

# Clinical Veterinary Diagnostic Laboratory



# CLINICAL VETERINARY DIAGNOSTIC LABORATORY

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# INTRODUCTION

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Welcome to CVM 6925, Veterinary Diagnostic Laboratory! This is a laboratory course designed as an introduction to diagnostic testing and laboratory techniques that are essential for “day one” competencies in a general veterinary practice setting. You will be actively engaged in a series of hands-on learning activities that take place in a traditional laboratory classroom (VSB 325), in a virtual teaching setting, through discussion-based debriefing sessions. Every effort has been made to make these sessions case-based learning activities that guide you through the journey of selection, practice, and clinical integration of common clinical tests such as fecal examinations, urinalysis, culture and sensitivity, hematology, and many more!

The course is set up as a “flipped classroom”. This means that for each of the laboratory units (denoted as a module), this book will guide you through a pre-laboratory reading. Each reading has “knowledge checks” along the way to help hone in important aspects of the reading or laboratory exercise to ensure that you have grasped the important points or can answer the most commonly asked questions that arise during the laboratory procedures. At the end of each reading, you will be guided back to the Canvas site to take a pre-laboratory/end-of-module quiz. Since we only have a small amount of time to carry out the in-class exercises, you must come prepared to class by reading the pre-laboratory readings, completing the knowledge checks, and completing the pre-lab/ module quizzes. These laboratory exercises and virtual events take tremendous coordination and resources to execute, therefore, additional time outside of the laboratory sessions to complete the activity is not available.



## MODULE I

# MODULE 1: LABORATORY SAFETY

### Module Objectives

1. Describe the biosafety protocols and waste disposal protocols for the laboratory
2. Describe the difference between BSL-2 and other BSL statuses
3. Model the appropriate PPE when you attend in person laboratories



# MODULE 1.1: GENERAL LABORATORY SAFETY

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## General laboratory safety

We are working with BSL-2 designated samples that may have zoonotic potential along with potentially harmful chemicals, thus it is important to:

- Place book bags under benchtop in plastic bins
- Avoid clutter on your benchtop
- No eating or drinking in the lab
- Wear all appropriate PPE (personal protective equipment)
- Goggles are available for those that would like eye protection
- Wash your hands after lab
- Clean up spills immediately
- Long hair should be tied back
- Must wear close-toed shoes
- If you are pregnant or planning on becoming pregnant, please let an instructor know so that we can get you fitted with the appropriate PPE

## Benchtop safety

Each benchtop has a **stainless-steel metal tray** that contains the following items:

- microbiology stains
- heat sterilizer
- microbiology loops
- flammable liquids
- miscellaneous laboratory items

***All experiments are to be performed and conducted in or over this tray. Not on the black benchtop.***

The tray is to protect you and contain biohazardous material, breakables, and chemicals in a small area

in the case of a spill or glassware break. Please keep workbooks, laptops, iPad, tablets, or anything but your experiment, out of this area, and on the **black** benchtop or stored in the plastic bins under your benchtop.

## Centrifuge safety

The centrifuge is a commonly used tool in our laboratory. It uses centrifugal force to separate substances in liquid or solid media according to particle size and density differences. Centrifugation may present two serious hazards: mechanical failure and dispersion of aerosols. Therefore, training on how to use the centrifuge properly and safely is essential for all individuals as part of Lab-Specific Training.

### Safe Procedures for Centrifugation

#### Before centrifugation

- Train each operator on proper operating procedures
- The following steps are taken care of by our laboratory staff
  - Use only rotors compatible with the centrifuge. Check the expiration date for ultracentrifuge rotors.
  - Check tubes, bottles, and rotors for cracks and deformities before each use.
  - Make sure that the rotor, tubes, and spindle are dry and clean.
  - Examine O-rings and replace if worn, cracked, or missing.
- When you (student) are using the centrifuges
  - **Never overfill centrifuge tubes (don't exceed  $\frac{3}{4}$  full).**
  - Always cap tubes before centrifugation.
  - Always balance buckets, tubes, and rotors properly.
  - Check that the rotor is seated on the drive correctly, close the lid on the centrifuge, and secure it.

#### During centrifugation

- Keep the lid closed at all times during operation. Never open a centrifuge until the rotor has stopped.
- Do not exceed safe rotor speed.
- The operator should not leave the centrifuge until full operating speed is attained and the machine appears to be running safely without vibration.
- Stop the centrifuge immediately if an unusual condition (noise or vibration) begins and check load balances.

## After centrifugation

- Allow the centrifuge to come to a complete stop before opening.
- Wear gloves to remove rotor and samples.
- Check inside of centrifuge for possible spills and leaks, clean centrifuge and rotor thoroughly if necessary.
- Wash hands after removing gloves.

## Centrifuging Infectious Materials

Follow the safety procedures above, plus:

- Always wear gloves when handling tubes or rotors.
- Always use sealed safety cups, safety buckets, or sealed rotors with O-ring as secondary containment if available.
- Fill centrifuge tubes, load into rotors, remove from rotors, and open tubes within a biological safety cabinet if biological safety cabinet is available.
- Wipe exterior of tubes or bottles with disinfectant prior to loading into rotor or bucket. Seal rotor or bucket, remove outer gloves, and transport to the centrifuge.
- Always wait at least 10 minutes after the run to allow aerosols to settle before opening the centrifuge. Check for possible spills or leaks. For spills of infectious materials, see [Centrifuge Emergency Procedures](#).
- [Decontaminate](#) centrifuge interior, safety cups or buckets, and rotors if tube breakage occurs. See [Centrifuge Emergency Procedures](#).
- Include centrifugation procedure and decontamination plan in lab SOPs.

Centrifuge safety follows the UMN Biosafety and occupational health protocols: Please follow this [link](#) for more information.

## Safety equipment

Fire extinguishers, eyewashes, and showers are located throughout the classroom (VSB 325 and AS/VM 104). Please familiarize yourself with their location in the laboratories. They are denoted by the **green banners** as shown below.



Emergency Eyewash Banner

## Material safety data sheets

As a student, you have a right to know any information about the safety and risks of exposure for any chemical used or accessible in the laboratory. Material Safety Data Sheets (MSDS) are available for your review if you have a concern or questions, feel free to contact the instructor (Dr. Burton).

## Knowledge check



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online here:

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# MODULE 1.2: BIOSAFETY AND BIOHAZARDOUS WASTE

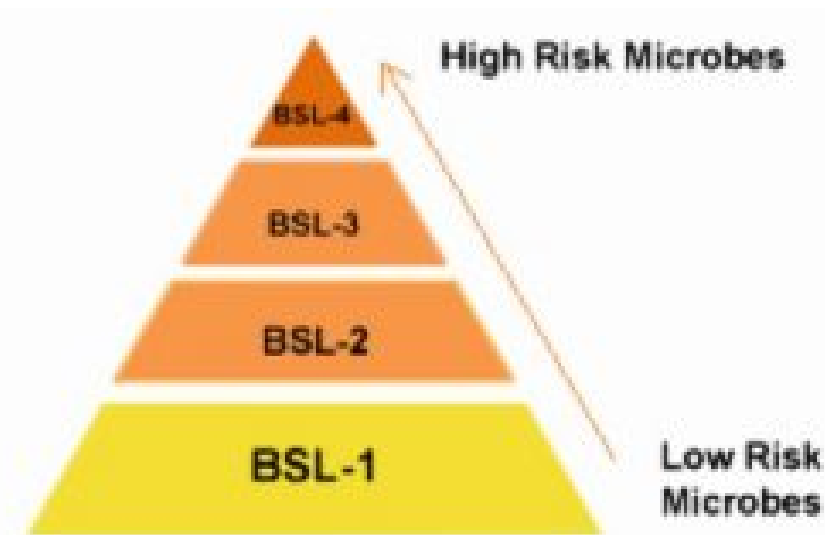
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## Biosafety

The CDC defines **biosafety** as “the application of safety precautions that reduce a laboratorian’s risk of exposure to a potentially infectious microbe and limit contamination of the work environment and, ultimately, the community.” The laboratory that you will be using for your experiments and procedures is a Biosafety level 2 (BSL-2) laboratory.

*What does being a BSL-2 laboratory means to you and your safety?*

The Centers for Disease Control and Prevention (CDC) and the National Institute of Health have established four levels of containment called Biosafety levels (BSLs). Each level has specific control measures for the containment of types of microbes and biological agents. The levels are assigned based on the evaluation of specific risks that the microbe or biological material may have on laboratory personnel and the community. The level of containment correlates with infectivity, the severity of disease, transmissibility, and the nature of the work conducted. Other risk factors that may also play in the determination of the BSL level include the origin of the microbe or agent and route of exposure.



Biosafety Levels (BSLs)

The CDC defines containment requirements for BSL-2 as the following:

“If you work in a lab that is designated a BSL-2, the microbes there pose moderate hazards to laboratorians and the environment. The microbes are typically indigenous and associated with diseases of varying severity. An example of a microbe that is typically worked with at a BSL-2 laboratory is *Staphylococcus aureus*.”

The containment requirements that we are required to abide by including the following.

## Laboratory practices

- Access to the laboratory is restricted when work is being conducted.

In our lab, this means that we do not allow individuals that are not enrolled or part of the teaching staff into the laboratory when we are conducting procedures.

## Safety equipment

- Appropriate personal protective equipment (PPE) is worn, including lab coats and gloves.
- Eye protection and face shields:
  - Eye protection must be worn when staff or students are handling risk group 2 (RG2) organisms at the benchtops
  - Face shields must be used when performing procedures that may create a splash hazard
- All procedures that can cause infection from aerosols or splashes are performed within a biological safety cabinet (BSC).
- An autoclave or an alternative method of decontamination is available for proper disposal.

In our lab, unless specified otherwise, you are required to wear clean scrubs (top and bottoms) or a long laboratory coat with long pants or a skirt along with closed-toed shoes with disposable gloves. You are responsible for the proper disposal of biological hazards into the appropriate waste containers (discussed later). You are required to disinfect your tables and

equipment before leaving the laboratory. The laboratory staff will dispose of the biological waste for decontamination.

## Facility construction

- The laboratory has self-closing doors.
- A sink and [eyewash](#) are readily available.

Every laboratory, including ours, regardless of the biosafety level follows the standard of microbiological practices. These practices include but are not limited to:

- Not eating, drinking, or applying cosmetics in the lab
- Washing hands after working with infectious materials and before leaving the lab
- Routinely decontaminating work surfaces

*If you leave the laboratory session without disinfecting your benchtops, disposing of your laboratory materials, or turning off and unplugging your microscope, your entire group will lose **2 points** that day.*

## Biohazardous waste

In this laboratory, we will be working with BSL-2 agents as part of your learning experience. To ensure everyone's safety and enjoyable learning environment, we have a rigorous biohazard waste policy.

### What is biohazardous waste?

**Biohazardous waste**, also called *infectious waste* or *biomedical waste*, is **any waste containing infectious materials or potentially infectious substances** such as blood. Of special concern are sharp wastes such as needles, blades, glass pipettes, and other wastes that can cause injury during handling. The waste reciprocals are labeled with the following red biohazard waste label.



Biohazard Waste Label

## 1. Sharps container

Each of your benches is equipped with a **sharps container**. Items that are sharp enough to puncture the skin and contaminated with unsterilized biological material should be placed in these bins.

Examples of items used in our lab that should be placed into the sharps container include:

- Glass Pasteur pipettes
- Glass slides
- Biologically contaminated broken glass

If they are overflowing or reaching capacity (3/4 full) please notify an instructor or teaching assistant.



Sharps Containers

## 2. Solid non-sharp biohazardous waste

Large biohazardous trash cans are located throughout the lab and are denoted by the large **orange** biohazardous waste sticker on their sides or lids. Lab consumables that have come in contact with

viable biological material that contains any laboratory material that is regarded as potentially infectious should be placed in the bins.

Examples of these types of items that should be placed in the biohazardous bins include:

- Gloves
- Culture flasks
- Plastic pipettes
- Pipette tips and well plates
- Wastes items contaminated with blood or other infectious or biological material



Biohazardous Bins

### 3. Liquid biohazardous waste

In some labs, we will be working with biohazardous liquids, mainly for **fecal** and **urine** procedures. On those days, the liquids should be placed in the liquid biohazard waste containers.

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## Knowledge check



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### Key Takeaways

- Experiments and procedures are done inside the silver tray on your bench
- The black countertop is your non-procedure/experiment workspace
- We wear approved laboratory attire when in the microbiology laboratory space
- You will be working with BSL-2 pathogens
- If you do not know how to dispose of waste product, please ask.

# MODULE 1.3: BEFORE LEAVING THE LAB EACH DAY

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## What do I do once I have finished my laboratory procedure?

- Throw out any used laboratory materials.
  - **2 points** will be deducted from your course total score each time you leave an un-tidy benchtop
- Disinfect your benchtop.
  - The disinfectant wipes are to be used on your silver trays, and the liquid disinfectant in the plastic bottles labeled disinfectant is used on the black laminate countertops.
- Disinfect your electronic devices
  - If you used any electronic devices to access the eManual instead of a printed version, all items must be disinfected prior to leaving the laboratory
- Disinfect writing instruments
  - If pens or other writing instruments were used during your experiment, these items should either remain in the lab at all times or should be disinfected if they will be taken out of the lab.
- Wash hands
- Do not forget your items in the bins
  - Many other undergraduate and graduate courses use this lab every day, so your things cannot be stored here

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## Knowledge check



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### Key Takeaways

- Experiments and procedures are done inside the silver tray on your bench
- The black counters are your non-procedure/experiment workspace
- We wear approved laboratory attire when in the microbiology laboratory space
- You will be working with BSL-2 pathogens
- If you do not know how to dispose of the waste products, please ask.

You have now reached the end of Module 1. If you are enrolled in CVM 6925, please go to the Canvas page and take the quiz: “Module 1: Laboratory safety quiz”. There is not an assignment that accompanies this module.



## MODULE II

# MODULE 2: INTRODUCTION TO COMMON FECAL DIAGNOSTIC PROCEDURES

### Module Objectives

1. Choose an appropriate diagnostic test based on clinical presentation and suspected parasitic infection.
2. Choose an appropriate fecal flotation solution based on the desired parasite ova you are aiming to recover.
3. Describe the procedure basics for the fecal diagnostic tests reviewed.
4. Describe the best collection technique for the fecal diagnostic test you have selected.



# MODULE 2.1: EXAMINATION OF FECES FOR DIAGNOSIS OF GASTROINTESTINAL PARASITISM

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## Introduction to gastrointestinal parasitism

All animals are subject to infection by internal parasites. Though the internal environment provides several advantages, a large hurdle the parasite must overcome is ensuring its survival by infecting new hosts. Some endoparasites deal with this problem by taking advantage of predatory, scavenging or blood-feeding behaviors of hosts by making infective stages available in tissues or blood.

Other internal parasites can infect reproductive or milk-producing organs, or even cross placental barriers, to ultimately infect new hosts. Then again, many internal parasites produce stages that are released into the environment, most commonly via excreta (usually feces and urine), that will hopefully (from a parasite's perspective) encounter and infect a competent host.

As a result, several endoparasitic infections can be diagnosed by finding characteristic ova, larvae, cysts, or oocysts through a routine examination of animal feces. Although a fecal examination seems straightforward, there are several issues that you will have to consider.

### **Considerations:**

- How should my clients, staff, or I handle fecal specimens?
- Am I more concerned about whether an animal is infected or if it has a high parasite burden?
- How can I increase the sensitivity of the fecal exam

In the diagnostic laboratory fecal laboratories, we will help you set up and correctly run fecal diagnostic exams, as well as assist you in diagnosing internal parasites.

## Considerations for using feces for the diagnosis of

# intestinal parasitism

By far, one of the most common samples submitted for parasite screening is feces as intestinal parasites consist of the majority of pathogenic veterinary parasites. While molecular techniques are beginning to gain popularity for characterizing intestinal parasitism, the mainstay for fecal evaluation remains fecal flotation, fecal sedimentation, dry and wet mount fecal cytology (e.g. direct fecal smear), and Baermann examination (for larvae).

## Principles for fecal collection and preservation

### 1. Fresh feces should be used for fecal exams

- A freshly voided sample or a rectal sample collected with a fecal loop or finger are ideal specimens for fecal examination.
- A small composite sample of the specimen should be placed in a suitably sized and airtight container (plastic cup with lid, glass jar, or a sealed plastic bag).
- Newspaper, tissue paper, paper towels, and cardboard boxes do not make satisfactory containers.

