)
- Manipulate the urine with:
  - Glucose
  - Distilled water
  - Feces
  - Blood components
  - Vinegar
  - Bleach

Artificial Urine Limitations

- Not for sediment analysis
- Ketones



Client: Artificial (BM)
Patient Name: Test
Species: Canine
Breed:

Gender:
Weight:
Age:
Doctor:

Test	Results	Reference Interval	LOW	NORMAL	HIGH

UA Analyzer (July 2, 2024 9:07 AM)

Collection	Free Catch
Color	Dark Yellow
Clarity	Slightly Cloudy
Specific Gravity	1.000
pH	6.5
LEU	25 Leu/µL
PRO	Negative
GLU	Negative
KET	Negative
UBG	Normal
BIL	Negative
BLD	Negative

7/2/24 9:07 AM: Lab Created

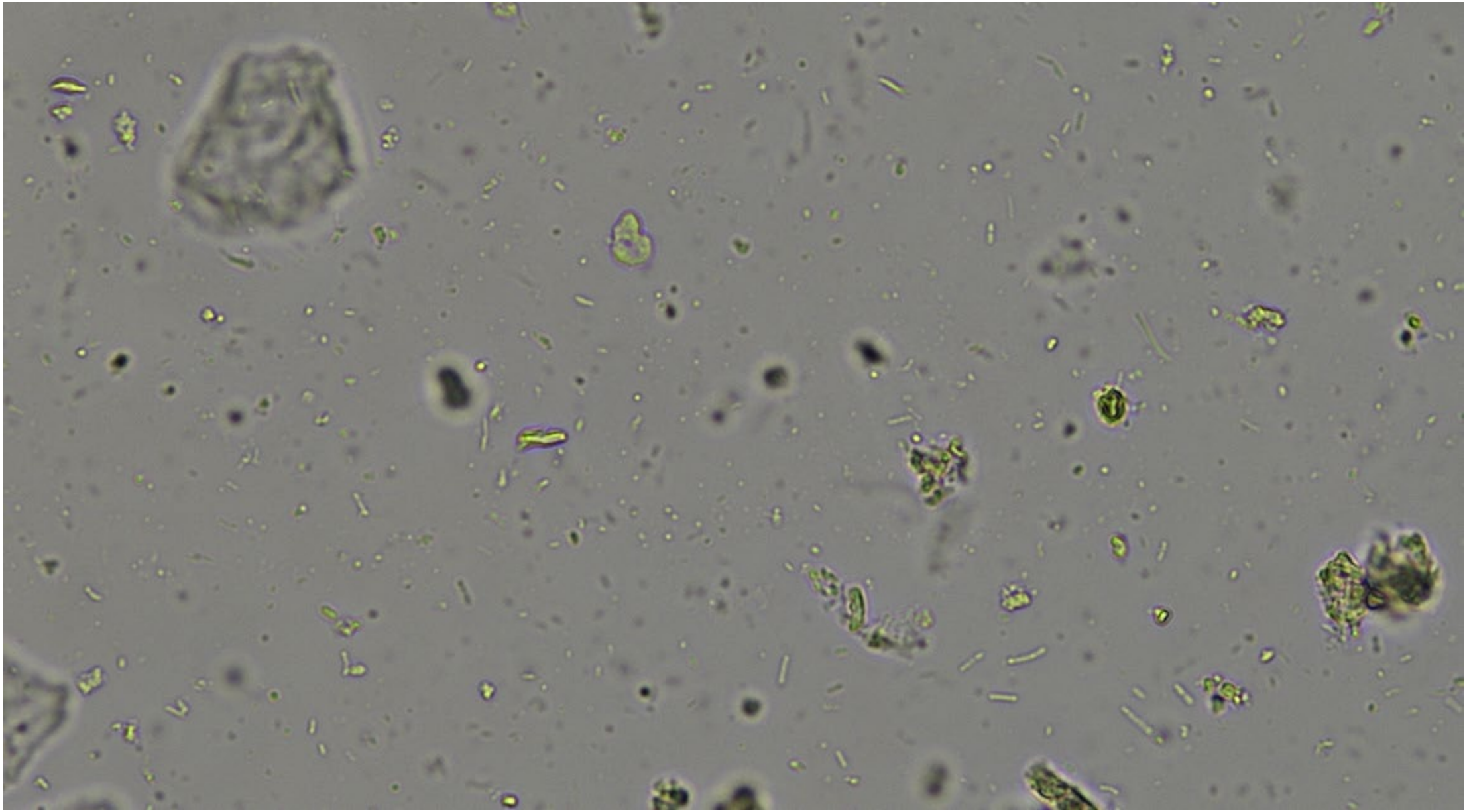
SediVue Dx (July 2, 2024 9:07 AM)

WBC	None detected
RBC	None detected
Bacteria	
Rods	None detected
Cocci	None detected
EPI	
Squamous	None detected
Non-squamous	None detected
Casts	
Hyaline	None detected
Non-hyaline	None detected
Crystals	
Unclassified	None detected
CaOx Di	None detected
Struvite	None detected
Amm Biurate	None detected
Bilirubin	None detected

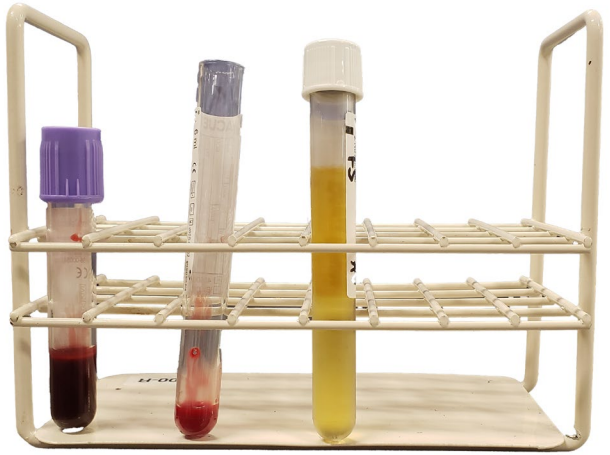
Potentially inappropriate concentration: Consider hydration status and, if persistent and inappropriate, renal disease, endocrinopathies, and medications.

Urinalysis Recipes

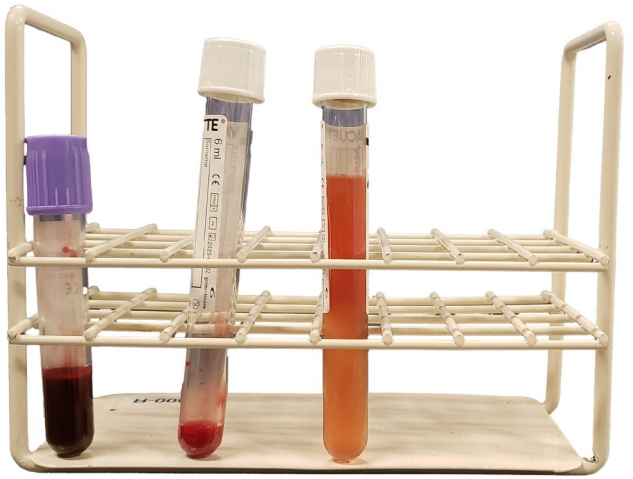
Abnormality to Mimic	Additive
Proteinuria	Serum or plasma
Acidic Urine	Vinegar
Alkaline Urine	Bleach
Glucosuria	15% glucose solution
Isosthenuria/Hyposthenuria	Distilled water or artificial urine
Bacteria present	Fecal material (VERY small amount!)
Hematuria/Pyuria	Cells from spun EDTA-tube