### 2. Feces should be preserved when same-day evaluation is not possible

- If the fecal exam is to be performed immediately, preservation is not always necessary.
- If the sample is to be kept until the next day or sent to a laboratory, place the sample in an airtight container with as much of the air removed (either squeeze the plastic bag or fill a small container).
- Refrigeration or the addition of a 10% formalin solution (1 part feces to 5 parts formalin) is highly recommended if there will be a delay in evaluation
  - Note: Many ova and oocysts will continue to develop in formalin-preserved feces.
  - Refrigerating or preserving feces minimizes bacterial and yeast contamination.
  - Samples should **not be frozen** because water expands when frozen, hence oocysts and ova will deform or break.
  - **Formalin-preserved samples cannot be used for fecal smear, larval culture or Baermann techniques** as these techniques require **living** organisms.

## Why is it necessary to collect fresh feces?

An important reason for obtaining *fresh feces* (or keeping samples refrigerated) is to facilitate nematode ova identification. Given **warm temperatures and time, strongyle-type eggs will develop into larvated**

**eggs, resulting in possible confusion with nematode eggs that are shed larvated in fresh feces** (i.e.: *Strongyloides* sp. (Threadworms) or *Physaloptera* sp. (Stomach worm) Additionally, by collecting fresh or rectal samples, you prevent finding free-living nematodes (those that live in the soil) which could be confused with veterinary parasitic larvae from migrating into the feces on the ground.

*Is there ever a case in which you would want to incubate the feces for a specified period of time and not refrigerate?*

In some cases, it is necessary to allow the egg or oocyst to develop for identification of specific structure or larval stages for more specific identification to the genus or species level. If you need to determine what coccidial genus is infecting an animal (e.g. *Eimeria* v. *Cystoisospora*), incubating the fecal sample for a few days will allow sporulation to occur (we can determine the genus and/or species based on the number of sporocysts within the ova). This technique is especially helpful when we are trying to determine if the parasite observed is spurious or parasitic.

### Case Example

A 6-month-old puppy presents to your clinic with a 48-hour history of diarrhea. On fecal flotation, numerous coccidian oocysts without an obvious micropyle are noted. To complicate matters you also find many *Cyniclomyces guttans* yeasts along with the coccidia. You know that these yeast are common commensals of rabbit GI tracts. After discussing with the owner you find out that the puppy love to eat rabbit poop. The last time you treated this puppy for *Cystoisospora* sp. as a puppy he had a terrible reaction and you are not keen on giving those drugs again. Knowing that the oocysts will quickly sporulate in the environment and *Eimeria* commonly have 4 sporocysts and *Cystoisospora* sp. has only 2 sporocysts you incubate the stool overnight and re-float. On the re-float, you find these oocysts contain 4 sporocysts, therefore they are *Eimeria* sp., an incidental finding and not the cause of diarrhea in this puppy.

# MODULE 2.2: DIRECT FECAL SMEAR (WET MOUNT)

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## Common fecal techniques used in diagnosing parasitism

In the next few pages, we will discuss common techniques used to recover parasite ova, or eggs, larvae, and motile forms (trophozoites) in feces. Many of these techniques will be practiced in our laboratory sessions over the next few weeks.

### Direct fecal smear

There are two separate techniques used for direct fecal smear analysis. These are the dry mount fecal cytology (DM) or wet mount fecal test (WM). Both are performed by collecting a small amount of fecal material directly from the lumen of the rectum. DM and WM are considered more sensitive than rectal scrapings for the detection of *Giardia* sp. and *Trichomonads*, as these protozoans by the time they reach the rectum are no longer associated with the mucosa.

Which one should I choose?

### Dry mount fecal cytology

This test is commonly selected when we are looking for abnormal bacteria or yeast organisms in feces. These samples are stained using a Romanowsky stain, such as Diff Quik, and evaluated at 100x, 400x, and 1000x magnification. *This is an uncommon method for evaluating feces for parasites.*

### Wet mount fecal test

Wet mount fecal test is the preferred method for the identification of motile protozoans, such as *Giardia* trophozoites, or in cases in which you have too little feces to use fecal flotation techniques. If you are looking for motile organisms, such as *Giardia* trophozoites, these samples must be reviewed within **30 minutes** of collection and are not suitable as a send-out test. This test is not indicated for the detection of bacteria or yeast.

## Wet mount fecal test procedure:



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**Video:** This video demonstrates the wet mount fecal test. In this video, they refer to this test as a “direct fecal smear” this is another common name for this test that you will encounter.

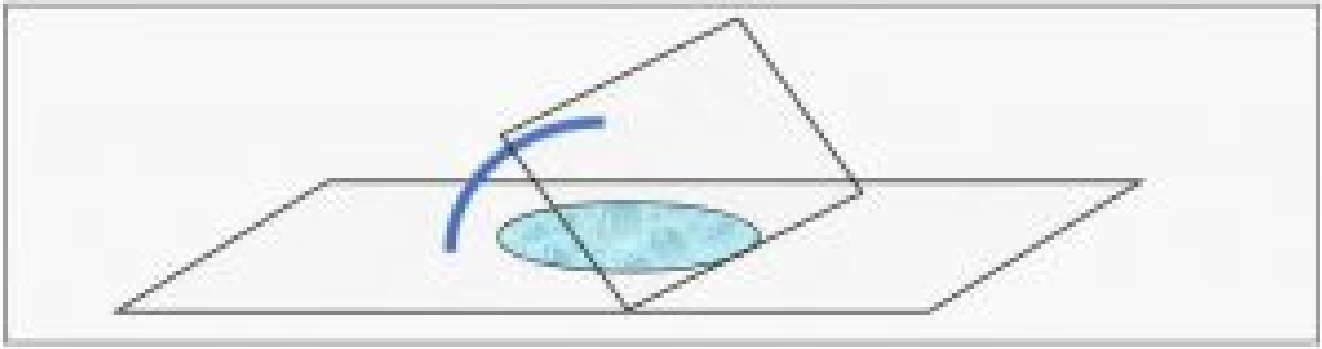
## Materials and equipment needed

- **Page 4** of your Parasitology Laboratory Techniques book
- Glass slides and coverslips
- Water (saline preferred)
- Microscope and light source
- Lugol’s iodine (helpful for identifying Giardia cysts)\*\*

## Technique

1. Take a small amount of feces (i.e., the amount of feces adhering to a rectal thermometer) and emulsify with a few drops of water on a glass slide. The resultant mixture should not be opaque (place the slide on top of this page – **if you can’t see the letters on this page under the mixture, the mixture is too thick**→ add more water or use less feces).
2. Add a coverslip and examine under 100x magnification (eyepiece 10x \* objective 10x = 100x total magnification) and 400x magnification. Be sure to reduce brightness (adjust light intensity) and increase contrast (adjust condenser to down position) so that eggs or trophozoites and their internal structures are easily seen.
3. Examine entire smear systematically for parasite ova, worm larvae, or protozoan structures.

\*\* Lugol’s iodine can be used to help identify Giardia & Hexamita cysts. Before placing the coverslip, add a drop to the mixture. Iodine will enhance the internal structures of the cyst but will kill Giardia and Hexamita trophozoites. **We do not have Lugol’s iodine in our laboratory.**



Coverslip placement for wet mount procedure

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## Knowledge check



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# MODULE 2.3: FECAL FLOTATION

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## Fecal flotation

Fecal flotation is by far the most common diagnostic procedure used to detect intestinal parasites in our veterinary species due to its low cost and is a relatively straightforward procedure to conduct. The majority of parasite ova/ eggs have a specific gravity (SG) that falls between 1.05 and 1.23, thus the fecal flotation relies on the ascension of eggs and ova using the difference in overall density to the flotation solution.<sup>1,2</sup> The most common flotation solutions used in veterinary medicine include saturated sodium chloride, sodium nitrate, sugar (e.g. Sheather's solution), magnesium sulfate, and zinc sulfate. In the perfect scenario, a fecal flotation solution would preserve egg and oocyst morphologic integrity while limiting the ascension of fecal debris; however, not all flotation solutions are created equal. For example, sugar solutions have an SPG of 1.25 resulting in Giardia cysts becoming distorted and difficult to identify for the novice, but a better overall ova yield than other solutions. In addition to choosing the best flotation solution for the parasites, a practitioner is wishing to recover, passive versus centrifugal flotation methods can yield vastly different results. In general, **centrifugal flotation techniques are considered more sensitive in recovering parasite ova.**

Centrifugation methods use centripetal motion to aid in the suspension of the helminths eggs and protozoan oocysts in a solution versus passive relying completely on the density and specific gravity for ascension.<sup>3</sup> The S.G. of flotation solutions should be checked periodically, at least monthly, or when opening a new bottle. You can easily check the S.G. of your flotation in your clinic using a hydrometer.

Sodium Nitrate (Fecasol)	33% ZnSO <sub>4</sub>	Saturated NaCl	Saturated MgSO <sub>3</sub> (Epsom)	Sheather's Sugar
S.G. 1.2-1.33 ✓ Common helminths & protozoan eggs/cyst ✗ Distorts Giardia ✗ Flukes ✗ Some unusual tapes & nematode eggs	S.G. 1.18 ✓ Common helminths & protozoan eggs/cyst ✓ Preferred for Giardia ✓ +/- lungworm larvae ✗ Less sen. for common tapes ✗ Flukes, unusual tapes & nematodes	S.G. 1.2 ✓ Common helminths & protozoan eggs/cyst ✗ Distorts Giardia ✗ Flukes ✗ Some unusual tapes & nematode eggs	S.G. 1.32 ✓ Common helminths & protozoan eggs/cyst ✗ Distorts Giardia ✗ Flukes ✗ Some unusual tapes & nematode eggs	S.G. 1.26 ✓ Common helminths & protozoan eggs/cyst ✓ ✓ Crypto ✓ Less damage ✗ Distorts Giardia ✗ Flukes ✗ Some unusual tapes & nematode eggs ✗ Sticky mess

The pros and cons of the most common types of fecal flotation solutions used in clinical practice. Adapted from Zajac & Conboy. Veterinary Clinical Parasitology. 8th ed. Pg. 6

In today's laboratory, you will practice both centrifugal and passive flotation techniques.

**A few questions to ask yourself as you practice these two techniques are the following:**

1. Which of the two techniques has a better recovery of ova?
2. What are the advantages and disadvantages of each technique?

## Simple or Passive Fecal Flotation Technique

**The following instructions are for the technique we will be using in the laboratory sessions.**

Fecalyzer® is a commercial kit that is widely used in primary care settings. The technique below describes the passive technique without the kit. ***In the laboratory, we will practice using the Fecalyzer® kit to recover parasite ova using the kit's instructions.*** Fecalyzer® kits use a commercially available flotation solution, Fecasol®. It is a NaNO<sub>3</sub> flotation solution with SpG 1.20 (not saturated) and will float most

common eggs and oocysts, but will distort *Giardia* cysts. In the laboratory today, we will use  $\text{ZnSO}_4$  instead of  $\text{NaNO}_3$ .

## Technique

1. Mix about 5 gm of feces with 20 ml of flotation solution in a plastic cup or other suitable containers.
2. Strain through a tea strainer into a second cup.
3. Swirl cup and decant fecal suspension into a centrifuge tube or other straight-sided vial.
4. Fill tube or vial with enough flotation solution so that the meniscus is just level with the top of the tube.
5. Place glass slide or coverslip on top of the tube and allow it to stand for at least 20 minutes.
6. Liftoff slide or coverslip, invert, cover drop with a coverslip and examine under 10X and 40x magnification, condenser in the down position, with low light. A drop of Lugol's iodine can be placed on the slide before placing the coverslip to enhance the identification of *Giardia* cysts.



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Video: This video demonstrates the passive fecal flotation technique using a Fecalyzer® kit. There are other methods for performing the passive fecal location that is outlined in your parasitology laboratory manual.

## Centrifugal Fecal Flotation Technique

**The following instructions are for the technique we will be using in the laboratory sessions.**

The centrifugal flotation technique you will practice in the laboratory is a two-step process with the first step being a “wash” to help decrease the amount of fecal debris. In the clinical laboratory setting, people often skip the water “wash”, but in this laboratory, since it is assumed that you are still trying to learn the difference between plant or fecal debris and parasites, we will perform the water wash.

## Technique

1. Mix a few grams of feces (~½ thumb-size) with a small quantity of water to make a well-mixed fluid suspension.
2. Strain through a tea strainer into a clean container.
3. Swirl container, pour filtrate into a centrifuge tube, fill tube to within 2 in. of the top, counterbalance **with another equally weighted tube of water**, and centrifuge at 2000 rpm for at least

2 minutes.

4. Pour off supernatant fluid and replace it with a few milliliters (~5 ml) of flotation solution (i.e. ZnSO<sub>4</sub>, NaCl, sugar, etc). Mix well with a wooden applicator to resuspend the pellet.
5. Fill centrifuge tube to within an inch of the top, **counterbalance with the flotation solution**, and centrifuge at 2000 rpm for at least 3 minutes
6. Gently touch a sterile wire loop to the surface of the fluid in the tube. Surface tension should cause a drop of surface liquid to adhere to the loop. Transfer drop to a slide, cover with a cover glass\*, and examine under 10X and 40x with the condenser down and reduced light.

\*A drop of Lugol's iodine can be placed on the slide before placing the coverslip to enhance the identification of Giardia cysts (we will not do this in the lab).



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Video: This video demonstrates the passive centrifugal flotation technique. This method is also outlined in your parasitology laboratory manual.

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## Knowledge check



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# MODULE 2.4: IS THIS A PARASITE EGG?

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## Did I find a parasite egg?

You have finally made it to the finish line, your condenser on your microscope is down and you focus on...something...but what is it? It is your job now to determine if this is a pathogenic parasite, **spurious** parasite, or simply a **pseudoparasite**.

## Parasite egg and pseudoparasite resources:

There are many resources available to you here is a list of a few:

1. Your parasite manual (See link in Module 2 assignment)
  2. CAPCvet parasite egg identifier phone app (free): [Apple Store](#) & [Google Play](#)
  3. [CAPCvet website](#)
  4. Clinician's Brief: Differentiation of Parasites and Pseudoparasites (See link in Module 2 Assignment)
  5. Averbach's Spores and Pollen Illustrations (Link available in "General Resources" tab and in the Module 2 Assignment)
- 

### Key Takeaways

- Fecal flotation is the main method for intestinal parasite screening
- We have other fecal tests to look for specific parasite ova, trophozoites, or larvae
- Fresh feces should always be used to perform these tests when possible
- Just because you find an egg, oocyst, larvae, etc. on a fecal examination, does not mean that it is pathogenic or the cause of disease in your patient
- Pollon is one of the most commonly misidentified structures as parasite ova

You have now reached the end of Module 2. If you are enrolled in CVM 6925, please go to the Canvas page and take the quiz: “Module 2: Intro to fecal quiz.” There is an assignment associated with this module and the laboratory session.



MODULE III

# MODULE 3: THE QUANTITATIVE FECAL EXAM

## Module Objectives

1. Using case data, correctly choose and be able to justify the appropriate quantitative flotation technique
2. Compare and contrast the quantitative techniques presented in this module
3. Describe the basic techniques and principles of each test



# MODULE 3.1: THE QUANTITATIVE FECAL EXAM

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## Quantitative fecal exams

The fecal smear (dry and wet techniques), sedimentation, and fecal flotation techniques can indicate if an animal is harboring internal parasites. However, these tests do not provide an estimate of the number of ova or oocysts in a known amount of feces, as they are **qualitative** tests. Egg-counting techniques, otherwise known as **quantitative** fecal exams, allows for an **estimate** of the number of parasite structures (eggs or oocysts) within a known amount of feces. Quantitative information can assist in determining anthelmintic efficacy, as well as estimating pasture contamination. However, *egg counts from an individual animal at a particular point in time are of limited value* – results will vary depending on factors affecting egg/oocyst production. On the other hand, quantitative approaches, when used in a well-designed experimental or clinical study, can provide significant information.

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## Knowledge check



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## Quantitative fecal techniques

The two most common quantitative fecal techniques used in veterinary medicine are the **McMaster's** and **Wisconsin techniques**. Over the years, scientists and diagnosticians have made improvements on the original

techniques described at their initial development and you will see these techniques referred to as “modified”. A specific mass of feces and volume of flotation solution are measured out before conducting the test for all quantitative techniques. We can estimate the total number of eggs per gram (epg) of parasitic ova in the fecal mass being floated by knowing the specific fecal mass and fluid volume. This number can help estimate pasture contamination of ova and when used before and after anthelmintic administration, help identify potential resistance in a nematode population. Setting up an experiment or clinical trial for the determination of anthelmintic resistance using specific experiment design and quantitative fecal techniques is called a fecal egg count reduction test (FECRT).

To learn more about designing a FECRT, please refer to p. 14 in your [Parasite Laboratory Manual](#).

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## Knowledge check



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# MODULE 3.2: THE MCMASTER'S AND WISCONSIN TECHNIQUES

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## The McMaster's and Wisconsin techniques

The McMaster's and Wisconsin techniques are both quantitative tests but do have some differences that are outlined in the chart below. Please refer to pages 10-12 in your parasite lab manual for specific laboratory methods.

**Table 3.1: Modified McMaster's and Wisconsin Techniques**

	<b>Modified McMaster's</b>	<b>Wisconsin</b>
<b>Sensitivity</b>	within 25-50 epg	within <1 epg
<b>When would you choose this test?</b>	Expected egg numbers are >100 epg	Expected egg numbers are <100 epg
<b>Method</b>	The flotation is a <b>dilution technique</b> and requires a special reusable slide (McMaster's slide) with a grid and two chambers. Only eggs that are found in the grids are counted and applied to an equation to determine epg	Double centrifugal float technique using a known amount of feces. All eggs under the coverslip are counted to determine epg. This is not a dilution technique.
<b>Flotation solution</b>	Depends, often Sheather's Sugar Solution	Depends, often Sheather's Sugar Solution
<b>Preferred technique?</b>	The technique of choice for quantifying protozoan ova or when an animal is heavily infected with parasites because it is a dilutional technique.	The technique of choice for quantifying helminth ova or when you expect that an animal has a low parasite burden.

## Knowledge check



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# MODULE 3.3: MODIFIED MCMMASTER'S TECHNIQUE

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## Modified McMaster's technique

In today's laboratory we will be performing the Modified McMaster's technique (see pg. 12 or video below) to **quantify** the number of epg in the feces provided. Once you have prepared your fecal mixture and allowed the mixture to float using a McMaster's slide for **30 minutes** you will begin counting the number of eggs found in each chamber.

**Please watch the following video before the laboratory to gain an understanding of how you will perform the procedure. We will not be reviewing this technique in class.**



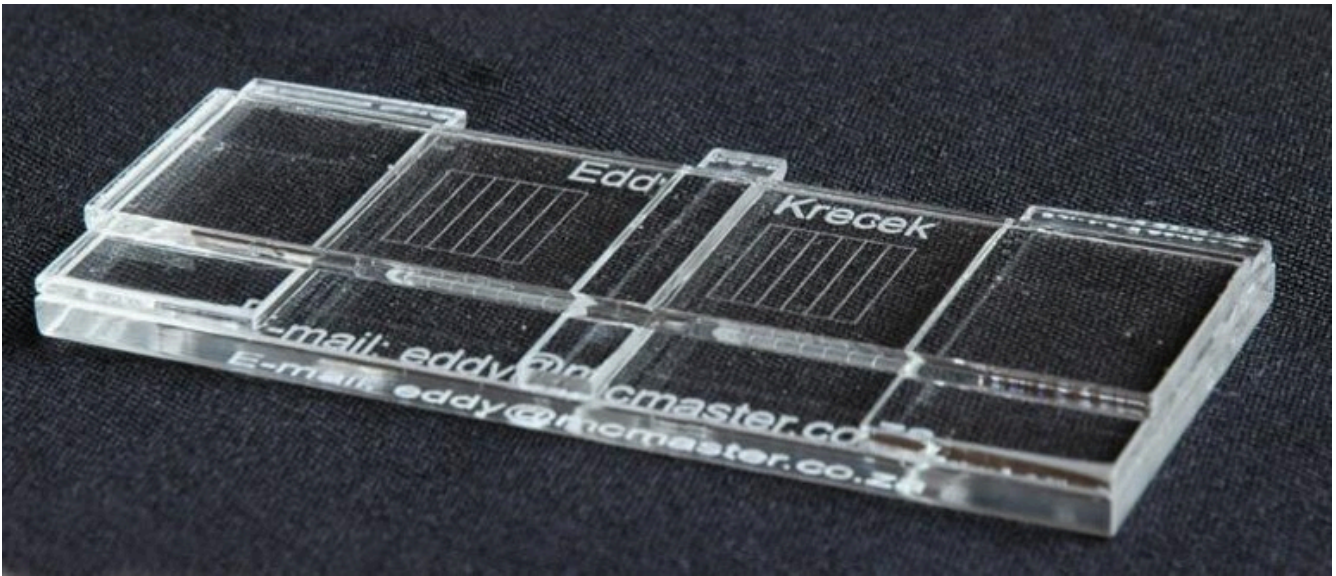
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**The next video describes the technique we use to count the eggs using a McMaster's slide. Please forward to 3 minutes 34 secs. We will not be reviewing this technique in class.**



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Below is an image of a McMaster's slide. Each chamber holds 0.15 mL of fecal material. Additionally, each chamber has 6 vertical lanes per grid. Thus, each grid is calculated so that you can enumerate the number of eggs or oocysts per 0.15 mL of fecal material.



McMaster's slide

You will count the number of eggs or ova seen in each chamber (the total of the 6 vertical grids). **Eggs or ova that touch the grid lines or are found outside of the counting chamber are excluded from the total number.** The total number of each type of ova is tallied for the calculation to the following formula.

Eggs Per Gram (EPG) Equation

$$\frac{(Eggs_{chamber\ 1} + Eggs_{chamber\ 2})}{0.3\ mL} \times \frac{total\ volume\ (mL)}{feces\ weight\ (g)} = epg$$

Let's look at an example:

**Fecal mass:** 2 grams

**The total volume of fecal solution:** 60 mL

Count	Chamber 1	Chamber 2	Total (Chamber 1+2)	EPG
<b>Strongyle-type</b>	5	2	7	700
<b>Coccidia</b>	30	40	70	7000
<b>Others (List)</b> <i>Nematodirus</i>	1	0	1	

## Case Example Calculations

Strongyle-type:

$$\frac{(5+2)}{0.3 \text{ mL}} \times \frac{60 \text{ mL}}{2 \text{ g}} = 700 \text{ epG}$$

Coccidia:

$$\frac{(30+40)}{0.3 \text{ mL}} \times \frac{60 \text{ mL}}{2 \text{ g}} = 7000 \text{ epG}$$

## Knowledge check



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### Key Takeaways

- The McMaster's and Wisconsin techniques are special types of fecal flotations that allow fecal egg quantification
- The McMaster's is a dilutional technique that is commonly used when a high egg burden is suspected
- The results from the two techniques are not interchangeable (e.g. you cannot compare results)
- These tests are validated for coccidia and Strongyle-type eggs
- These tests should be interpreted over several time points at the herd level to guide antiparasitic recommendations

You have now reached the end of Module 3. If you are enrolled in CVM 6925, please go to the Canvas page and take the quiz: "Module 3: Quantitative fecal quiz." There is an assignment that accompanies the in-person laboratory for this module.



## MODULE IV

# MODULE 4: POLYMERASE CHAIN REACTION (PCR)

### Module Objectives

1. Paraphrase the basic PCR procedure
2. Describe what the PCR is specifically detecting
3. Correctly interpret data from real-time PCR
4. Describe what a cycle threshold (Ct) and how this relates to patient test results



# MODULE 4.1: POLYMERASE CHAIN REACTION (PCR)

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## Introduction to PCR

Polymerase chain reaction (PCR) tests are some of the most common types (often the gold standard) of diagnostic testing for the detection of infectious diseases. While this reaction is predominately performed as a send-out test to a reference laboratory, I suspect that patient side PCR testing will become available and a part of everyday practice within your veterinary career. What exciting times we live in!

These next set of pages will guide you through the steps of how the sample is prepared, analyzed, and the results that are generated from a general PCR reaction.

## What is Polymerase Chain Reaction (PCR)?

The polymerase chain reaction (PCR) is a common laboratory technique that amplifies a particular region of genetic material (DNA or RNA transcribed into complementary DNA) by making millions of copies in a sample. This is advantageous from a clinical standpoint as we often have samples that contain very little of an organism that is not in high enough concentration to be easily cultured or we have organisms that are in abundance but cannot be easily cultured using routine methods (i.e. *Mycobacterium* sp., *Mycoplasma*, *Lawsonia* sp., viruses and/or parasites in general).

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## Knowledge check



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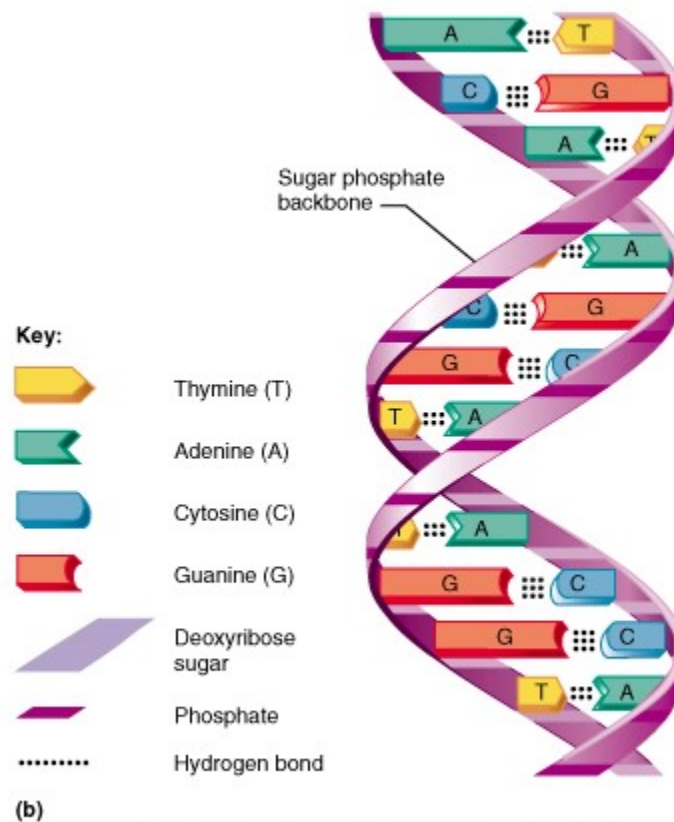
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## How does PCR work?

As mentioned previously, PCR is a technique that amplifies genetic material in a sample, but how exactly is that genetic material amplified?

PCR is essentially using the same replication principles that occur in living organisms but executing them in an artificial setting. As a reminder of that process let's review a little biology.

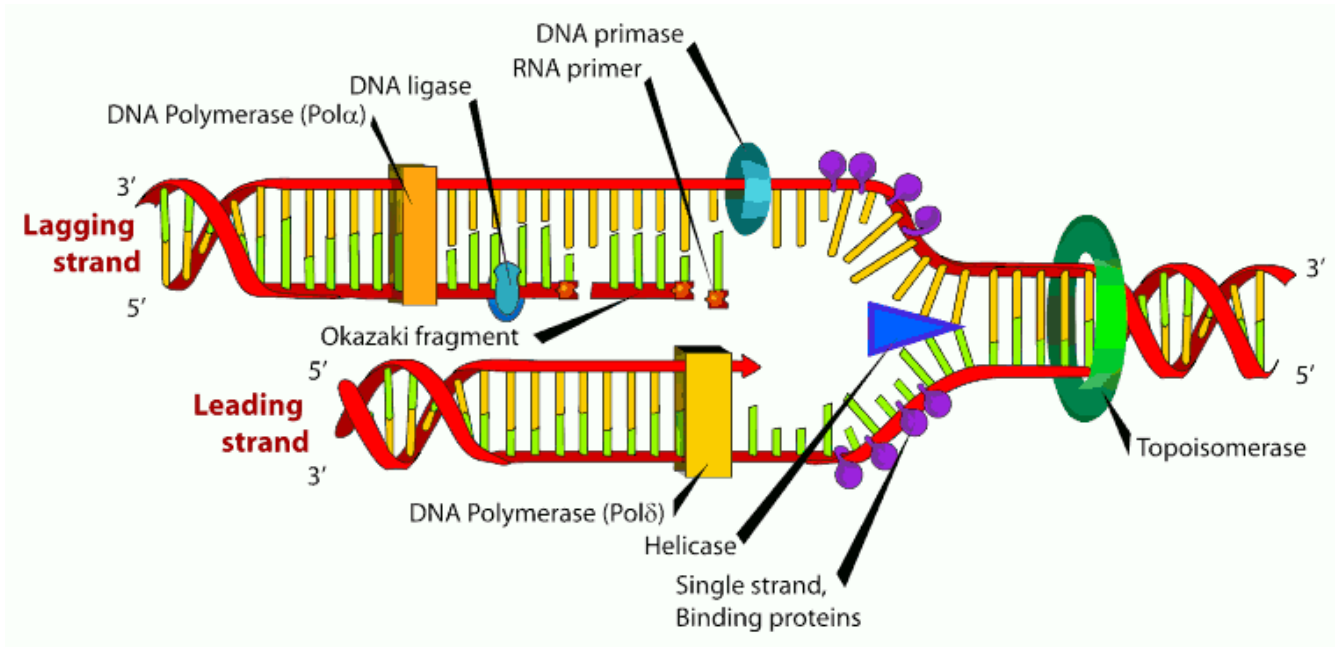
DNA is a molecule composed of two strands of genetic material that coil forming a double helix structure comprised of a sugar-phosphate backbone and individual nucleotides residing in the center of the structure.



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### DNA molecule

In normal biological replication cycles, when a portion of the DNA is needed to be replicated or transcribed to make another cell, proteins, etc., the DNA needs to be “unzipped” to allow for a DNA polymerase and RNA primers to attach and transcribe the targeted portion of the DNA strand. (See below)



## DNA Transcription

These same processes occur in vitro during the PCR's “*sequential cycles of synthesis*” following these 3 basic steps.

- 1. denaturation** or breaking apart of the two strands of DNA using extreme heat
- 2. primer annealing:** linking primers targeting specific sequences to the template DNA
- 3. primer extension** by the polymerase & synthesis of new strands

Let's take a closer look at these steps.

## Knowledge check



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# MODULE 4.2: DENATURATION, ANNEALING, AND PRIMER EXTENSION

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## PCR Ingredients

Before starting the PCR reaction, there are 4 key ingredients for the reaction to take place.

### 1. *Taq* polymerase

Similar to DNA replication that occurs under normal biological conditions, the PCR requires a DNA polymerase to make the new strands of DNA. The polymerase that is used in the PCR reaction is called “*taq* polymerase”, as it was originally isolated from the heat-tolerant bacterium *Thermus aquaticus* that normally live in hot springs and hydrothermal vents. Because of its lifestyle, it is most active at a temperature around 70°C (158°F).

### 2. Primers

A primer is a short sequence of nucleotides that provides the starting point for the DNA polymerase (*taq*) to attach to. These primers are used to flank the region of the DNA that we want to be copied. These primers bind to the complementary strand of the DNA template. The primers are created artificially in a laboratory setting and added to your sample during the preparation process. This means that scientists and researchers must identify unique sections of the DNA code to create a specific primer for each disease.