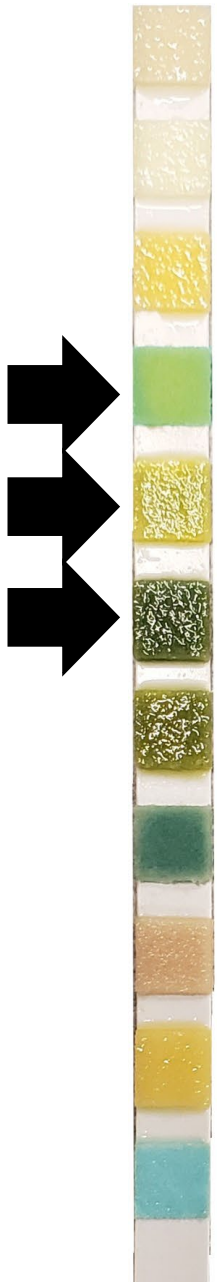
Urine Mix Example

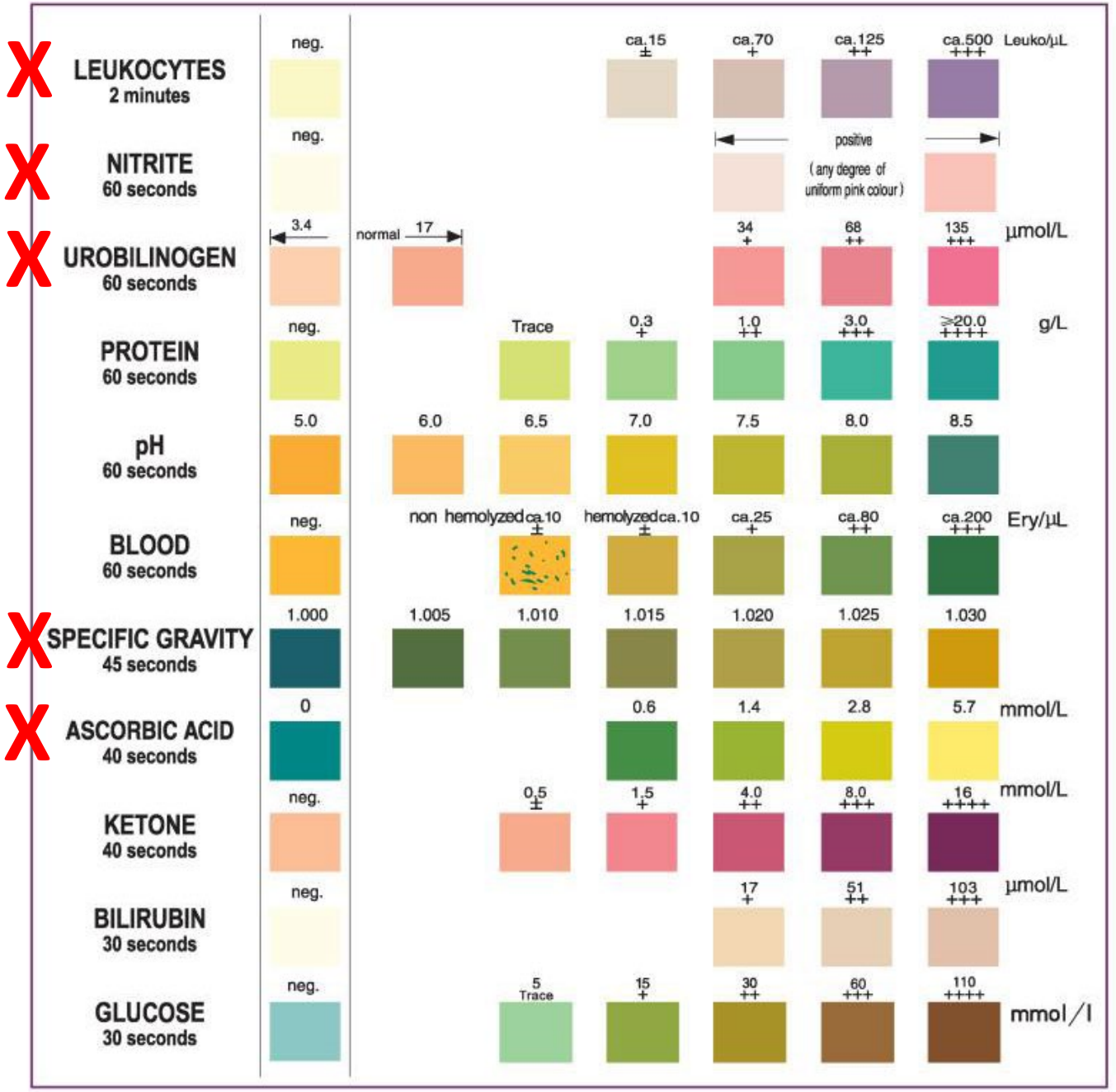


1 Tiny
Drop BC

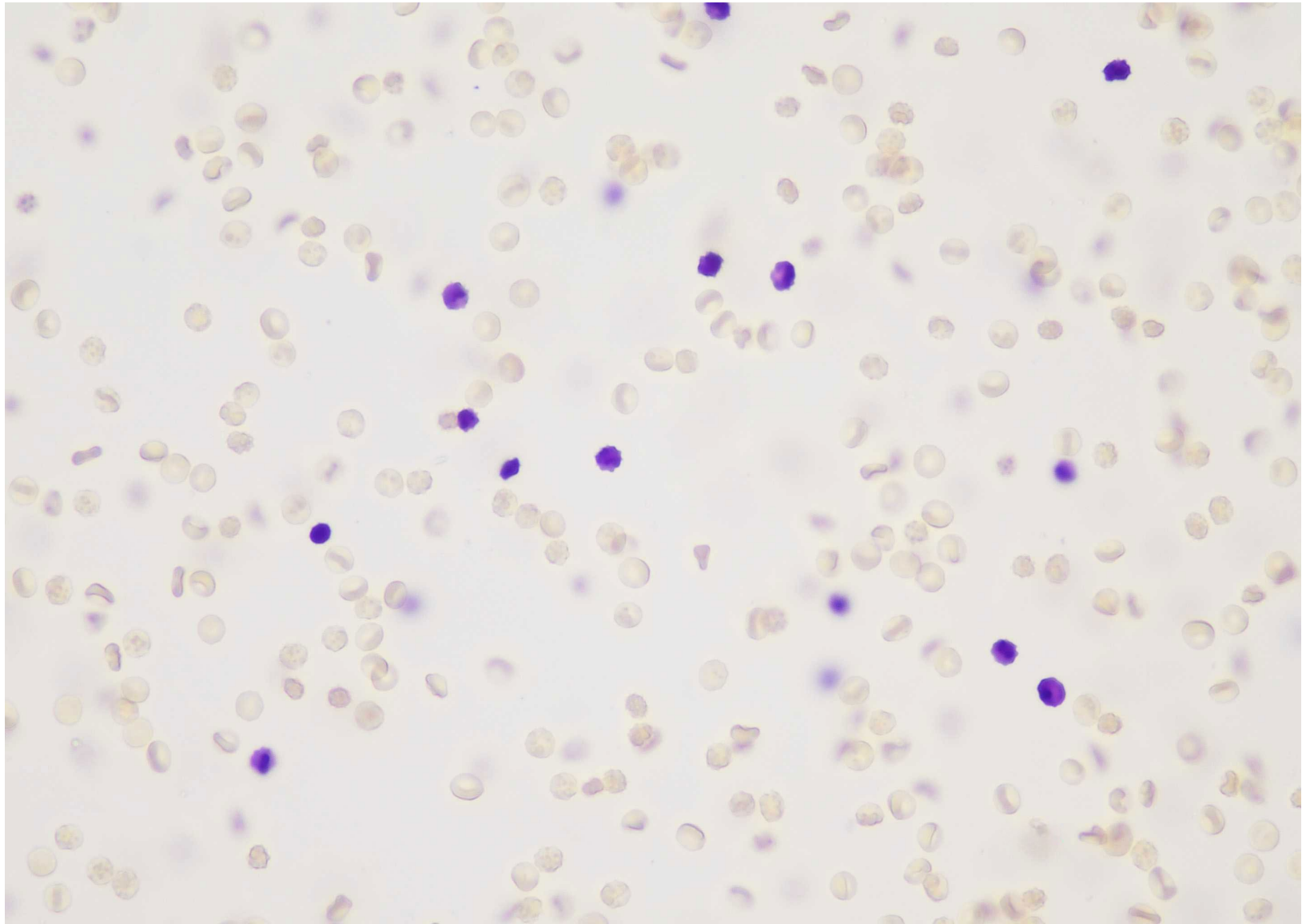


Urine Mix Example

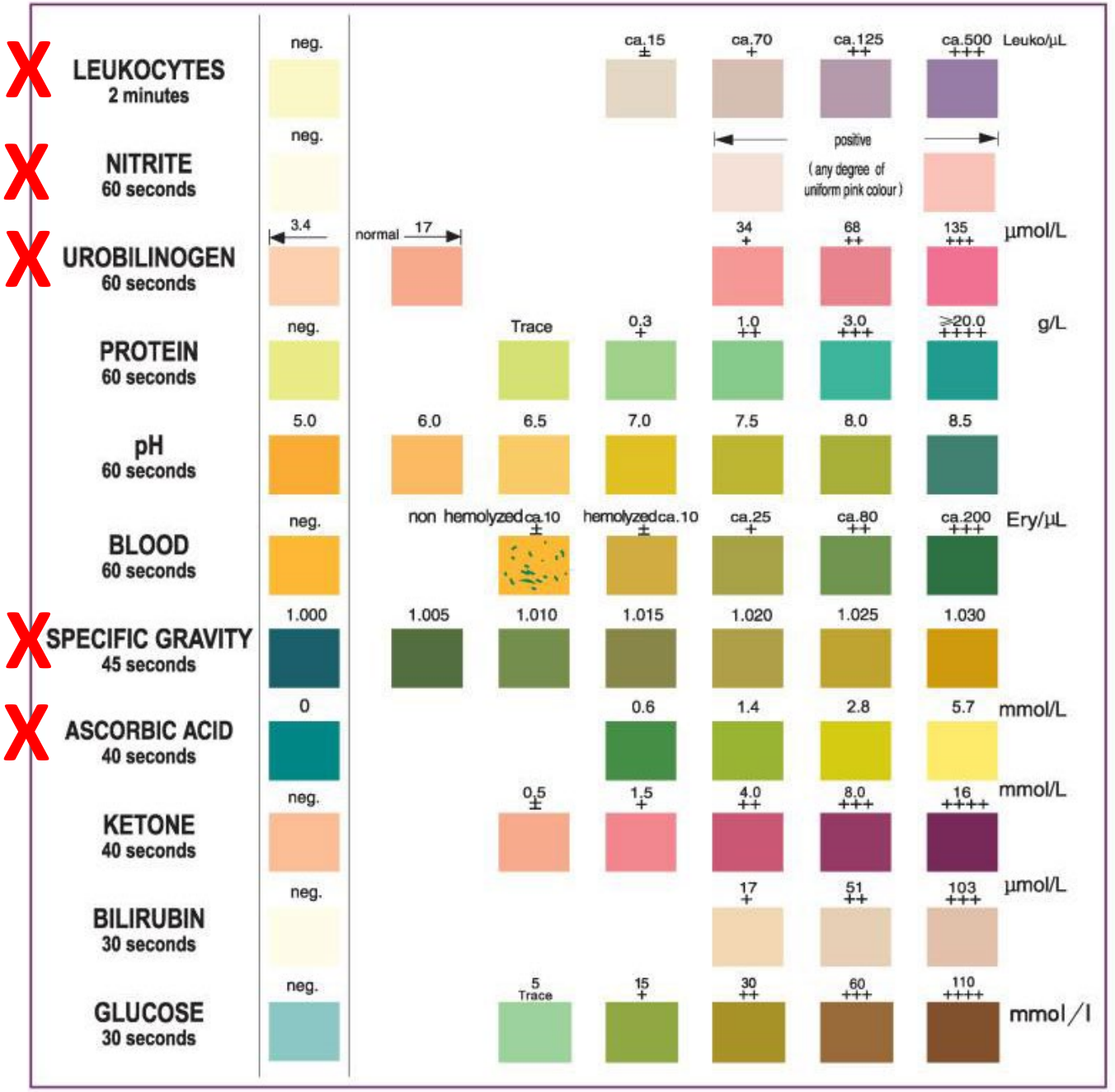




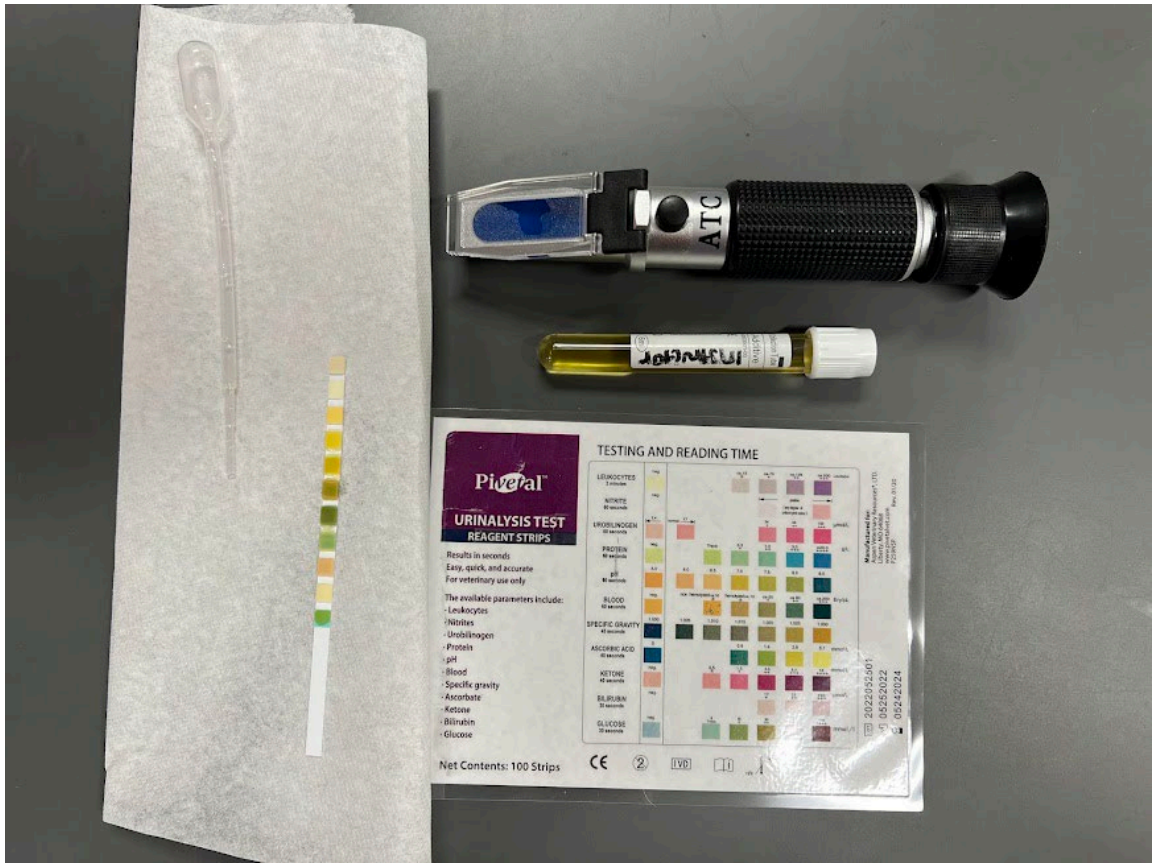
Urine Mix Example




Plasma
Bleach
Glucose (2gtt)
Glucose (4gtt)


LEUKOCYTES
2 minutes
neg.
ca.15 ±
ca.70 +
ca.125 ++
ca.500 +++
Leuko/µL
NITRITE
60 seconds
neg.
positive
(any degree of uniform pink colour)
UROBILINOGEN
60 seconds
3.4
normal
17
34 +
68 ++
135 +++
µmol/L
PROTEIN
60 seconds
neg.
Trace
0.3 +
1.0 ++
3.0 +++
≥20.0 ++++
g/L
pH
60 seconds
5.0
6.0
6.5
7.0
7.5
8.0
8.5
BLOOD
60 seconds
neg.
non hemolyzed
ca.10 ±
hemolyzed
ca.10 ±
ca.25 +
ca.80 ++
ca.200 +++
Ery/µL
SPECIFIC GRAVITY
45 seconds
1.000
1.005
1.010
1.015
1.020
1.025
1.030
ASCORBIC ACID
40 seconds
0
0.6
1.4
2.8
5.7
mmol/L
KETONE
40 seconds
neg.
0.5 ±
1.5 +
4.0 ++
8.0 +++
16 ++++
mmol/L
BILIRUBIN
30 seconds
neg.
17 +
51 ++
103 +++
µmol/L
GLUCOSE
30 seconds
neg.
5 Trace
15 +
30 ++
60 +++
110 ++++
mmol/l


Urine, Artificial
with Ketone


Piveral
URINALYSIS TEST
REAGENT STRIPS
TESTING AND READING TIME
Net Contents: 100 Strips

UA Assessment Results

Collin College
VTHT 2331 – Clinical Pathology II

Urinalysis Results Form

Student name: _____ Date & time of collection: 7/3/2024 12:00pm

Patient identification: "Molly" Species: Canine

Breed: Border Collie Age: 10yr Sex: Female Intact

Method of urine collection: Cysto centesis Volume of urine collected: 6mL

Storage method: Room Temp OK Date & time of results: 7/3/2024 12:35 pm

Gross analysis of urine: light yellow color, clear, Very Mild ammonia odor ✓

Specific gravity by refractometer: 1.008 ✓

Biochemical analysis:

Leukocytes (WBC/µL)	Neg
Nitrite	Neg
Urobilinogen (µmol/L)	Neg
Protein (g/dL)	Negs
pH	7.5 ✓
Blood (RBC/µL)	Ca. 25 ery/µL ✓
Specific Gravity	1.010 ✓
Ascorbic Acid (mmol/L)	1.4 mmol/L
Ketone (mmol/L)	Neg
Bilirubin (µmol/L)	Neg
Glucose (mmol/L)	15 mmol/L ✓

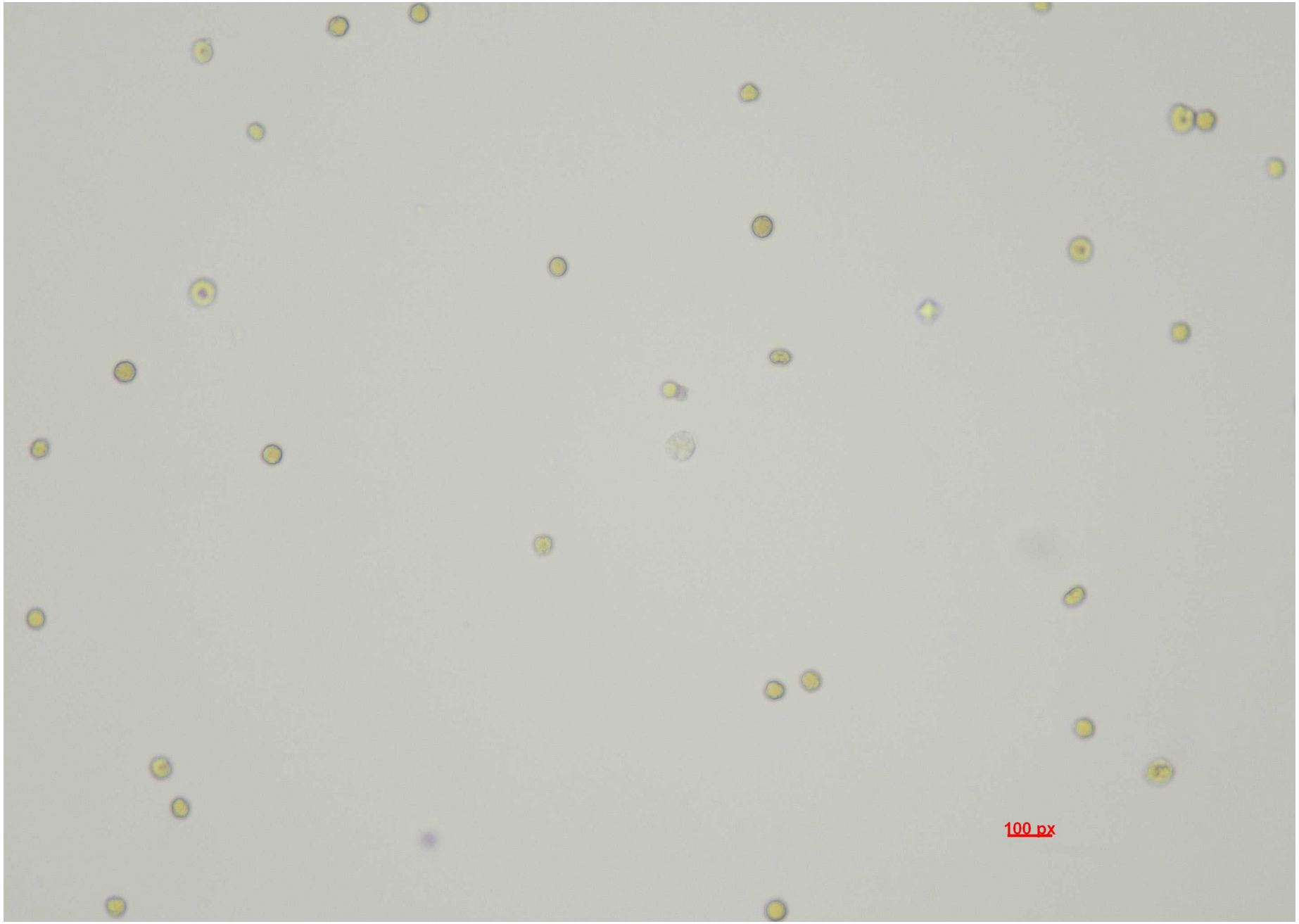
Urine sediment analysis (describe and quantify all observations):

Sample volume: 6mL 6.5mL?

Findings: Moderate Red Blood cells found per HPF

One Calcium oxalate Dihydrate crystal found on HPF

One squamous epithelial cell found per HPF


100 px

Standardized Assessment

Skill: **Serology**

- Save your expired kits: HW, 4Dx, blood-typing/cross-matching, etc.
- Save your positive cases
- EDTA-blood will generally keep for 2-weeks under refrigerated conditions
- Separate serum or plasma for longer storage for use in most tests
  - Aliquot into smaller tubes so that they only freeze-thaw once
  - Mix into fresh blood to make a positive test

How to Aliquot Serum or Plasma

Freeze: Indefinitely
Thaw: Once and use

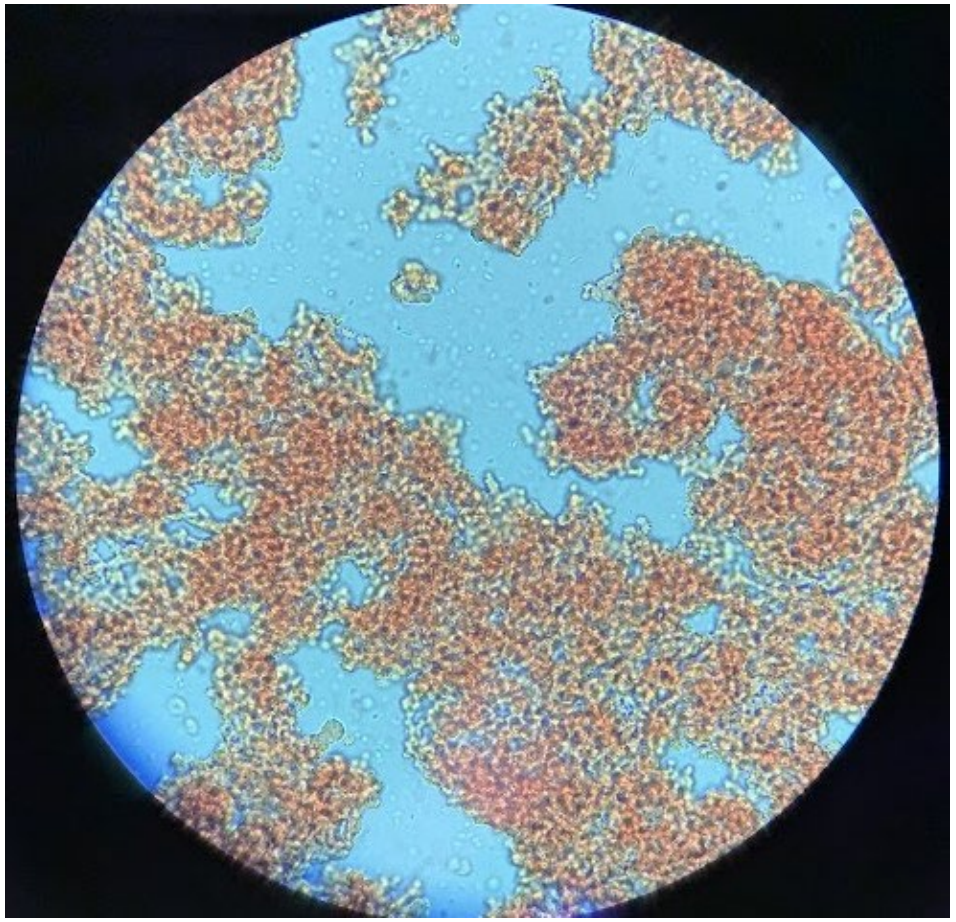
How to Mix Serum with EDTA-Blood

- One aliquot of defrosted serum can be used in multiple EDTA-tubes depending on quantity of antigen or antibody