### 3. Template DNA

This is the strand of DNA the *taq* polymerase is going to use for amplification

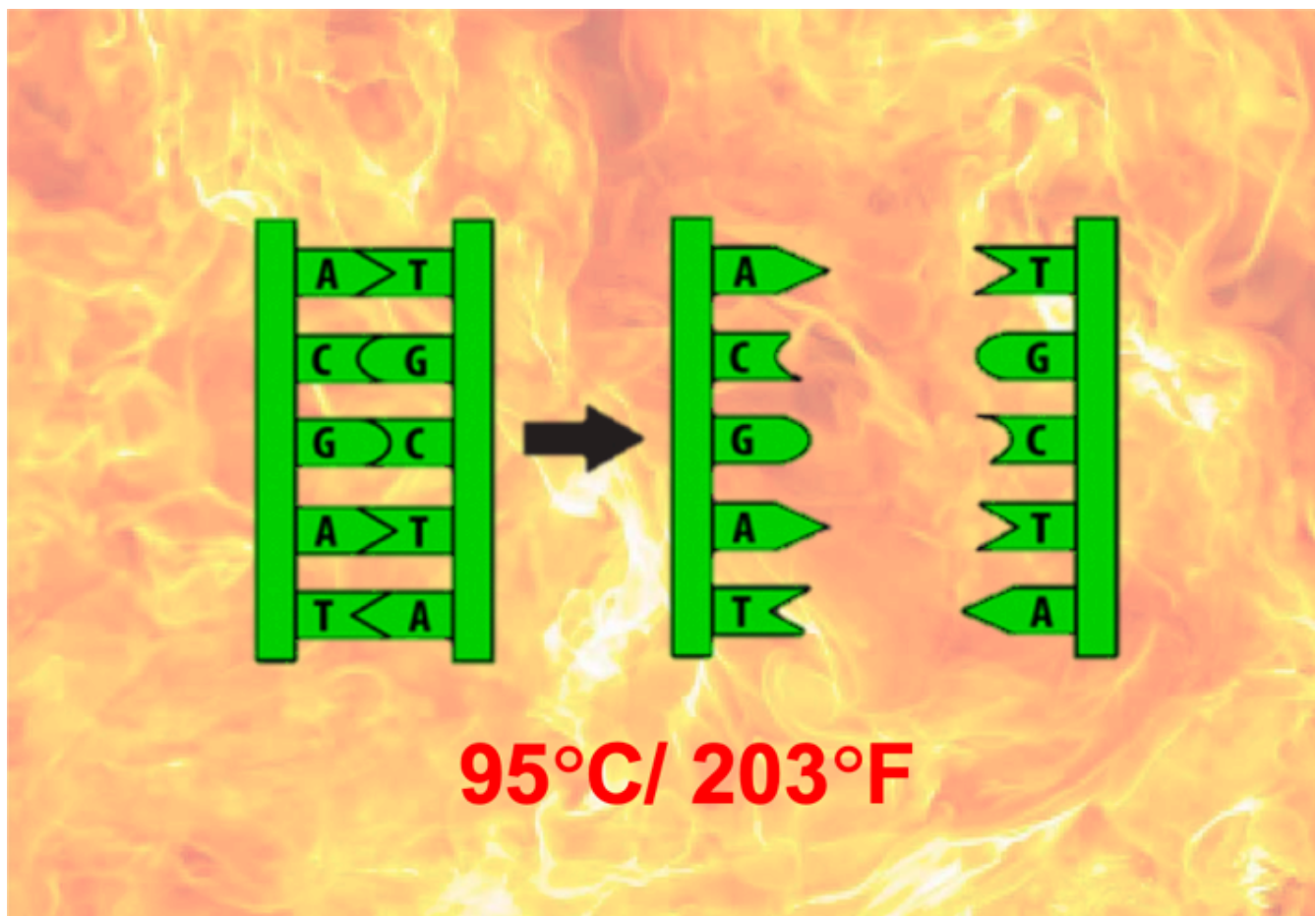
## 4. Nucleotides

Free nucleotides are added to the sample for the polymerase to use to synthesize the new strand of DNA aka the building blocks of the reaction.

## PCR: 3 basic steps

### Denaturation

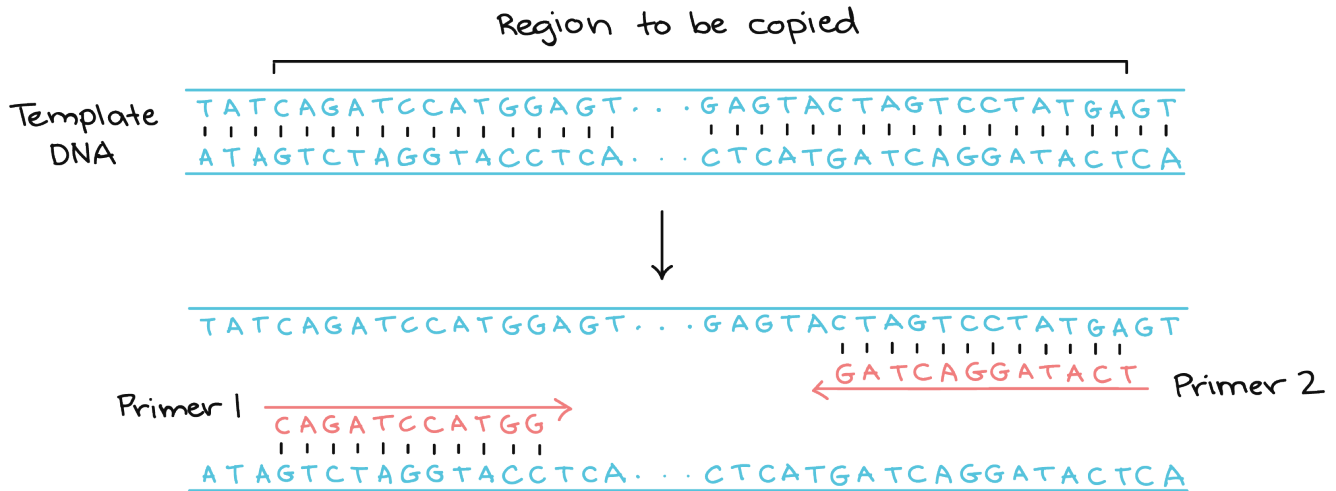
In vitro, we cannot replicate all of the biological intricacies that occur in the nucleus of a cell, however, we do know that under extreme heat, that the double helix will break apart. Therefore, the first step of the PCR reaction is to subject the DNA to **extreme heat** (95°C/ 203°F) to break apart the two strands forming the **template DNA**.



DNA denaturation under extreme heat

## Annealing

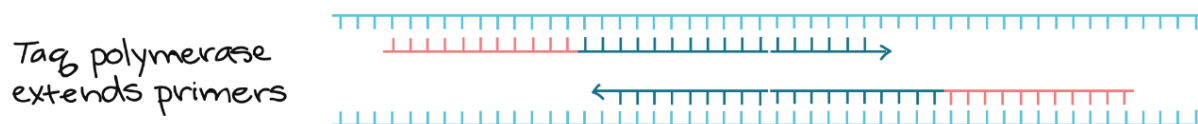
Now that we have successfully denatured the DNA, the temperature is too high for the **primers** to attach the **DNA template** so the reaction is **cooled down** to temperatures between 55-65°C (131-149°F).



Primer annealing under cooler temperatures

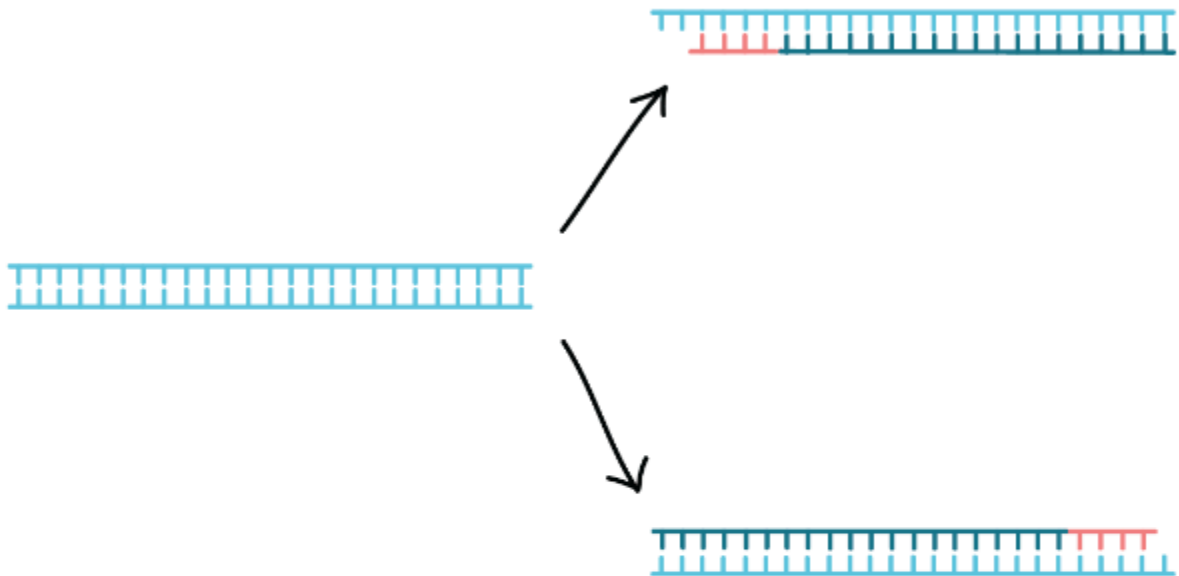
## Primer extension

Once the primers have attached to the DNA template we are now ready for the *taq* polymerase to attach and begin adding nucleotides to create a new double-stranded piece of DNA. As mentioned above the *taq* polymerase is active at temperatures around 70°C (158°F), so the reaction is **heated** from 55-65°C to 70°C to allow activation of the *taq* polymerase.



Primer extension under higher temperatures

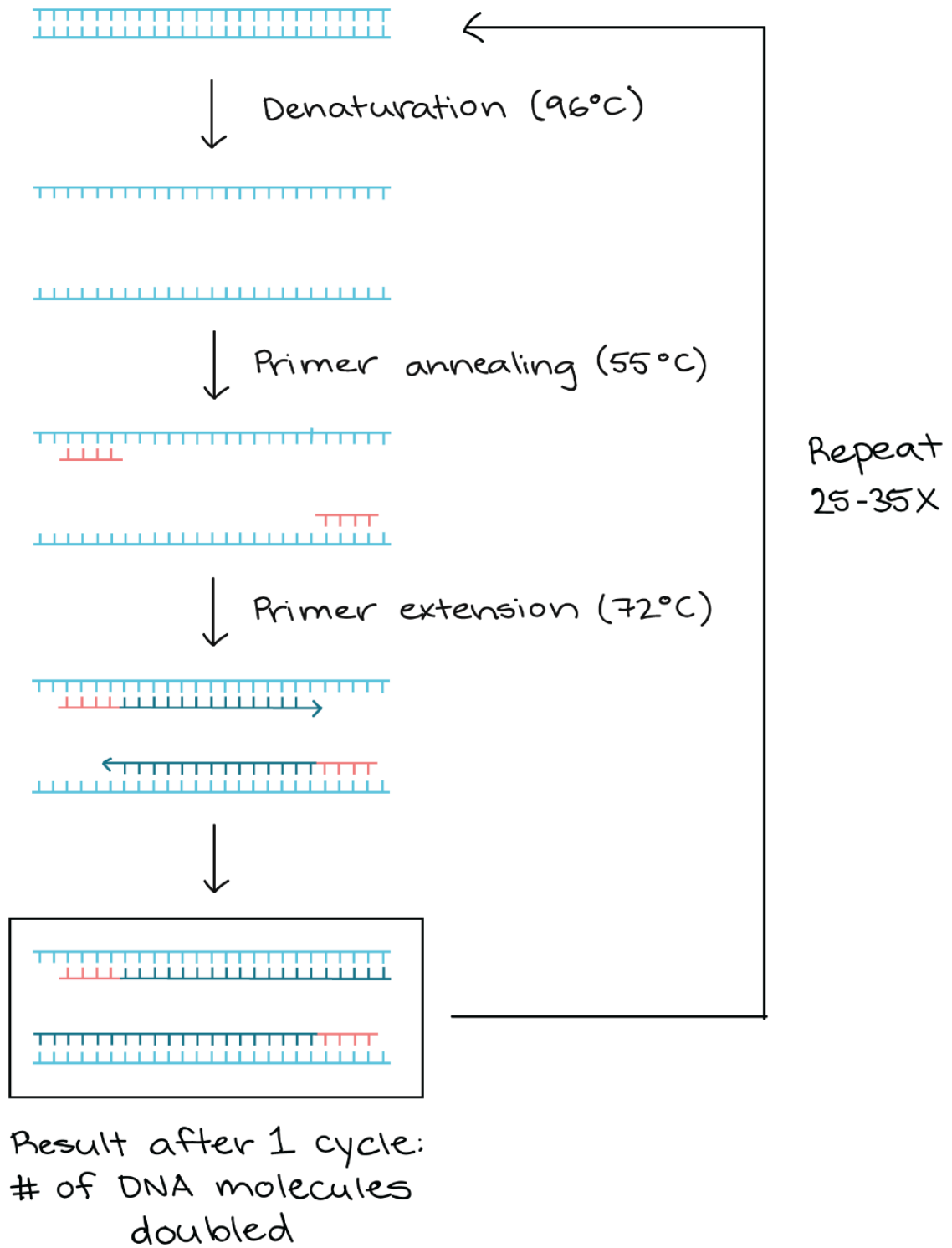
These **3 steps** together make up a PCR **single cycle**. Therefore, at the end of a single cycle, if we started with one double strand of DNA in a sample we will end up with two at the end of the cycle, doubling the number of DNA strands we began with.



cycle: 1

#### A single PCR cycle

In a typical PCR reaction, the cycle is repeated 25-40 times and takes approximately 2-4 hours, depending on the length of DNA needing to be copied (see image below). The reason for this is that after the first cycle, it is no longer only the original DNA template that is being used for amplification, but also the new strands that are used in the reaction too. Additionally, we do not just add one *taq* polymerase or primer to the sample, we add several to float around the reaction, so the **number of DNA molecules can roughly double with each round of cycling**.



The number of DNA strands doubles with each round of cycling

## Knowledge check



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# MODULE 4.3: INTERPRETATION OF RESULTS

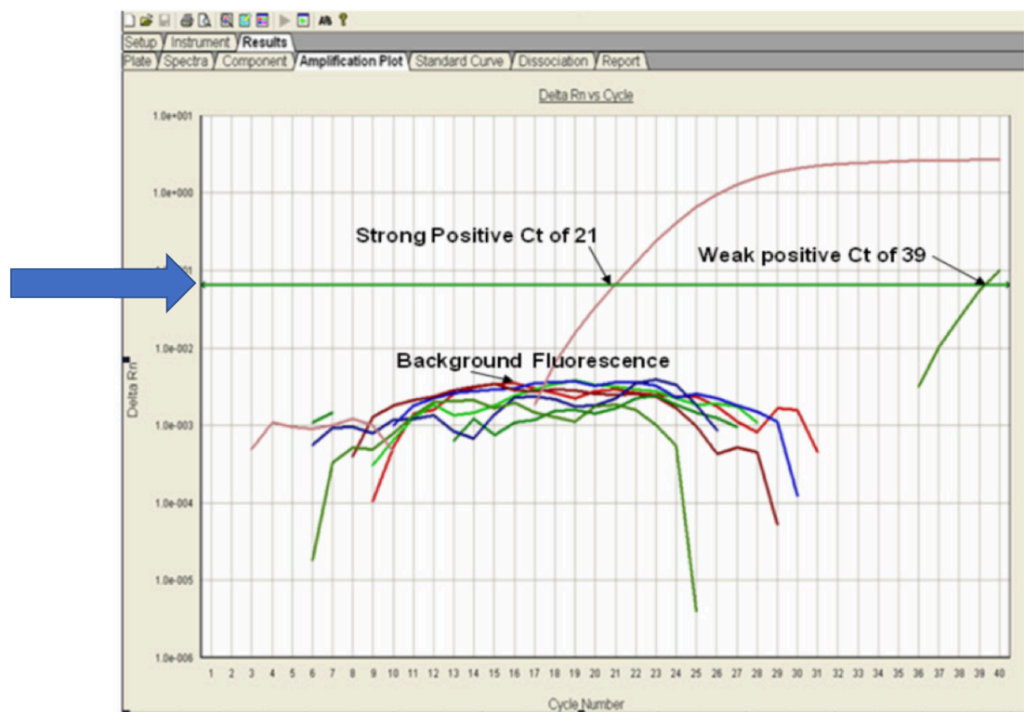
## Interpretation of results

In veterinary medicine, one of the most common PCR reaction modalities is Real-Time PCR (RT-PCR) in which sample amplification is detected by the accumulation of a fluorescence signal after every cycle (thus we can monitor it in real-time). This is in contrast with conventional PCR, where we can only verify amplification using gel electrophoresis after the 40 cycles.

The value or result that is generated and reported to the veterinarian is called the **cycle threshold (Ct)**. The Ct is defined as the number of cycles required for a fluorescent signal to cross the threshold (i.e. exceed background level). This means that the Ct level is **inversely proportional** to the amount of DNA in the sample. In other words, the **lower** the Ct level, the **greater** the amount of the target DNA is present in the sample.

Let's take a look at an example of the graph (below) generated in the lab from an RT-PCR reaction.

Baseline level of fluorescence needed to rule out background noise



Graph generated from an RT-PCR reaction

Let's orient ourselves to the graph above. This graph has several samples running at once for comparison of data. Each colored line represents a single sample except for the straight green line (denoted) which is the threshold of fluorescence needed to exceed rule out background noise.

In this laboratory, they run each sample for 40 cycles (X-axis), and along the Y-axis is the fluorescence level emitted by the DNA amplified.