Making Cross-Matching Chaos

Plasma from EDTA-blood

EDTA-whole blood

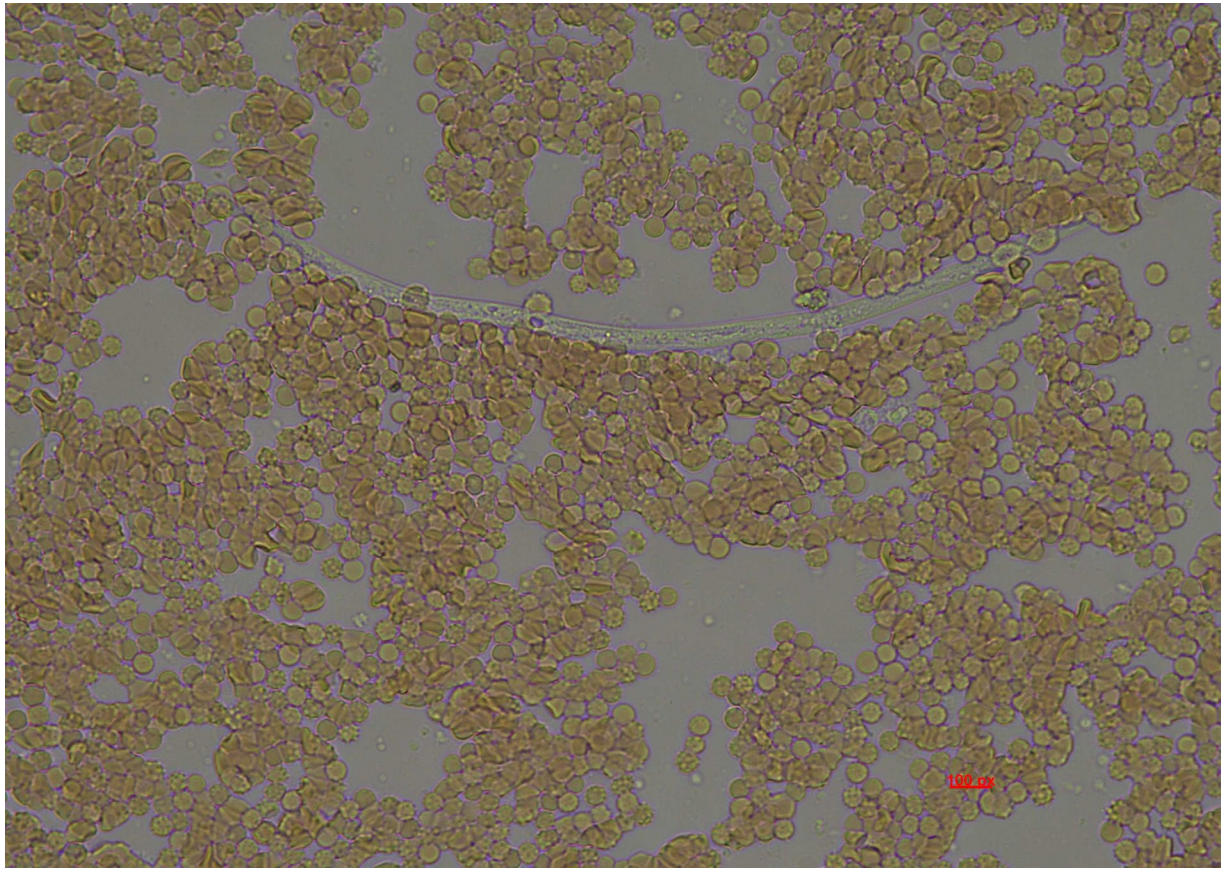


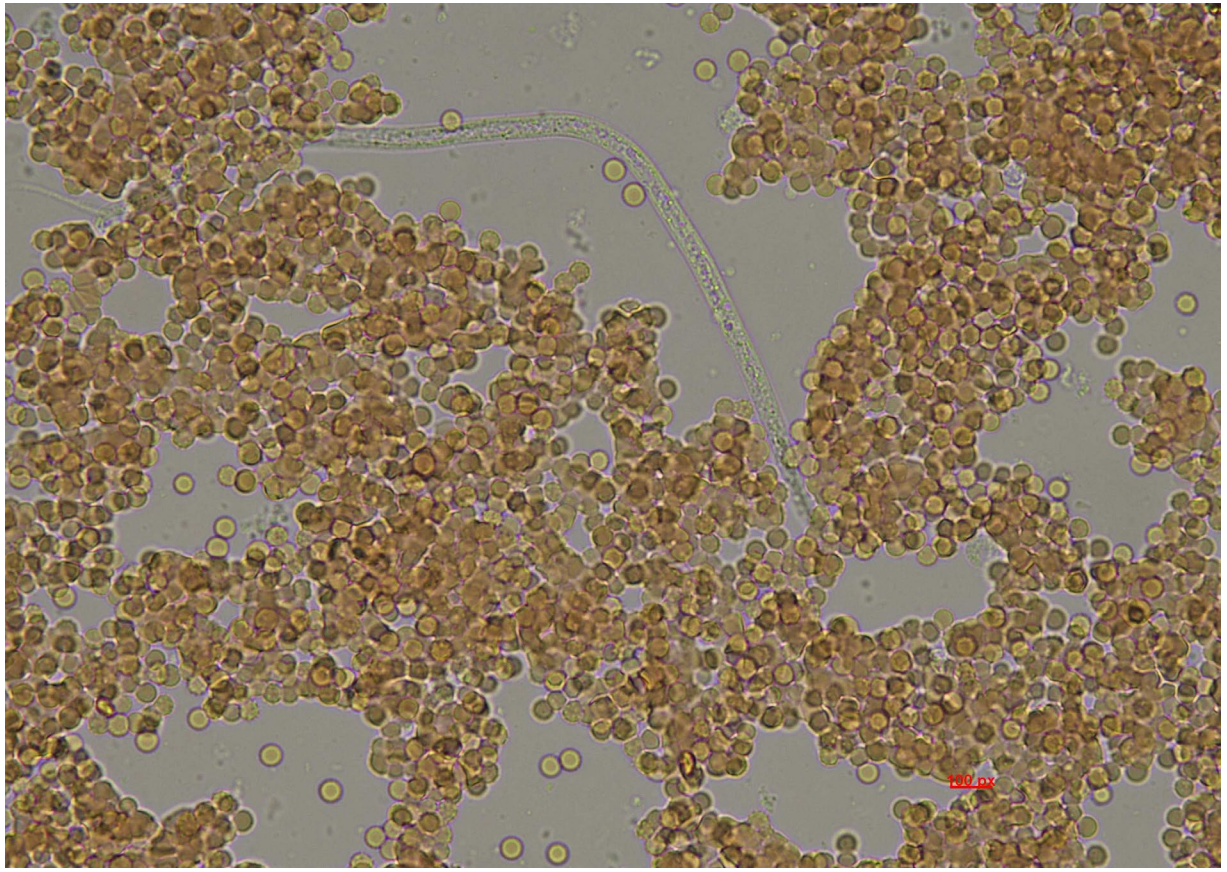
Heartworm Testing

- Experiment to keep microfilaria alive
- Room temperature vs. refrigerated
- Unfed vs. fed every six days (2 drops fresh EDTA blood)

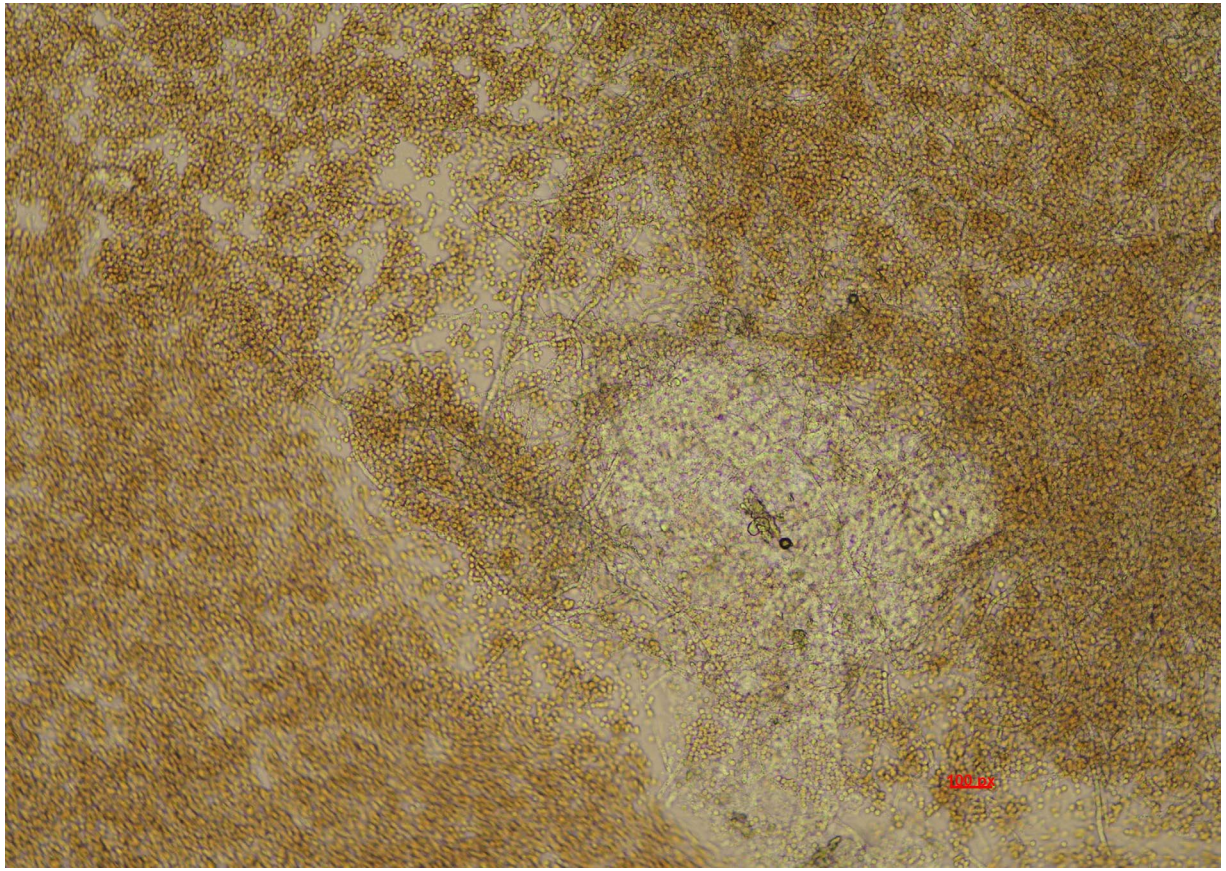
Survival Time	Room Temp	Refrigerated
Unfed	Day 14	Day 28
Fed	Day 21	Day 28