Taking a look at the data, we have two samples that penetrate the fluorescence threshold and considered true positive reactions. For the red sample, it took 21 amplification cycles (Ct) to reach the fluorescence threshold and for the green sample, it took 39 Ct's. Since it took fewer cycles to detect the DNA for the red sample, we can say that the red sample contains more DNA than the green sample. This is specific for this run, samples from two different runs should not be compared (e.g. sample A from run 1 had a Ct of 20 and sample B from run 2 had a Ct of 30 does not mean that sample B has less DNA than sample A).

For this sample type, the reference laboratory has set up a reference interval for the evaluation of the data. These reference intervals are laboratory and sample source-specific so you cannot compare quantified PCR results across laboratories or different source types.

## Results for the above reactions

### Red sample:

Ct: 21

### Green sample:

Ct: 39

## Laboratory Reference Interval Interpretation

Cts < 29 are strong positive reactions indicative of abundant target nucleic acid in the sample Cts of 30-37 are positive reactions indicative of moderate amounts of target nucleic acid Cts of 38-40 are weak reactions indicative of minimal amounts of target nucleic acid which could represent an infection state or environmental contamination

Based on the laboratory reference intervals we can say that the **red** sample is a **strong positive** and we are confident that there is a large amount of the targeted DNA present in this sample. For the **green** sample, it was a **weak positive** and there is a small amount of the targeted DNA present in this sample, or it is a product of environmental contamination. This should be interpreted with caution. What we cannot determine for PCR is if this DNA detected is from a viable or alive organism or not only that there is targeted DNA present.

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## Knowledge check



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# MODULE 4.4: REAL-LIFE EXAMPLE

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## Clinical Scenario

You are working in a large shelter and there has been a recent outbreak of kennel cough. You decide to run an RT-PCR Respiratory panel to get a better understanding of the potential respiratory pathogens in the shelter and aid in the development of isolation, control, and vaccination protocols. You chose 4 sick dogs to run the RT-PCR respiratory panel on. The following are results from the Pneumovirus RT-PCR data on those 4 sick dogs.

### Pneumovirus RT-PCR

Item	Result
1      1575568 5 - Canine Mixed Dog Spayed	Negative Ct value: Undetermined
2      1577981 - Canine Terrier, Nos Male	Positive Ct value: 36.0251
3      1577133 - Canine Staffordshire Bull Terrier Male	Positive Ct value: 20.1314
4      1578544 - Canine Labrador Retriever Male	Positive Ct value: 37.0096

RT-PCR respiratory panel results for 4 sick dogs

## Reference interval interpretation

**Table 4.1: Reference interval interpretation for RT-PCR respiratory panel**

Cycle Threshold (Ct)	Interpretation
Ct < 29	Strong positive
Ct 30-37	Moderate positivity
Ct >38	Weak positive

## Knowledge check



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### Key Takeaways

- PCR is an amplification technique that allows for the detection of genetic material in very small amounts
- PCR testing is not always the best choice as an infectious disease screening tool
- This test is very sensitive and very specific
- High CT values may be an indicator of a false positive

You have now reached the end of Module 4. If you are enrolled in CVM 6925, please go to the Canvas page and take the quiz: “Module 4: Intro to PCR quiz.” There is not an assignment or in-person laboratory associated with this module.

## MODULE V

# MODULE 5: INTRODUCTION TO MICROBIOLOGY STAINS

### Module Objectives

1. Compare and contrast the general appearance of Gram-positive, Gram-negative, and acid-fast bacteria when using Romanowsky, Gram, and acid-fast stains.
2. Describe the counterstains for Gram and acid-fast stains.
3. Create clinical scenarios for each of the recommended stains.



# MODULE 5.1: CYTOLOGICAL STAINS

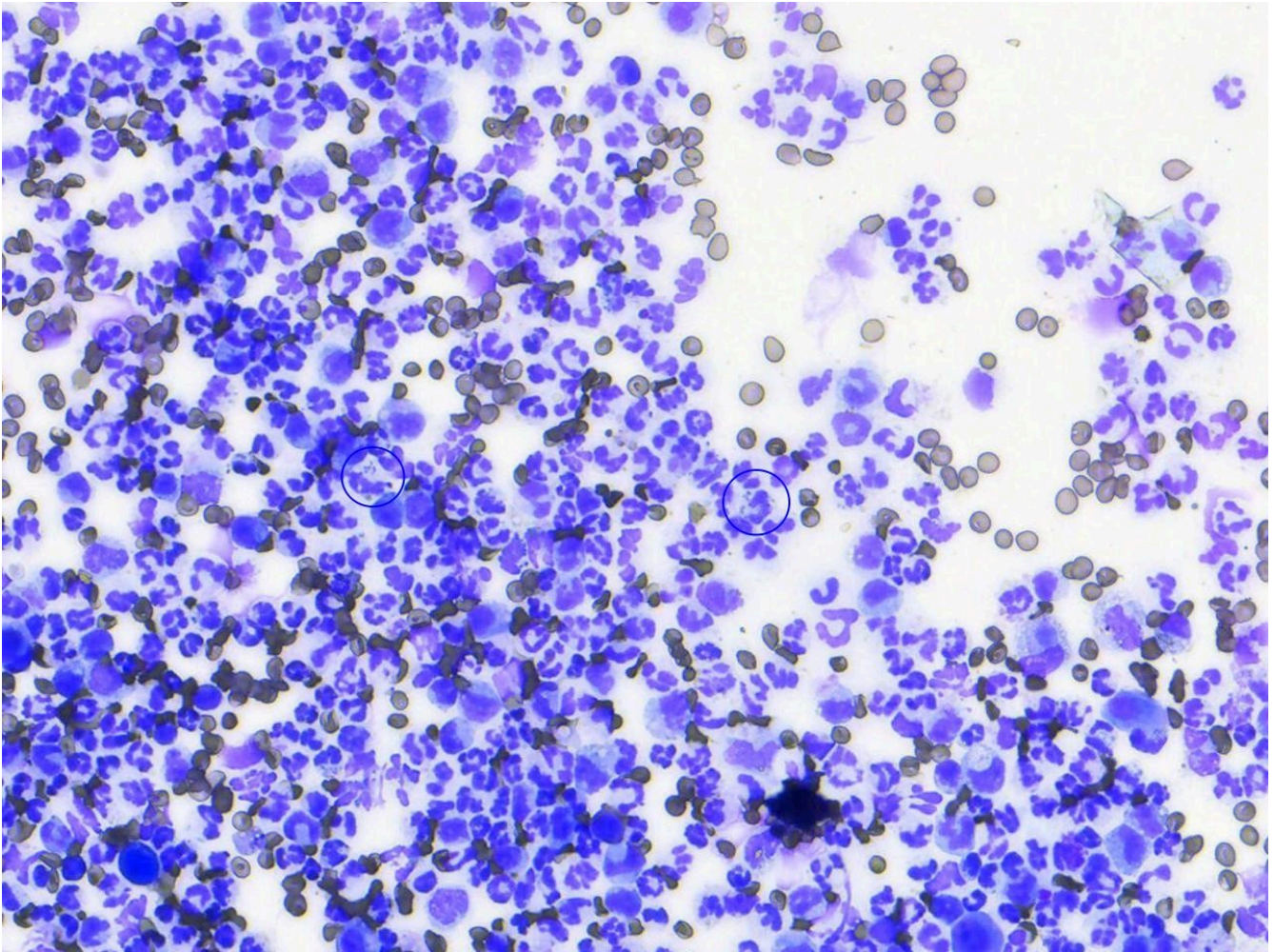
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## Introduction to stains

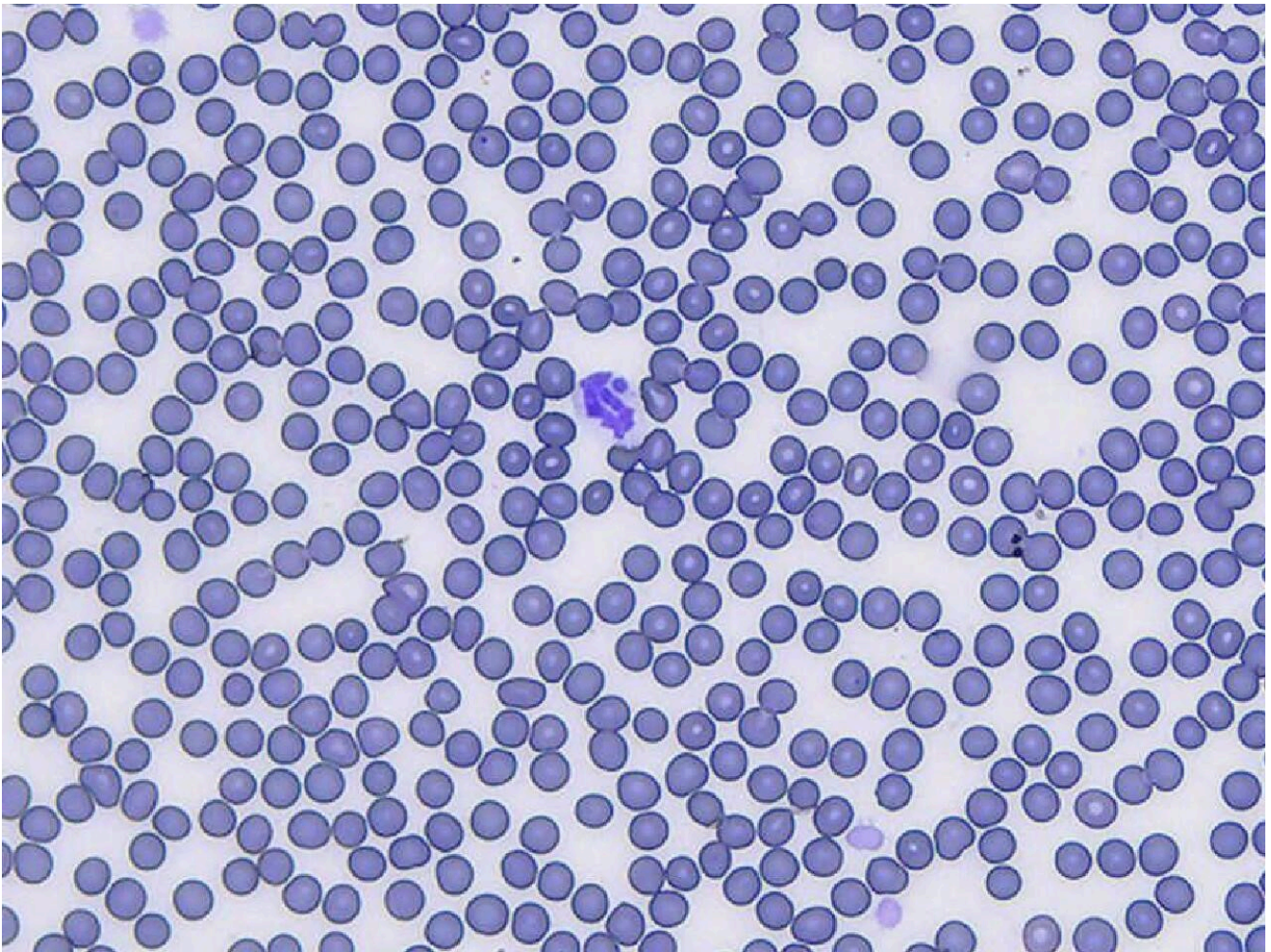
There are myriad of stains that are available in veterinary medicine for the diagnosis of various diseases. These stains can be divided into two broad categories based on specific goals. These include those that are used for cytology or evaluating cellular morphology and microbial stains.

## Cytological stains: Romanowsky type stain

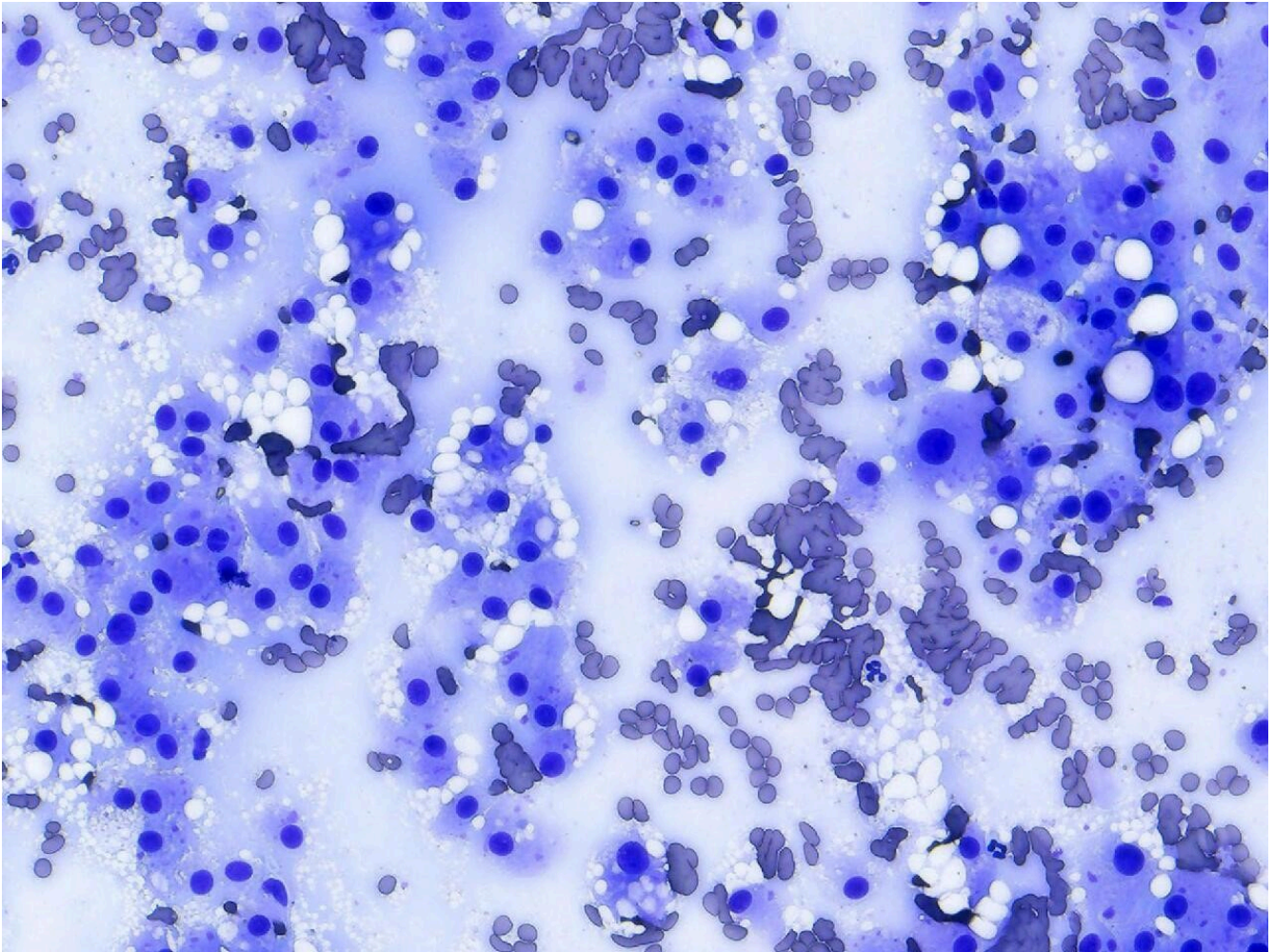
In veterinary medicine, the most common cytological stains are Romanowsky-type stains, such as Diff Quik or Wright-Giemsa stains. The goal of cytological staining is to highlight cellular morphology, specifically nuclear and cytoplasmic details. While these stains do stain common microbes and can be especially useful for identifying protozoal, microfilaria, and fungal agents, these stains are particularly useful in characterizing host structures, such as inflammatory cells (neutrophils and macrophages), neoplastic cells, or resident cellular components. All bacteria, regardless if Gram-positive or negative stain **blue or deep purple** using Romanowsky types stains.



Fine-needle aspirates from an abscess in a dog. In this image, you can see that there is marked suppurative inflammation characterized by many degenerate neutrophils and low numbers of macrophages. Degenerate neutrophils containing phagocytized bacteria are circled. 100x objective, Diff Quik.



Canine blood smear with a single segmented neutrophil that contains a single *Anaplasma phagocytophilum* morula. The morula is the blue, circular structure on the northern end of the cell. You can appreciate that the bacteria in the abscess and the bacterial morulae in this smear are the same blue color. 100x objective, Diff Quik.



Liver aspirates from a dog with well-differentiated hepatocellular carcinoma. You can see that the neoplastic hepatocyte nuclei are round, 2x the diameter of an RBC, and placed in the center of the cells. The cytoplasm is the pale blue color that surrounds the nuclei. Sometimes the cytoplasm has variably sized lipid droplets within the cytoplasm. 50x objective, Diff Quik

## Procedure:

In the laboratory, you will be given step-by-step instructions on how to perform the Diff Quik procedure. Here is a video that also explains the steps.



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## Knowledge check



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# MODULE 5.2: MICROBIAL STAINS

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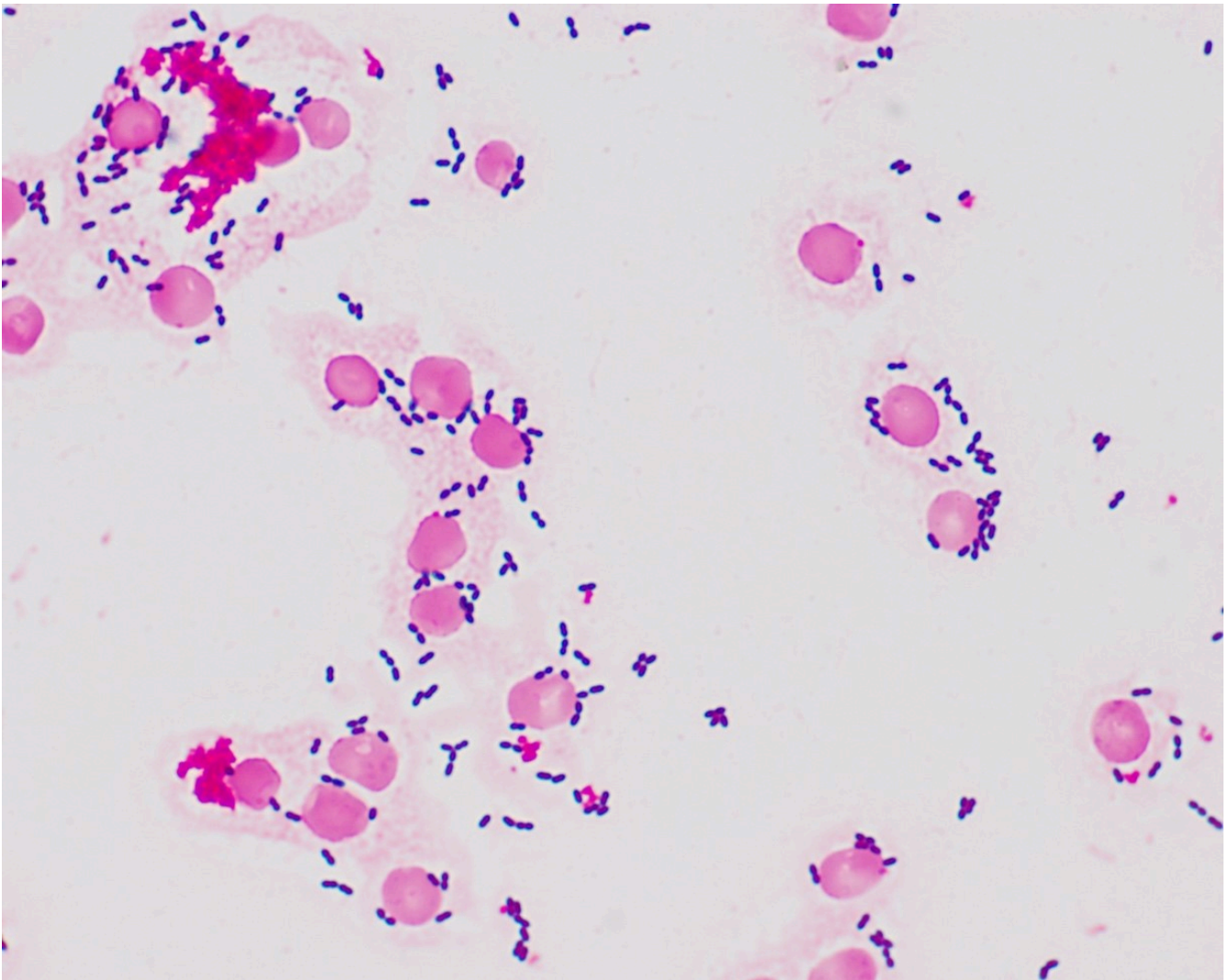
## Microbial stains

A few of the common microbial stains used in veterinary medicine include Gram stain and Acid-Fast stain. In our laboratory, we are going to focus on Gram's stain and acid-fast.

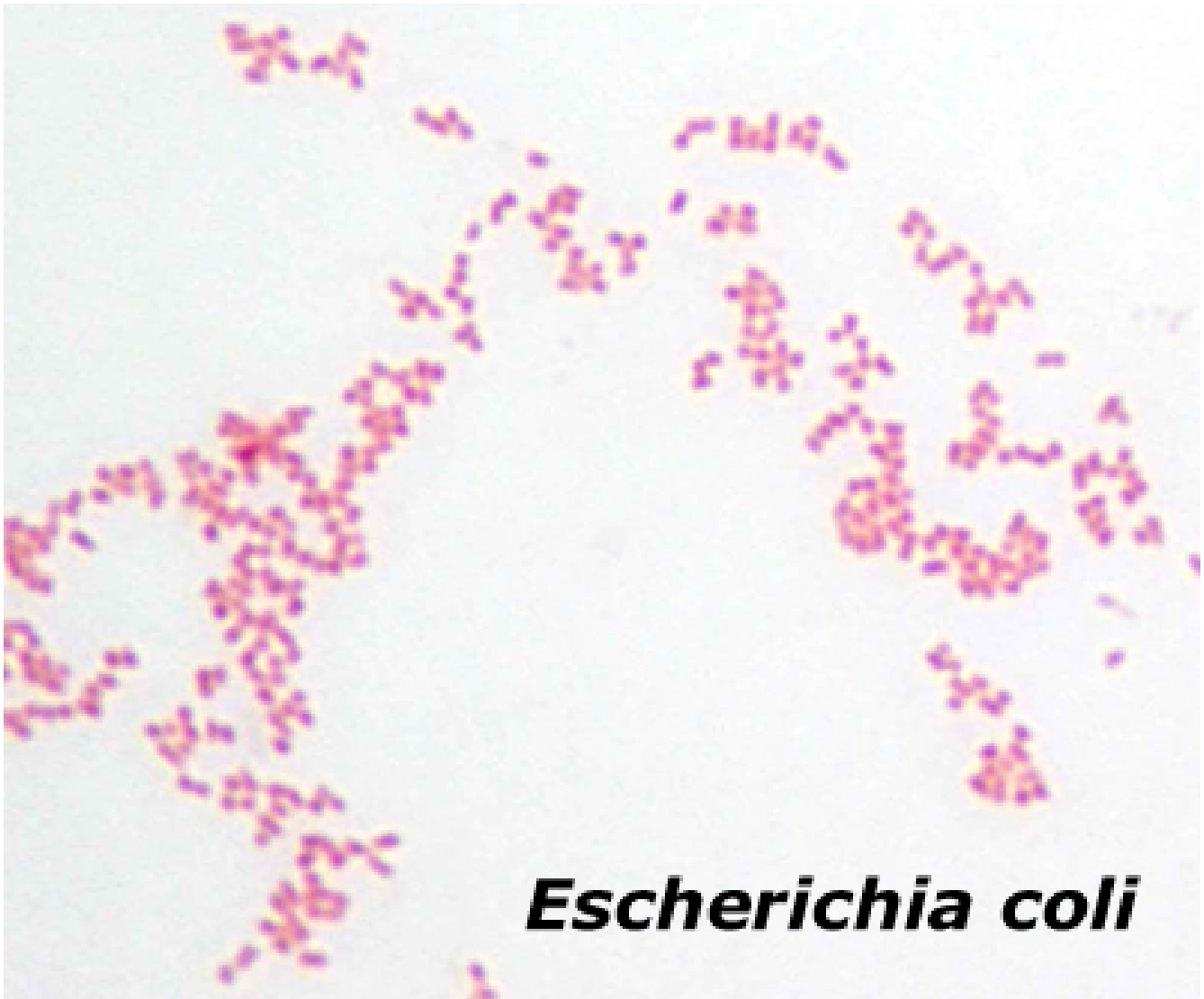
### Gram stain

Gram stain is a common technique used to separate bacteria into two large categories; Gram-positive and Gram-negative based on cell wall characteristics. The thick peptidoglycan layer in the Gram-positive bacteria's cell wall retains the crystal violet stain giving a **deep purple** color. Gram-negative bacteria have a thinner peptidoglycan layer that does not retain the crystal violet after a decolorization step and application of a red dye counterstain called Safranin, resulting in their **red** appearance. In veterinary medicine, we can use knowing if a bacterial population is Gram-negative or positive to help guide initial antimicrobial therapy while waiting for pending culture results.

It is important to remember that these color patterns are originally based on cellular properties following *isolation from pure bacteria culture*. While these features also remain in vivo, antimicrobial administration and phagocytosis of bacteria break down their cell wall resulting in inconsistent Gram staining features. I.E. Gram-positive bacteria may appear Gram-negative.



Canine blood smear that is stained with a Gram stain. In the image, you see many deep purple diplococci that are free and associated with RBCs (the large red circular structures). Note that the RBC picks up the Safranin counterstain. WBC will have a similar staining feature EXCEPT the nuclei will stain Gram-positive. WBC nuclei make great internal controls. (100x)



## ***Escherichia coli***

*E. coli* bacteria harvested from a pure culture. In comparison to the blood smear above, you can see the pale red appearance of the Gram-negative bacteria in this image. (100x)

### **Procedure**

In the laboratory, you will be given step-by-step instructions on how to perform a Gram stain. Here is a video that also shows the steps.



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# Knowledge Check



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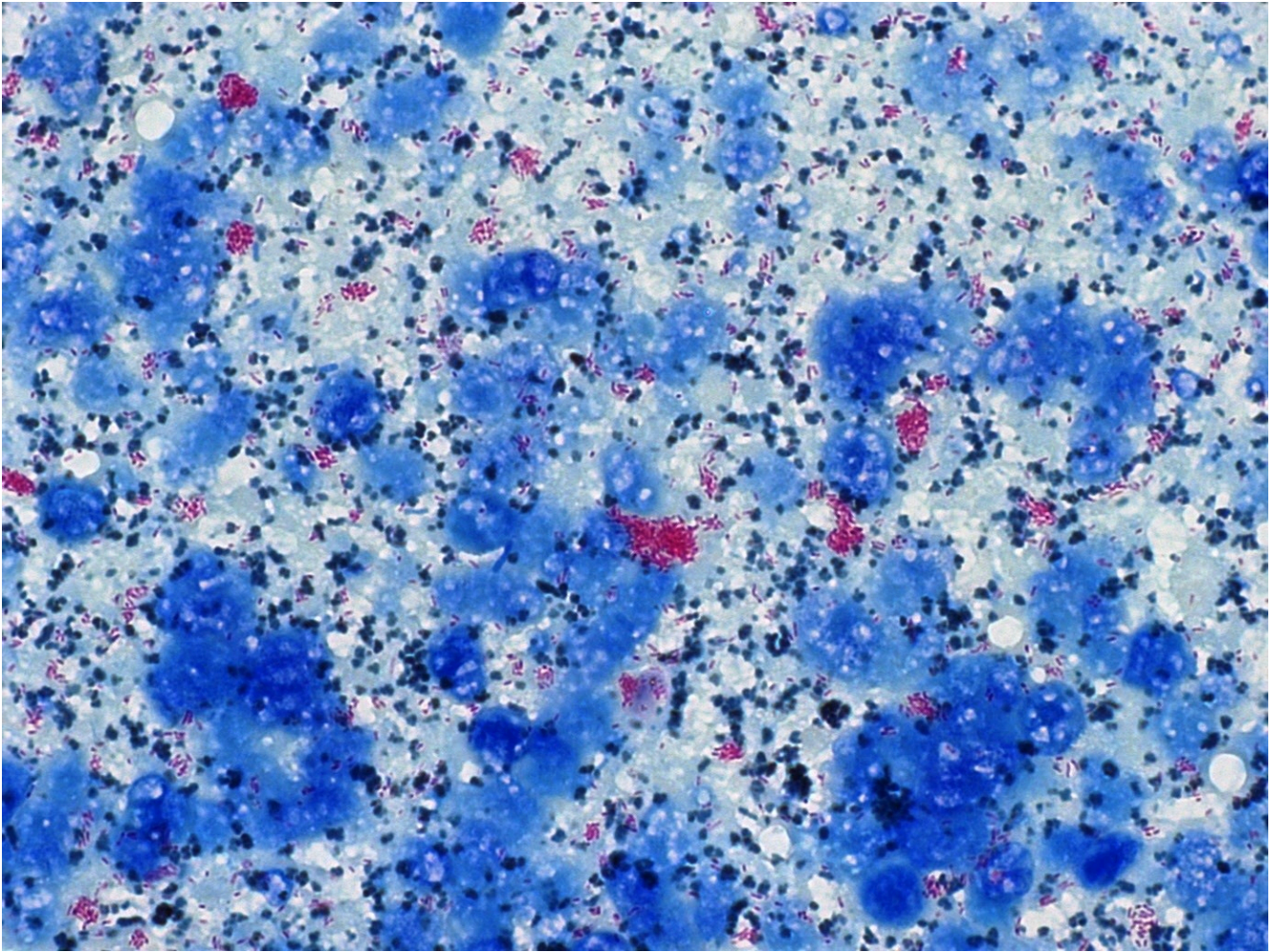
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## The acid-fast stain

Certain bacteria, most notable *Mycobacteria*, have **lipid components within their cell walls** that make them resistant to taking up stains such as Romanowsky-type stains and Gram stains. Thus, acid-fast stains are used to differentiate acid-fast bacteria from non-acid fast bacteria.

The principle of these stains is the **carbol fuchsin** solubilizes the lipid present in the cell wall resulting in the bacteria appearing **pink** or **red** and retain that color after decolorization with alcohol. Non-acid fast organisms lack the lipid material in their cells wall following decolorization leaving them colorless. The sample is then stained with a counterstain, most commonly **methylene blue** or **malachite green**. Only decolorized cells absorb the counterstain and take its color and appear **blue** or **green** while acid-fast cells retain the **pink** or **red** color.



In the image above from a fine needle aspirate of a skin lesion, you see a tremendous amount of cellular debris that is picking up the methylene blue counterstain. Amongst the cellular debris is many individualized and aggregates of rod-shaped, acid-fast bacteria that appear bright red.

## How the acid-fast technique is performed in a laboratory



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## Summary of stains

Below is a summary of the staining characteristics of bacteria with each stain:

**Table 5.1: Summary of stains**

	Gram-positive	Gram-negative	Acid-fast
Romanowsky-type	Purple	Purple	Colorless
Gram Stain	Purple	Red	Colorless
Acid Fast	Green/Blue	Green/Blue	Red

## Knowledge check



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### Key Takeaways

- Cytological stains, such as Romanowsky stains, are designed to highlight cellular features (i.e. nucleus, cytoplasm, rod, cocci)
- Microbiology stains are often indicated when infectious agents, commonly bacteria, are seen using cytologic stains

- The most commonly used microbiology stains in veterinary medicine help differentiate bacteria based on cell wall characteristics

You have now reached the end of Module 5. If you are enrolled in CVM 6925, please go to the Canvas page and take the quiz: "Module 5: Intro to stains quiz"

## MODULE VI

# MODULE 6: BLOOD SMEAR TECHNIQUE AND RETICULOCYTE COUNTING

### Module Objectives

1. Identify on a blood smear the 3 "anatomic" structures of the film (body, monolayer, and feathered edge)
2. Paraphrase why larger cells migrate to the feathered edge
3. Recite how you can recognize the monolayer using a microscope
4. Differentiate between an aggregate and punctate reticulocyte
5. Use the terms polychromatophil and reticulocyte appropriately based on the stain used



# MODULE 6.1: PREPARING A DIAGNOSTIC BLOOD SMEAR

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## Preparing a diagnostic blood smear

Making a blood smear or film is a skill that cannot be acquired in a single day. This skill must be practiced over and over again. However, hopefully, this exercise will give you the appreciation of how valuable hiring someone competent in making blood smears is and how challenging it can be to perform an accurate WBC and platelet count off of a sub-optimally prepared smear.