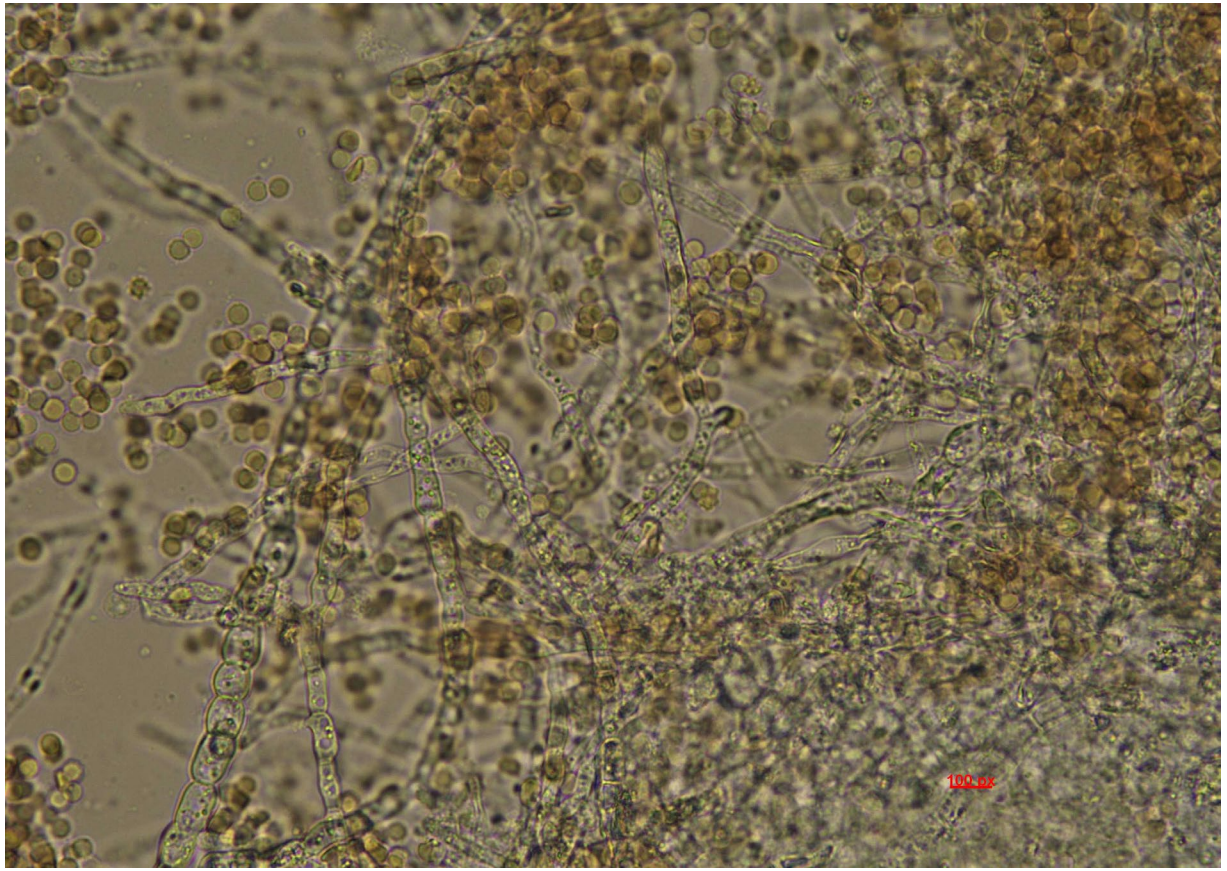
Three weeks after collection!





Room Temp Specimens





Standardized Assessment

Skill: **Fecal Analysis**

- Can be difficult to standardize:
  - Ova hatch
  - Lack of uniformity between areas of the feces

- Organic assessments:
  - Review a slide before the assessment and review the slide made by the applicant
    - But slides dry out and form crystals...

Standardized Assessment

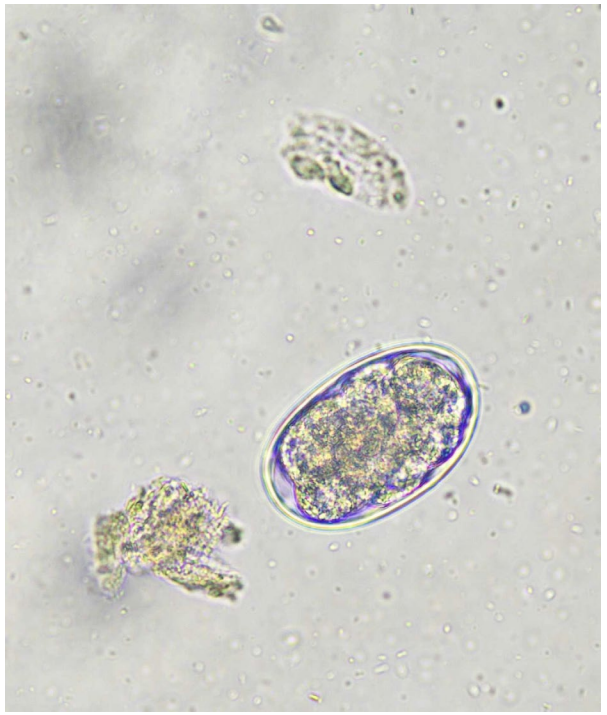
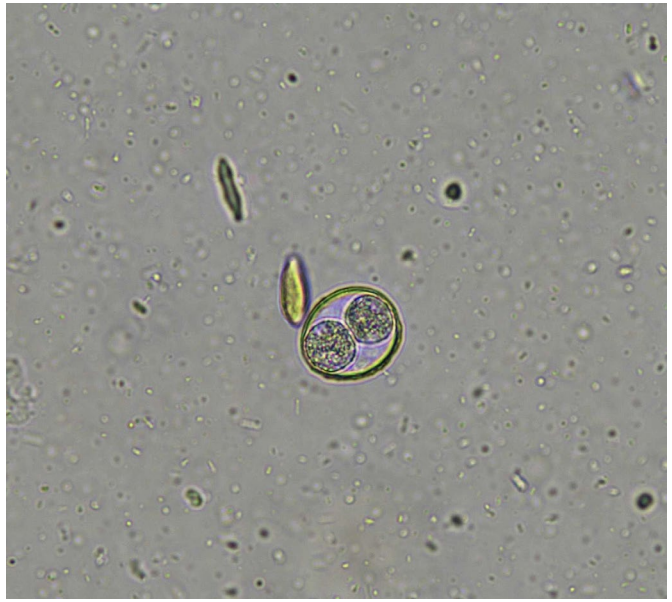
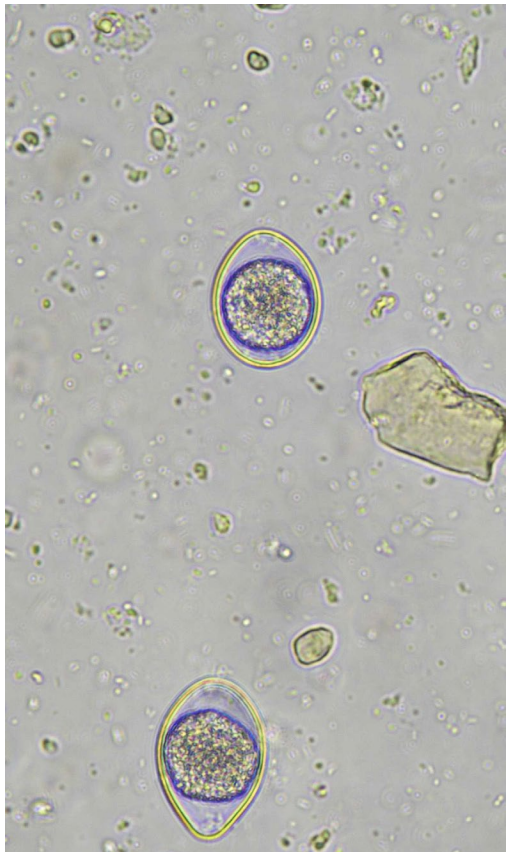
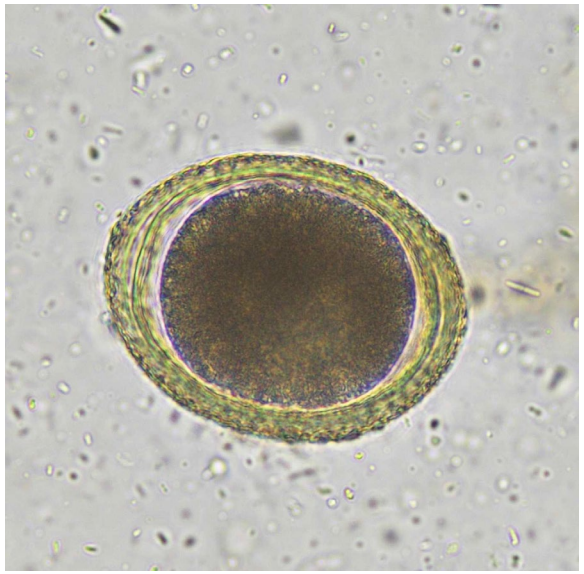
Skill: **Fecal Analysis**

- Collect samples from local shelters and identify positive samples
  - Can combine multiple samples into poop soup

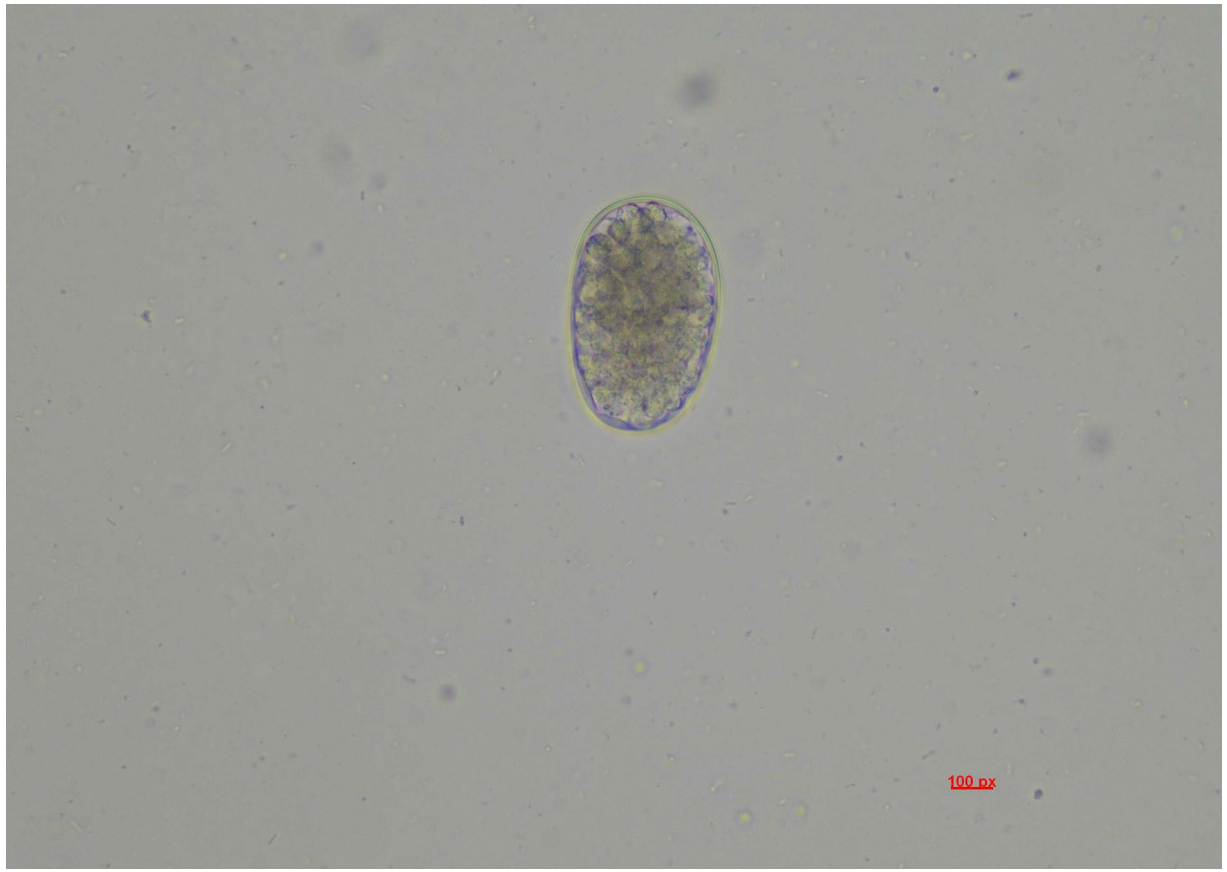
- It’s possible to preserve feces with 10% formalin
  https://www.cdc.gov/dpdx/diagnosticprocedures/stool/specimencoll.html