All blood smears have 3 essential components:

### 1. Feathered edge

The very distal region of the blood smear is formed at the end of your blood distribution. Under magnification, this area appears similar to the anatomy of a feather with the individual barbs. The feathered edge is useful for looking for platelet clumping, microfilariae, large neoplastic cells, and other large cells that are heavy and drifted to the edge.

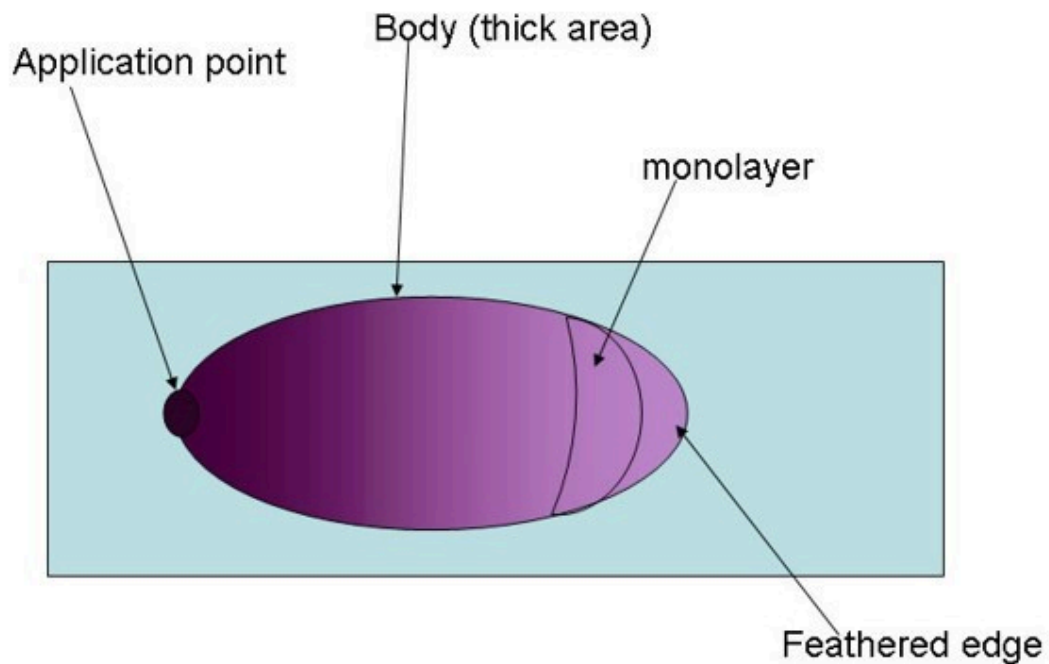
### 2. Monolayer

The monolayer is the zone in which there is an equal distribution of erythrocytes (RBC) and leukocytes (WBC), and lies between the body and the feathered edge. Blood smears are shaped similar to a feather, so the monolayer itself is shaped in an arch. *This region is where we perform 100 WBC count, platelet counts, and evaluation of erythrocyte morphology (i.e. schistocytes, spherocytes, acanthocytes, etc.).* When performing a 100 nucleated cell count you will need to adjust to the monolayer as you go (i.e. you cannot just move straight across your slide)

### 3. Body

It can help you identify agglutination or rouleaux, but these findings should always be confirmed in the monolayer as abnormalities seen in the body will also be observed in the monolayer.

# Zones of a blood smear



Zones of a blood smear

## Knowledge check



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# MODULE 6.2: BLOOD SMEAR PROCEDURE

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## Criteria to determine acceptability:

How do I know if I am reviewing a diagnostic slide?

### Acceptability Criteria

1. The smear is smooth, with no ripples due to the jerky movement.
2. Holes do not appear in the blood smear.
3. Extends at least 2/3's of the slide.
4. The smear is spread across (side to side) both sides of the slide to the edge.
5. Rainbow sheen at the end of the slide. (feathered edge and monolayer)
6. Smear begins 0.5 inches from the base of the slide or 4mm from the frosted edge
7. The slide is labeled with the patient identifier and type of sample (in this case, "blood film")

It is important to note that this technique is optimized for a **healthy** patient. If an animal is anemic, then you will need to *increase* your angle to ensure the appropriate spreading of the blood. Conversely, if the animal is hemoconcentrated (most commonly from dehydration), you will need to **decrease** your angle.

Another important thing to remember is that the erythrocytes and white blood cells are ***in suspension*** in your EDTA tube. This means that as your tube sits on your benchtop, the WBC and RBCs begin to settle to the bottom of the EDTA tube. It is imperative that you ***gently*** rock the tube back and forth to re-suspended the RBCs and WBC's.

**What happens if you do not re-suspend your blood sample and allow the cells to settle?**

- May artifactually make your patient appear anemic
- Heavy cells, such as neoplastic cells, will settle to the bottom and may not end up on the smear
- May artifactually make your patient look leukopenic
- Will skew your WBC differential as WBC settles to the bottom at a different rate.

***This same settling phenomenon occurs on the drop of blood you place on your glass slide as well! So be sure not to let your drop sit for more than a few seconds without spreading your sample.*** Once you place the drop of blood on the slide, be sure to be ready to spread the blood to avoid settling of cells on your slide.

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## Knowledge check



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## How to make a blood smear

In the laboratory, we will not be practicing the blood smear technique, as this is a task typically performed by veterinary paraprofessionals. It is outlined here for your reference in the future.

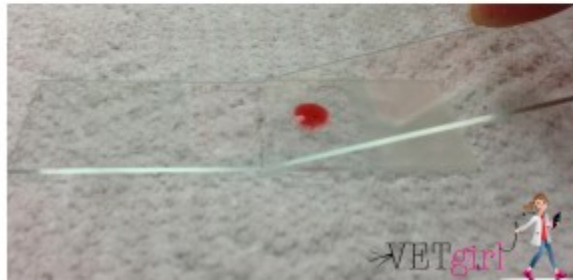
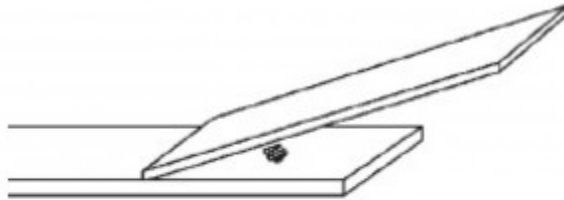
Supplies needed for preparing a blood smear:

- 2 glass slides
- Immersion oil
- Blood sample

- Microscope

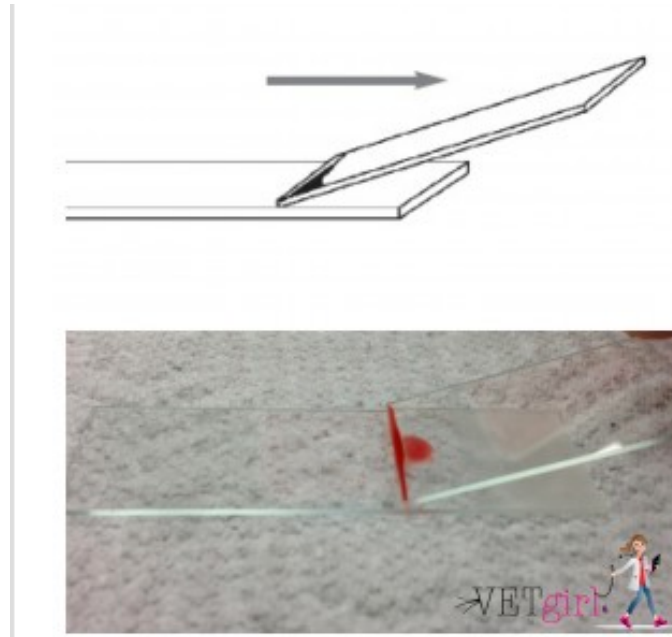
## Steps

1. On one slide (the sample slide) place a small drop of *well-mixed* blood. If the blood is not well mixed the WBCs will settle to the bottom of the EDTA tube or syringe.



Positioning the spreader slide behind the drop of blood

2. Use the second slide as a spreader slide
3. Place the end of the spreader slide on the sample slide so that the short-sided edge of the spreader is below the drop of blood
4. Hold the spreader slide at an angle of 30-45° (relative to the sample slide) and bring the spreader slide back against the drop of blood so that the blood spreads in a thin line via capillary action



Blood spreads in a thin line via capillary action

5. Rapidly-but gently –drag the spreader slide along the entire length of the sample slide in one fluid motion
6. If the technique was performed correctly, the smear should end before the end of the sample slide in a “feathered edge”
7. Air-dry the sample slide (NEVER HEAT FIX). Fix and stain (Diff Quik) the slide



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## Knowledge check



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online here:

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### Key Takeaways for Blood Smears

- The feathered edge is where large cells and large organisms migrate to (i.e. neoplastic cells, microfilaria)
- The monolayer is the zone in which there is an equal distribution of erythrocytes (RBC) and leukocytes (WBC) and lies between the body and the feathered edge.
- An acceptable blood smear from a healthy animal will have all 3 zones.
- The monolayer is where we perform 100 WBC count, platelet counts, and evaluate erythrocyte morphology
- Depending on the PCV/HCT, the angle at which you hold the slider may need to be increased or decreased
- Always mix your blood before preparing a blood smear

# MODULE 6.3: EVALUATING ERYTHROCYTE REGENERATION USING MANUAL RETICULOCYTE COUNTS

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## Manual reticulocyte counts

Assessment of regeneration is the very first step in the evaluation of anemia to determine if the anemia is regenerative or non-regenerative in dogs and cats. We do not routinely use manual reticulocyte counts in other species such as equids, small ruminants, camelids, or bovids because they either do not release immature RBC's into circulation when anemic or they do so inconsistently. Specific causes of regenerative and non-regenerative anemia will be discussed in your Clinical Pathology Course (Hematology), but in this laboratory, we will practice how to assess erythrocyte regeneration in our patients using **new methylene blue stain**.

In both normal cell turnover and anemia, immature erythrocytes are released into circulation. Reticulocytes are immature, non-nucleated RBCs that contain RNA and continue to synthesize hemoglobin even after they lose the nucleus (**metarubricyte** stage). The term "reticulocyte" is synonymous with the term "polychromatophil" observed on Romanowsky stain (Diff Quik, Wright Giemsa, etc.) and many of our benchtop hematology analyzers will provide either a reticulocyte percentage or a reticulocyte count. In cases in which you suspect regeneration and your analyzer does not provide a reticulocyte count or you suspect a concurrent **Heinz body** anemia, we use New Methylene Blue (NMB) stain to perform a manual reticulocyte count. Heinz bodies also stain with NMB stain.

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## Knowledge check



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# MODULE 6.4: RETICULOCYTE PROCEDURE

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## Reticulocyte smear

In the laboratory, you will be preparing a reticulocyte smear from patient blood for practice. This patient is likely not anemic so you will be also provided a pre-made canine reticulocyte smear for practicing reticulocyte enumeration. You are welcome to try to enumerate reticulocyte from the provided blood but it may not be the most rewarding experience.

## Supplies needed:

Brightfield Microscope with a **100x objective lens**

Specimen: Whole (K3) EDTA blood

## Procedure

1. Mix 3 drops of New Methylene Blue with 2 drops of well-mixed whole (EDTA) blood
2. Incubate the mixture at room temperature for 15 minutes
3. Resuspend mixture by mixing gently, but thoroughly
4. Make 3 blood smears
5. Allow to air dry

## Counting reticulocytes

After your sample has air dried you will perform a manual reticulocyte counting using the **100x objective**.

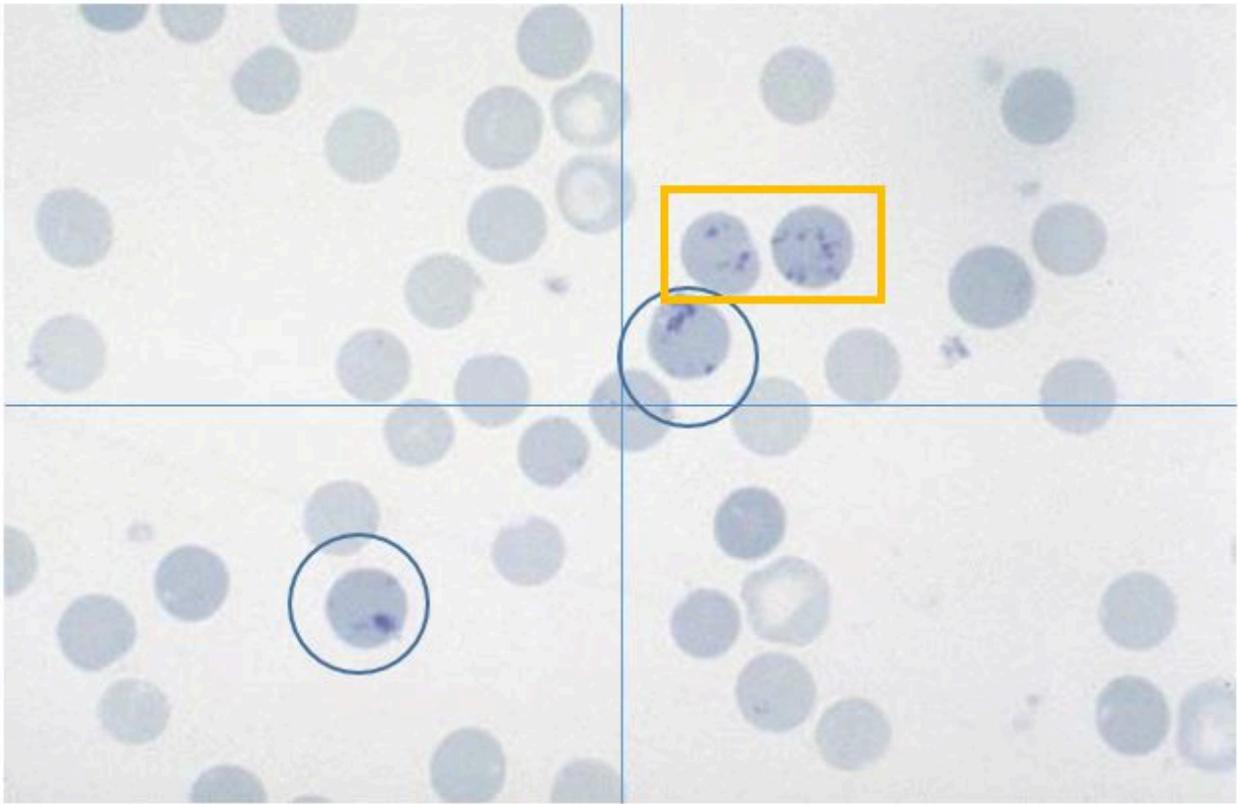
1. The first step for either a cat or dog is to identify a region of the blood film preparation where the RBCs are evenly distributed.
2. On a piece of paper, make two columns. Label one aggregate reticulocytes and the other RBCs. (See the example below. Each box represents a 1000x magnification field on your scope. )
3. Canine- Find an area on the blood smear regions of the slide that the RBCs are evenly distributed.  
Count the total aggregate reticulocyte per 1000 RBCs. *Dogs only release aggregated reticulocytes so count*

*all cells with stippling.*

- 4. Feline- In cats, the erythrocyte maturation process is a little different than dogs. Aggregate reticulocytes mature into punctate reticulocytes within 12-24 hours; punctate reticulocytes circulate for at least several days (7-10 days) before all the RNA is lost. *The reticulocyte count in cat blood should only include the percentage of **aggregate** reticulocytes*; punctate reticulocytes are not included in the standard reticulocyte count because they do not reflect the most recent bone marrow response (e.g. an anemic cat with only punctate reticulocytes is not actively regenerating at this time, but has shown some bone marrow regeneration in the past 7-10 days). Count only the total number of aggregate reticulocytes per 1000 RBCs.
- 5. Below is an image of a feline blood film. Count all the RBC in the field (including aggregate reticulocytes), then count all the reticulocytes. In this image, there are ~42 total RBC (not counting those along the edges that are cut off) and 2 aggregated reticulocytes

Table 6.1: Counting Aggregate Reticulocytes

Aggregate reticulocytes	Total RBCs
2	42



Feline blood film

**Yellow** square = Punctate; **Blue** circle = Aggregate

Reference interval: Feline aggregates: < 60,000 retic/ uL Canine aggregates: <80,000 retic/uL

6. You will continue moving randomly to 100x fields (even if they do not have reticulocytes) until you have reached 1000 RBCs. See the example below.