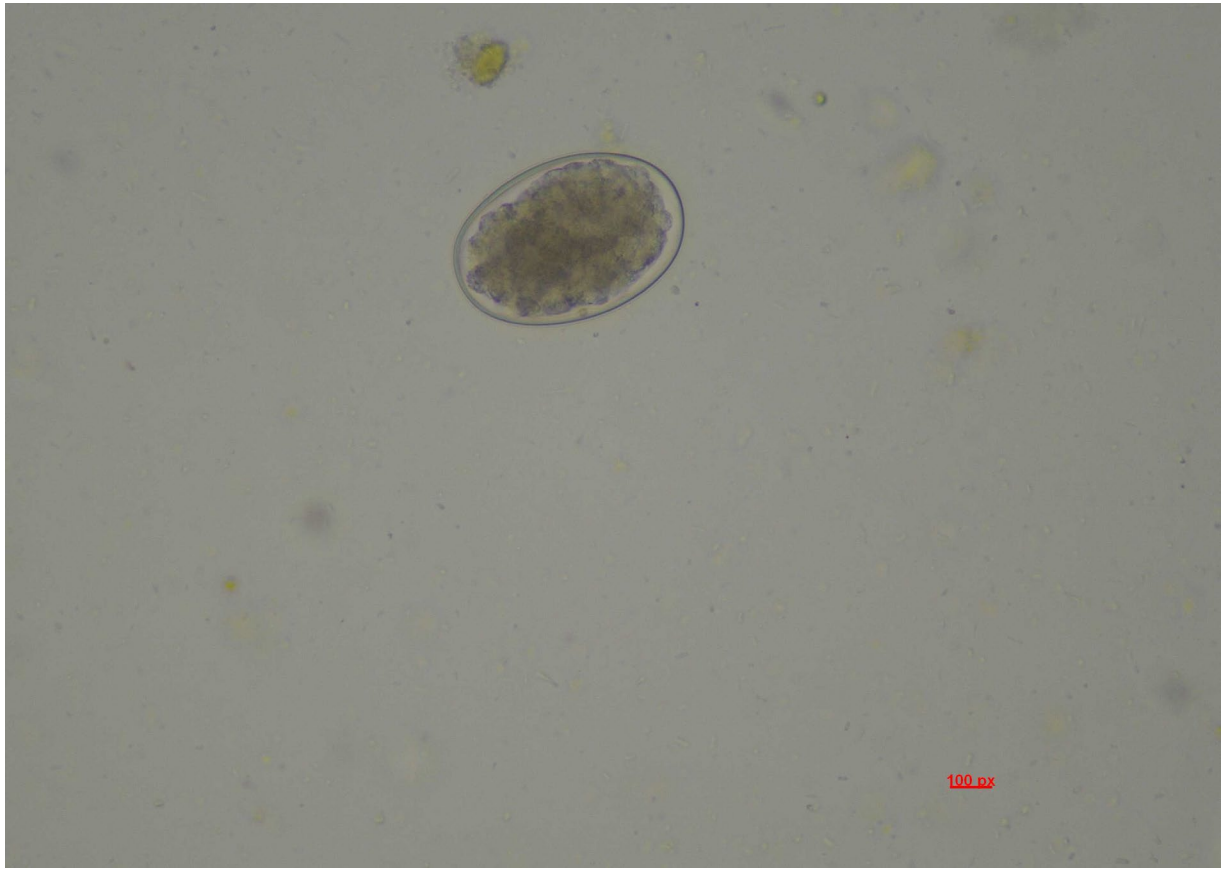
Parasite ID

- Experiment to see how long we can still identify ova/oocysts
- Day 0

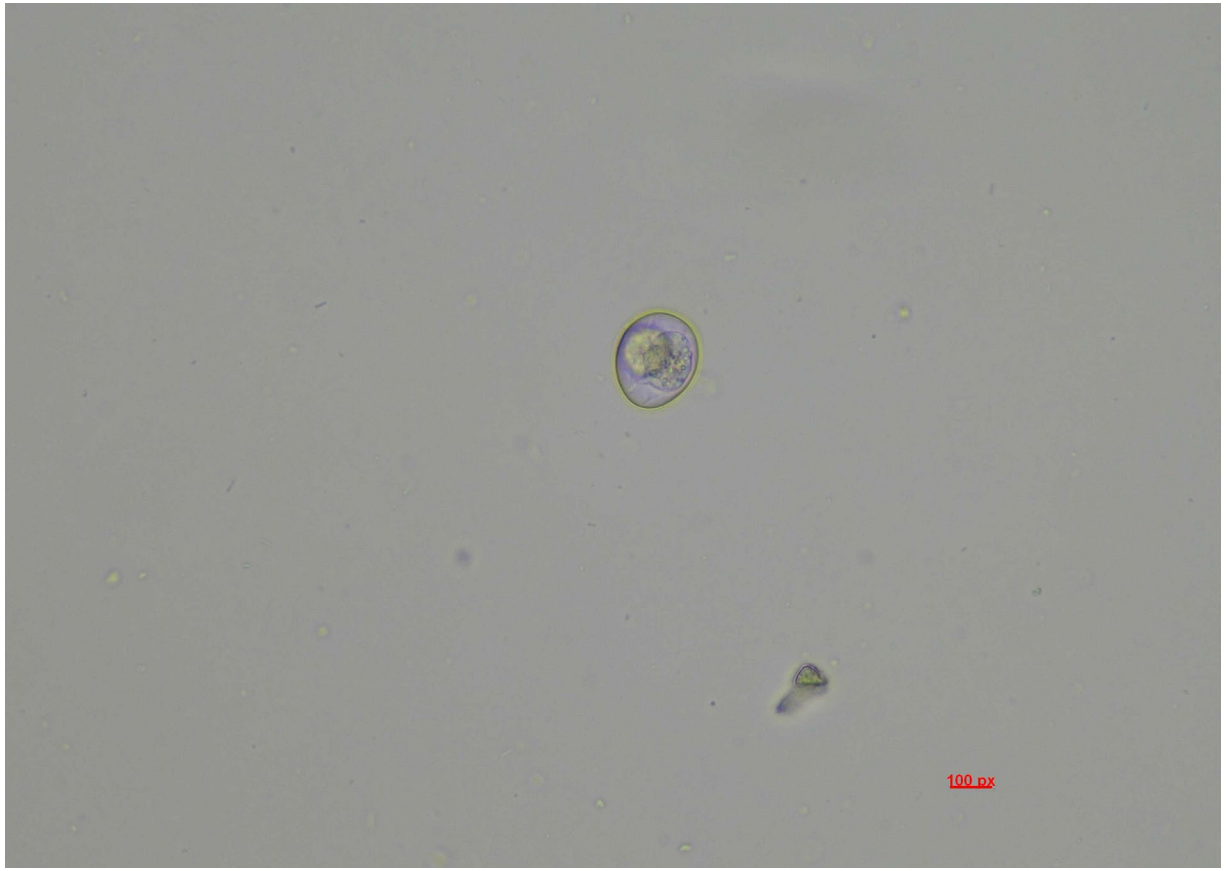





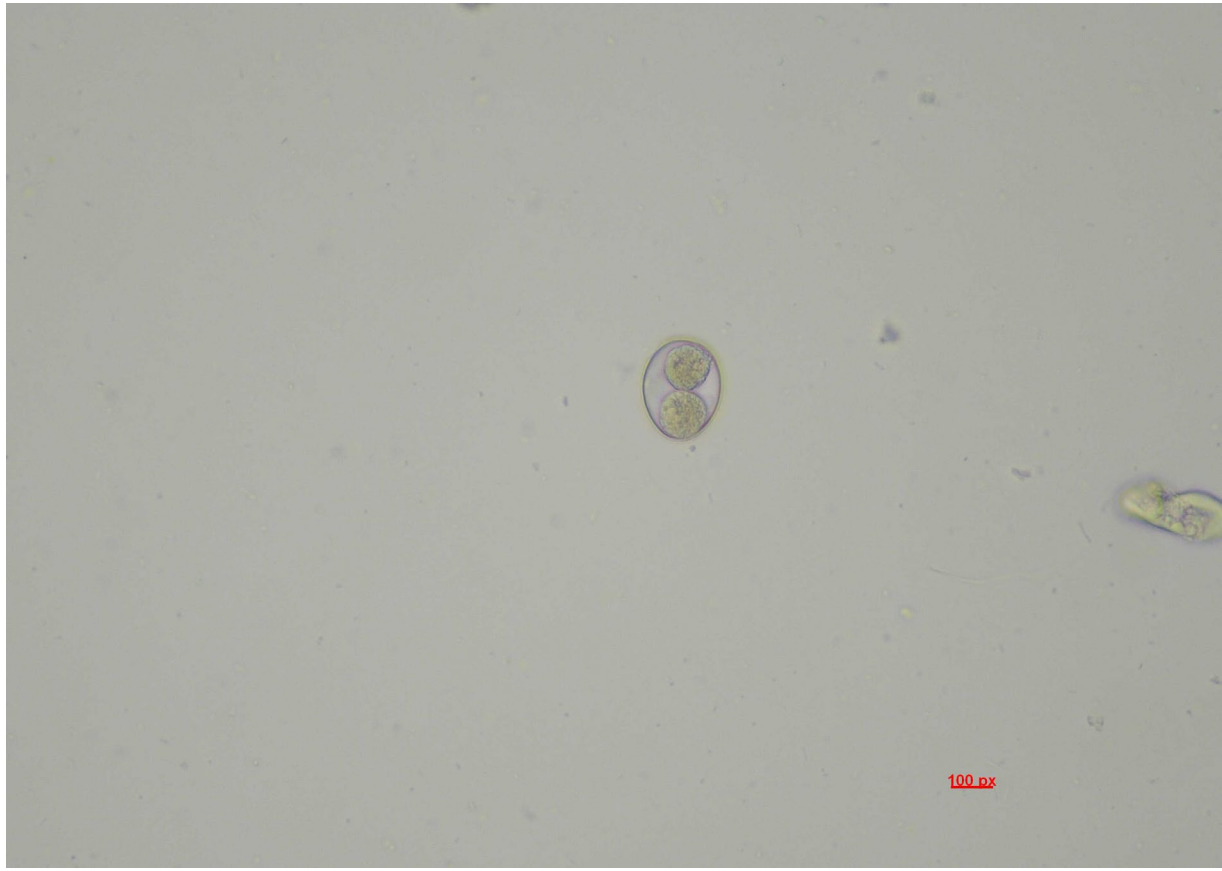
Control vs. Formalin: Day 52



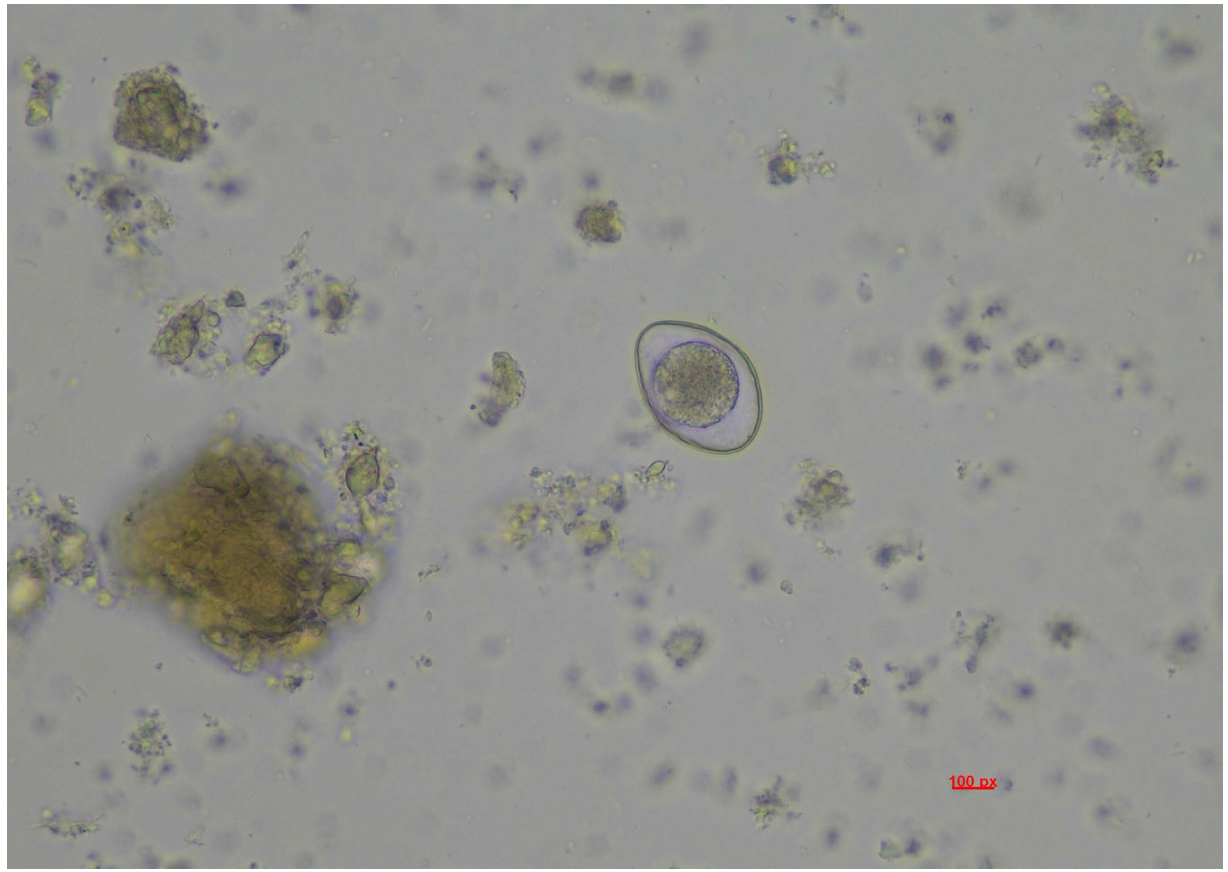


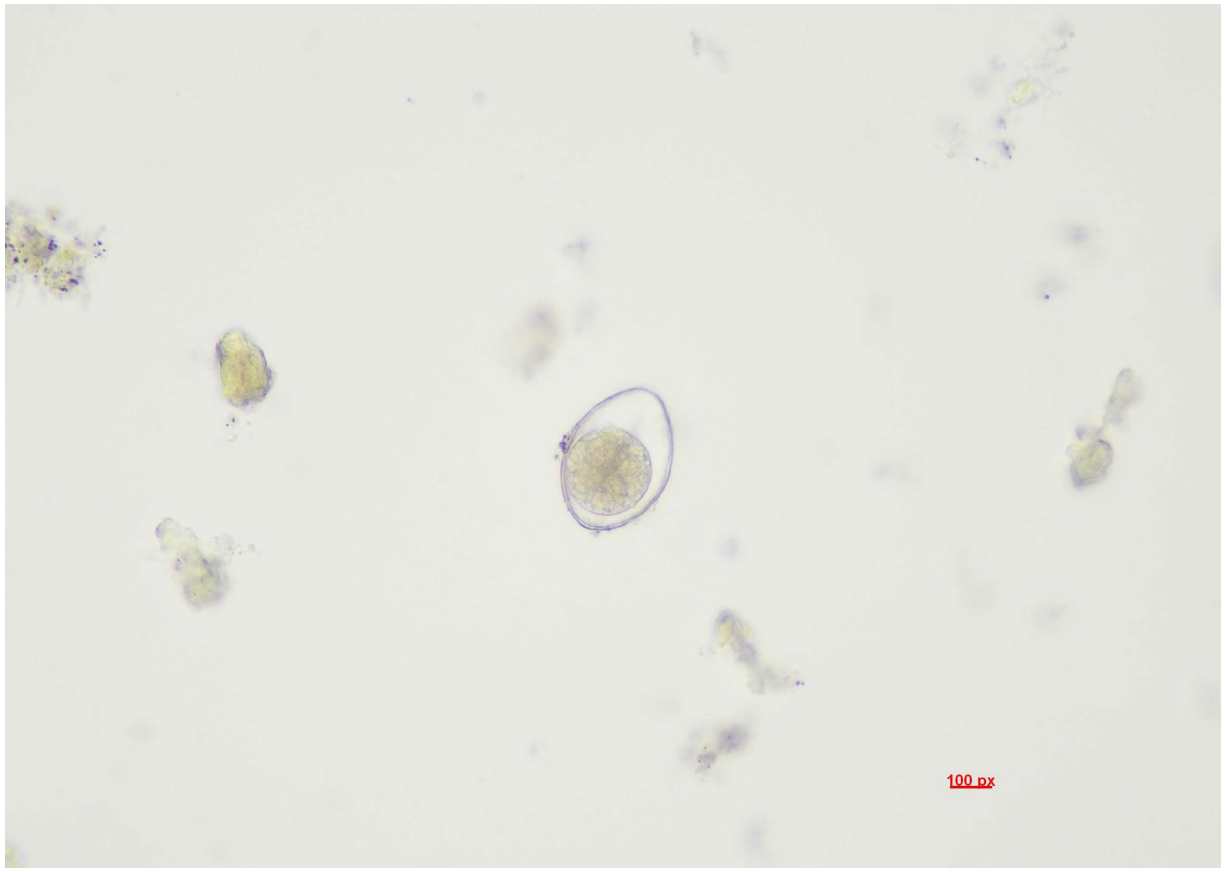
Control vs. Formalin: Day 52



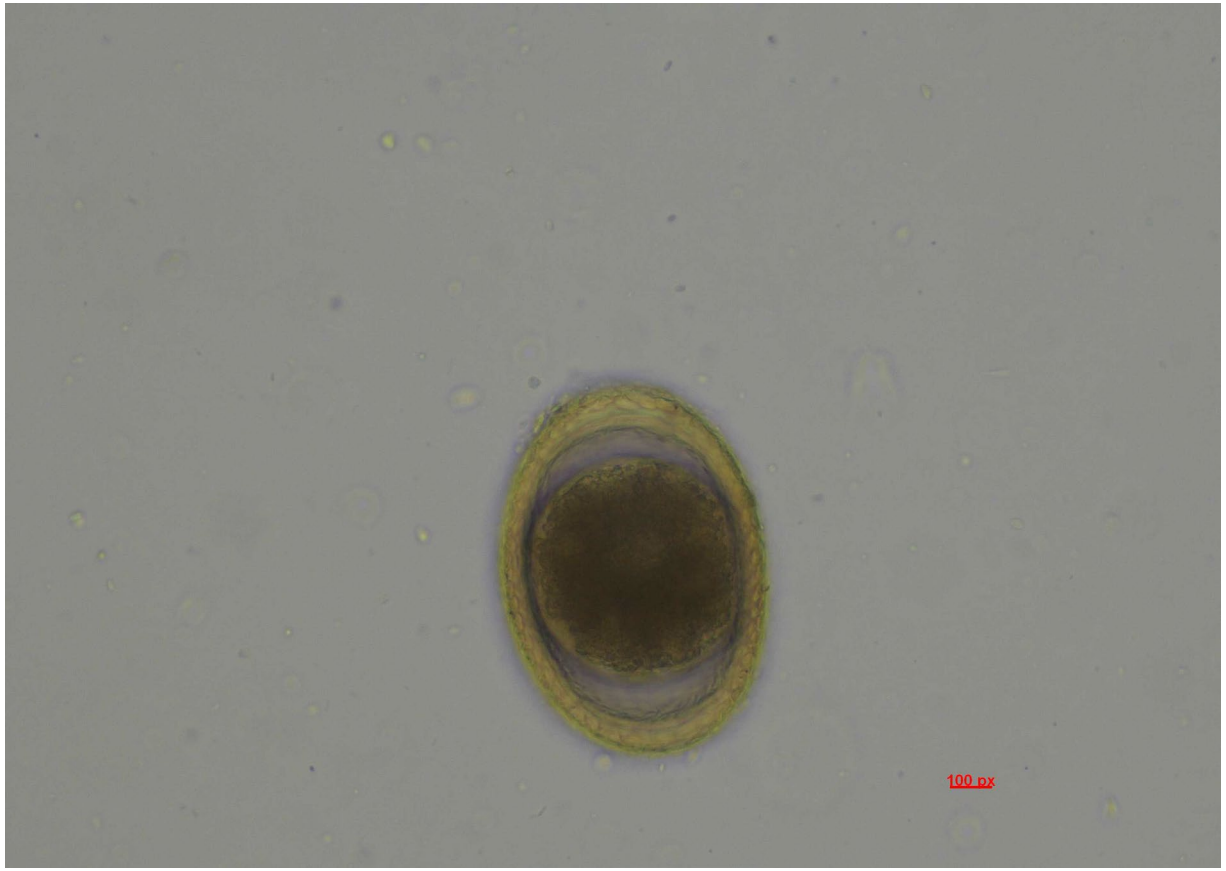


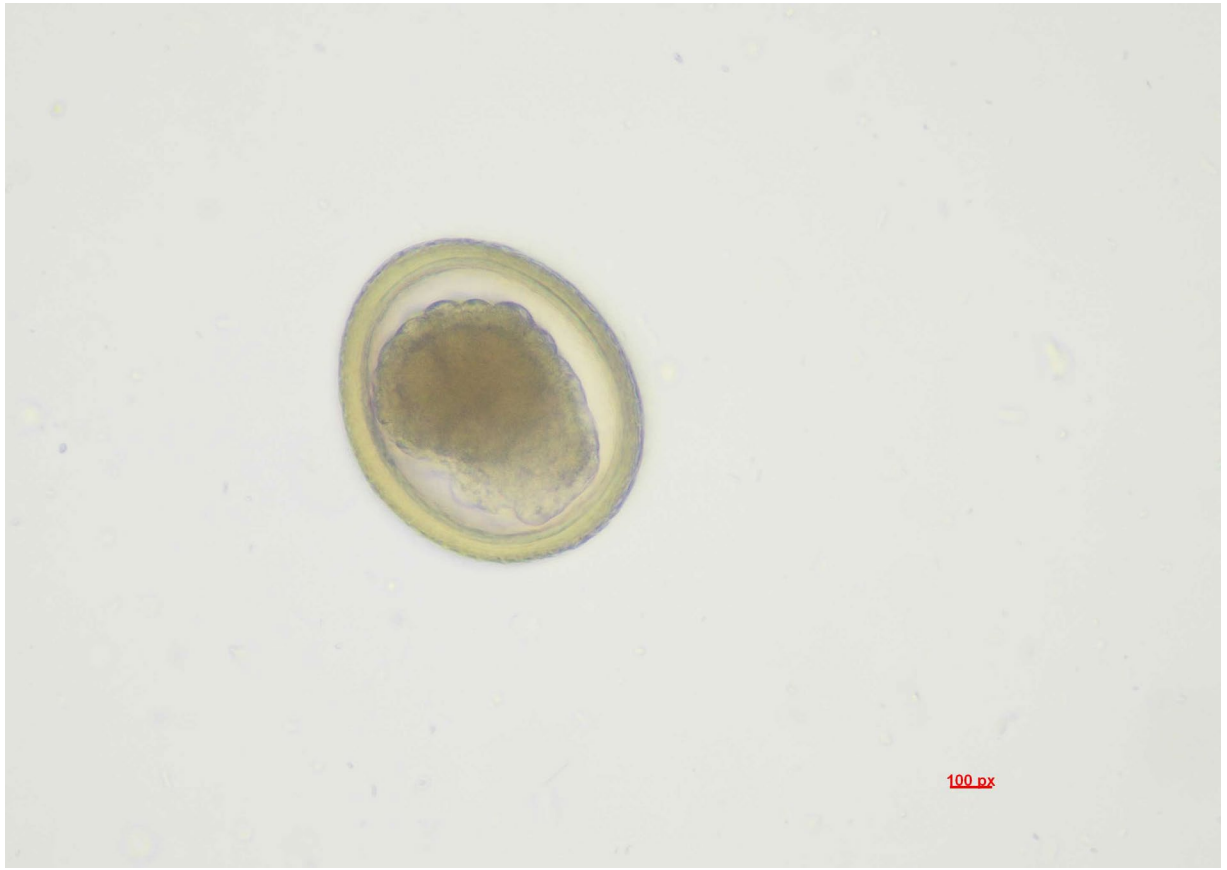
Control vs. Formalin: Day 52





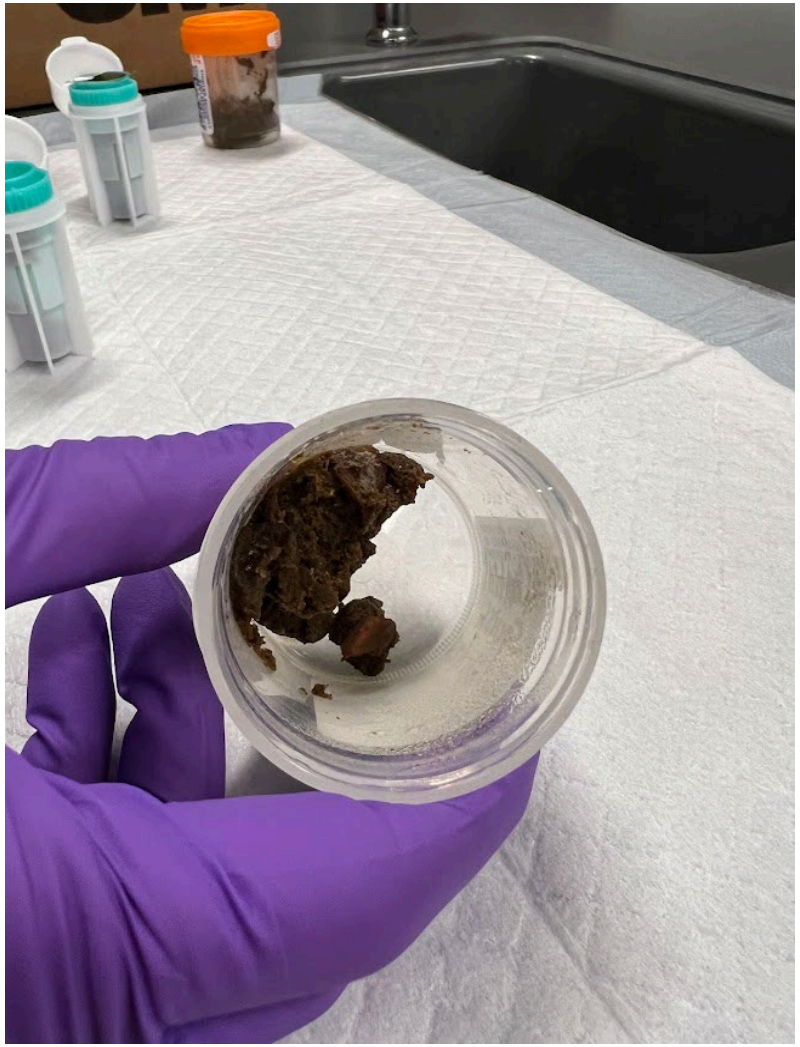
Control vs. Formalin: Day 52

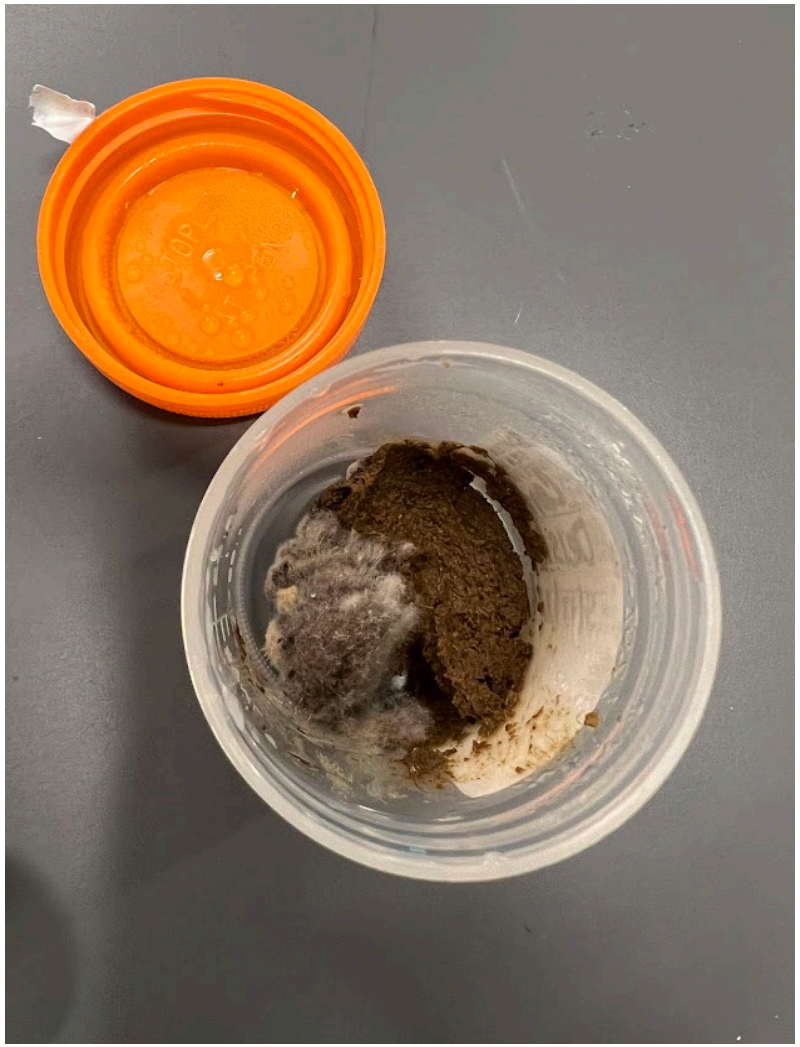




Gross Control Samples

- Day 52



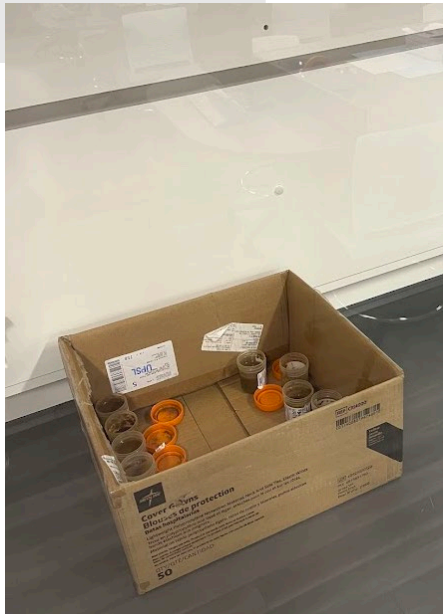


Formalin-Fixed Feces

Pros	Cons
Eliminated molding	Time-consuming
Eliminates foul odors	Biohazard
Guaranteed positive samples	Inaccurate gross assessment
No morphology changes	

Steps:

- Mix feces with 10% formalin at a 1:1 ratio
- Refrigerate for long-term storage
- Allow formalin to evaporate in hood for 7-21 days (depending on volume/SA)



Standardized Assessment

Skill: **QC/Troubleshooting**

- Close observation necessary

- Level 1: Standard QC/QA
- Level 2: Packaging a sample for shipment to outside lab
- Level 3: Task of finding a test and listing the requirements; Repairing CBC machine after clot

Feedback

- Immediate vs. follow-up
  - The sooner the better
- Be genuinely enthusiastic about what you've learned about them in the process
- Areas with deficits should be approached as an opportunity for growth
- Provide space for the student to ask additional questions

Questions?