**Table 6.2: Counting Aggregate Reticulocytes Continued**

Aggregate reticulocytes	Total RBCs
2	42
5	54
9	45
0	50
1	49
3	59
0	48
2	52
1	45
4	52
0	42
1	61
2	36
5	62
6	60
7	44
1	51
0	52
3	45
2	47
<b>Total</b>	<b>Total</b>
54	996

## Calculation

In this feline patient, you have counted 54 aggregated reticulocytes per 1000 cells. The next step is to turn this into a percentage, also known as the reticulocyte %.

Reticulocyte % Equation

$$\frac{\# \text{ reticulocytes} \times 100}{\text{total \# RBCs counted}} = \text{Reticulocyte \%}$$

Case Example Calculation

$$\frac{54 \times 100}{996} = 5.42\%$$

## How do I interpret this?

Whenever we generate a percentage, you need to ask yourself. What is this a percentage of? For example, 5% reticulocytes in an animal with a PCV or HCT of 60% is different than an animal with a PCV or HCT of 12%.

There are several ways we can determine a value for the total number of erythrocytes. Generally, we use the **absolute reticulocyte count equation**, but in instances when we only have a PCV, we will use the corrected reticulocyte equation. Both equations account for the severity of anemia, but the absolute is considered more accurate. For a more thorough description of the two calculations, please see the [eClinPath](#) website.

## Absolute Reticulocyte Count Equation

$$\text{Absolute Reticulocytes}(/uL) = \text{reticulocyte \%} \times \text{RBC count (in million/uL)}$$

## Case Example Calculation

In our patient, we gathered the following data from the CBC.

- HCT: 15%
- RBC count: 1.5 million RBCs

$$\frac{5.42\%}{100} = 0.0542$$

$$0.0542 \times (1.5 \times 10^6) = 81,300 \text{ reticulocytes} / uL$$

## So what does this mean?

This value can help us determine the degree of regeneration to better understand the patient's regenerative response to anemia. We have species-specific reference intervals for these values.

**Table 7.3: Degree of regeneration and absolute aggregate reticulocytes**

Degree of regeneration	Feline Absolute aggregate reticulocytes (/uL)	Canine Absolute aggregate reticulocytes (/uL)
None	< 60,000	< 95,000
Mild	80,000	100,000
Moderate	100,000	300,000
Severe	≥ 200,000	≥ 500,000

Based on the table using the [eClinPath website](#) values (these values may vary by geographic region and are considered more of a guideline), our cat has approximately 81,300 reticulocytes per uL, so this cat has a mild regenerative response. How we interpret if this mild response is appropriate or expected will depend on the clinical signs and duration of anemia. This will be discussed more in your medicine and clinical pathology courses.

## Knowledge check



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### Key Takeaways for Evaluating Regeneration

- Reticulocytes are immature RBCs that are the stage just before a mature RBC and after a metarubricyte (nRBC)
- We can use supervital stains, such as new methylene blue to stain the retained ribosomal RNA for reticulocyte enumeration
- We use the term “polychromatophil” when we observe immature RBCs when using Wright Giemsa or DiffQuik. We use the term “reticulocyte” when we use new methylene blue to view and evaluate RBCs.
- We only count aggregate reticulocytes in cats and both punctate and aggregate in dogs
- Large animals tend to not have reticulocytes in circulation, even when very anemic

You have now reached the end of Module 6. If you are enrolled in CVM 6925, please go to the Canvas page and take the quiz: “Module 6: Blood Smear and Retics quiz.”

## MODULE VII

# MODULE 7: HEMATOGENOUS INFECTIOUS DISEASE

### Module Objectives

1. Correctly identify the body, monolayer, and feathered edge of a blood smear
2. Explain why large organisms are often found along the feathered edge or the transition between the monolayer and feathered edge
3. Correctly identify common infectious diseases of the blood
4. Memorize which cells these organisms infect
5. Recall the vectors for the organisms found in the blood
6. Explain the relation between the organism and the clinical signs based on the pathophysiology of the organism



# MODULE 7.1: BLOOD SMEAR EVALUATION FOR INFECTIOUS DISEASE

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## Blood smear evaluation for infectious disease

When evaluating a blood smear for infectious agents, you follow the same flow as you would for acute leukemia or immune-mediated hemolytic anemia. As a reminder, the blood smear is comprised of 3 different “zones”; the **feathered edge**, **monolayer**, and the **body**. The smear exam begins with a “flyover” at low magnification (10x objective) of the entire slide. While this quick evaluation may seem like a waste of 30 seconds, it is by far the most important step of any blood film evaluation. At low magnification, you can identify large cells, confirm a leukocytosis described by your hematology analyzer, and infectious components that might otherwise be missed. Classic infectious examples of these are *Dirofilaria immitis* or *Acanthocheilonema reconditum*, both microfilariae found in the blood of dogs in the United States and seen best at low magnification.

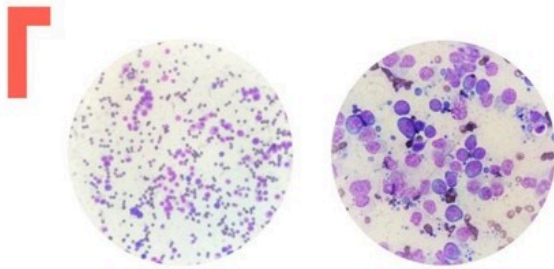
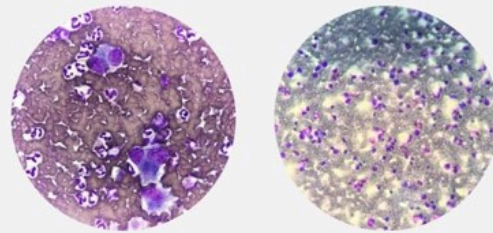
Once scanning the entire slide using the 10x objective, you will move on to the 40x objective to evaluate the WBC's. WBC's that contain phagocytized bacteria (bacteremia), rickettsia organisms (*Anaplasma* or *Ehrlichia* sp.), fungi (*Histoplasma* sp.), or protozoal organisms (*Leukocytozoon*) are easiest found in the monolayer (See figure below on how to locate the monolayer) and the feathered edge. **The organisms result in the WBC's being slightly heavier and they end up migrating towards the feathered edge.** Additionally, extracellular organisms that are  $>10\text{ }\mu\text{m}$  are evaluated at this level. Examples of an extracellular organism seen best at high power include *Trypanosoma* sp. (*T. cruzi*, *T. congolense*, *T. vivax*, *T. brucei* subsp. *brucei*, and *T. simiae*)

Last, the blood smear is evaluated within the monolayer at the 100x objective (oil immersion). At high magnification, organisms that are  $<3\text{ }\mu\text{m}$  (most neutrophils are  $15\text{ }\mu\text{m}$ ) are easiest to visualize. Examples of these organisms include; *Babesia* sp., *Cytauxzoon felis*, *Mycoplasma* sp., and several others.

## Non monolayer

high cell density

- ✗ Regions with too many cells overlapping make it difficult to pick out individual cells



## Monolayer

low cell density

- ✓ Regions with increased space between cells allow for easier visualization of individual cells

Locating the monolayer

### Key Takeaways

- Step 1: Evaluate the entire slide at low power using your 10x objective lens
- Step 2: Evaluate the WBC in the monolayer high power using you 40x objective lens (no oil)
- Step 3: Evaluate the RBCs and platelets at high power using your 100x objective lens (immersion oil required)

## Knowledge check



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# MODULE 7.2: BLOOD SMEAR EXAMPLES

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## Blood smear examples

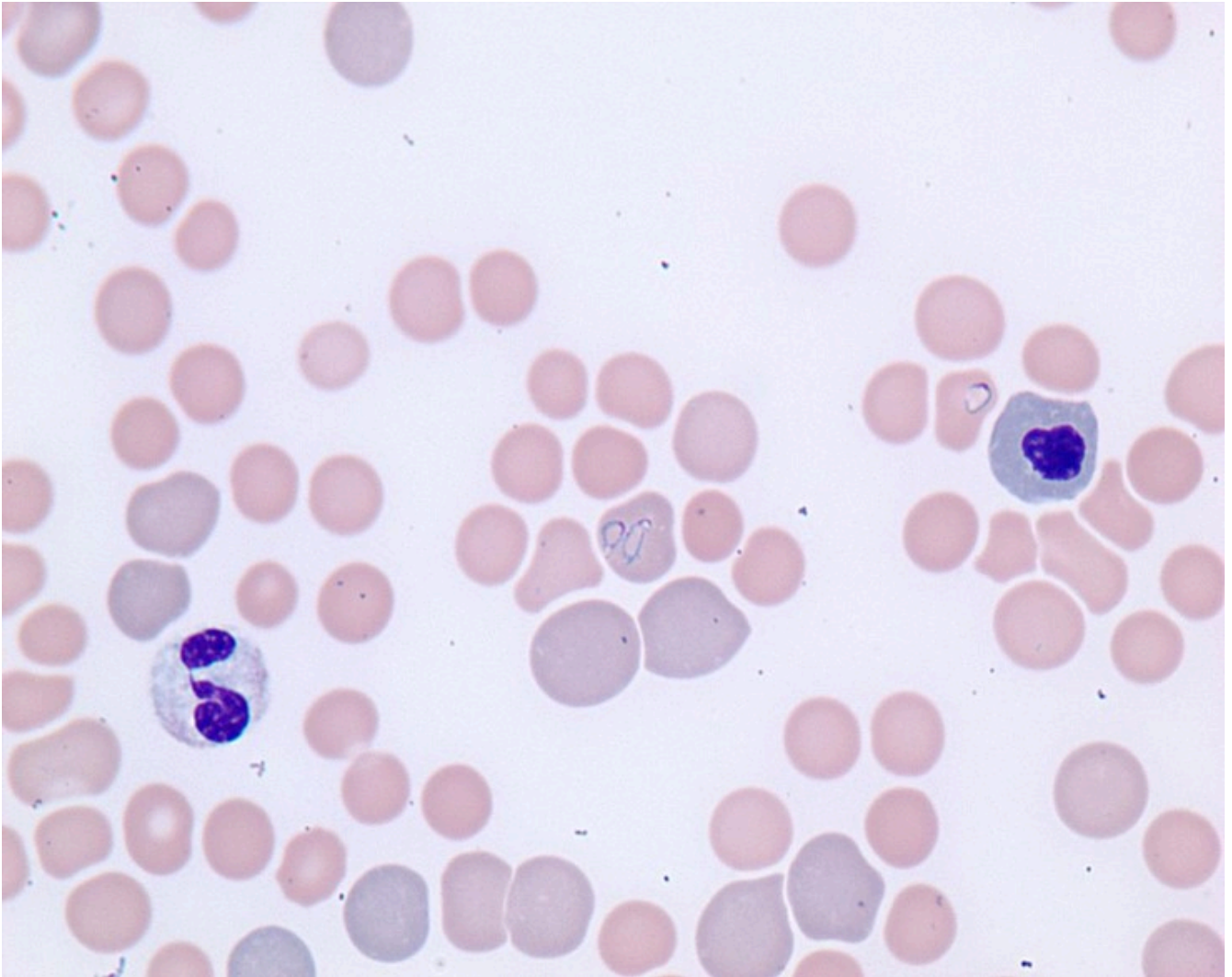
Here are a few examples of common organisms found on the blood film of veterinary patients in the United States:

**Table 7.1: Common organisms found on the blood film of United States veterinary patients; \*picture of the organism in the text below**

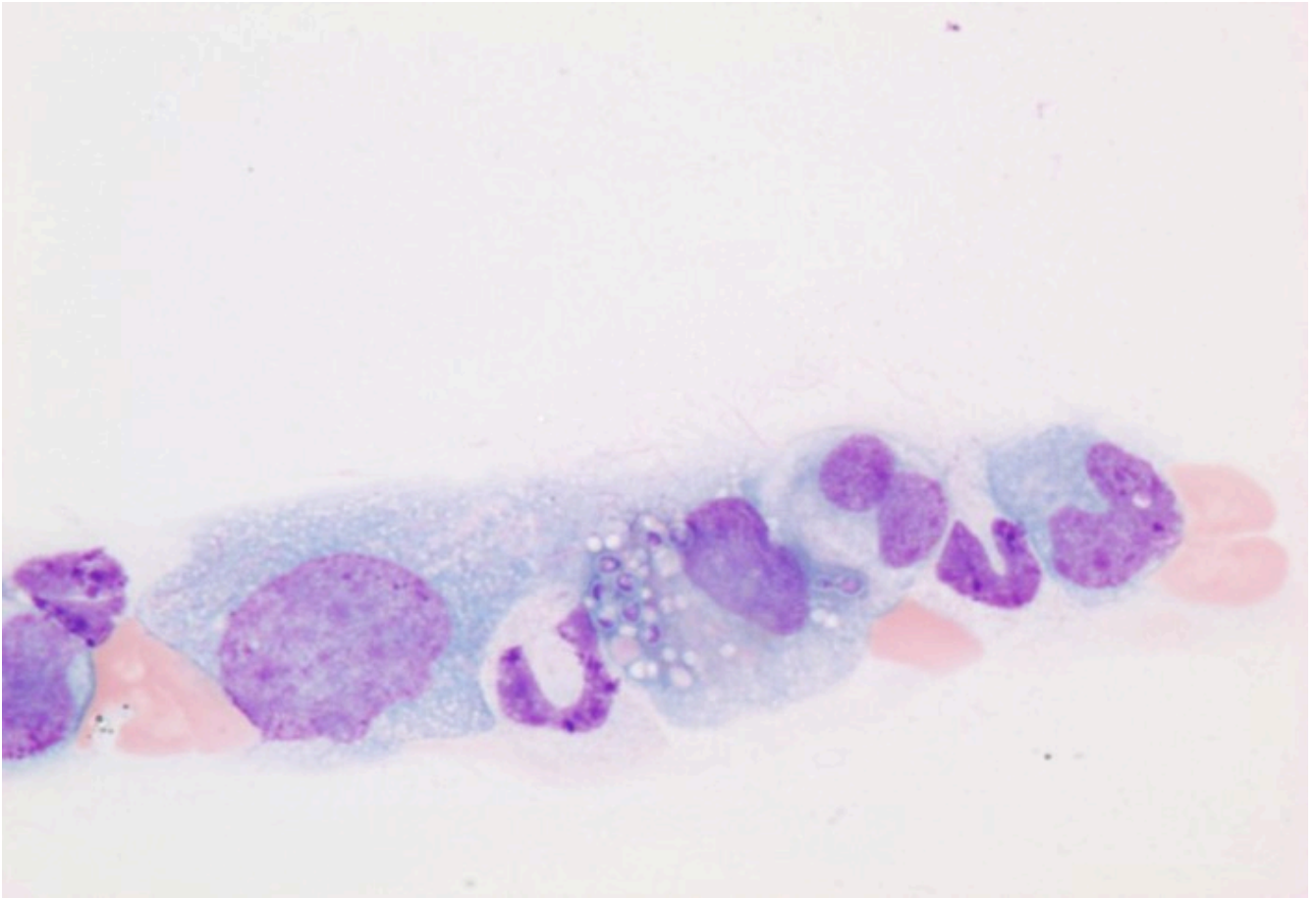
Organism	Species found in	Where it is found (intra/ extracellular)	Magnification easiest found at	Location on blood smear
<b>Parasitic</b>				
<i>Dirofilaria immitis</i>	Canine, Feline	Extracellular	10x	Feathered edge
<i>Trypanosoma sp.</i>	Canine, Bovine	Extracellular	40x	Monolayer
<i>Babesia sp.*</i>	Canine, bovine	RBC	40x, 100x	Monolayer
<i>Cytauxzoon felis*</i>	Feline	RBC (piroplasms), monocytes (schizonts)	100x	Monolayer (piroplasms), Feathered edge (schizonts)
<b>Bacteria</b>				
<i>Anaplasma phagocytophilum</i>	Canine, equine	Neutrophils, eosinophils	40x	Monolayer
<i>Anaplasma marginale*</i>	Bovine	RBC	40x, 100x	Monolayer
<i>Ehrlichia ewingii</i>	Canine	Neutrophils, eosinophils	40x, 100x	Monolayer
<i>Ehrlichia canis</i>	Canine	Monocytes, lymphocytes	40x, 100x	Monolayer
<i>Mycoplasma sp. (M. haemofelis, M. haemolamae, M. canis)</i>	Canine, feline, camelids, many others	Surface of RBC's	100x	Monolayer
<b>Fungal</b>				
<i>Histoplasma capsulatum*</i>	Canine	Monocytes	40x, 100x	Feathered edge, sometimes monolayer

## Visual atlas

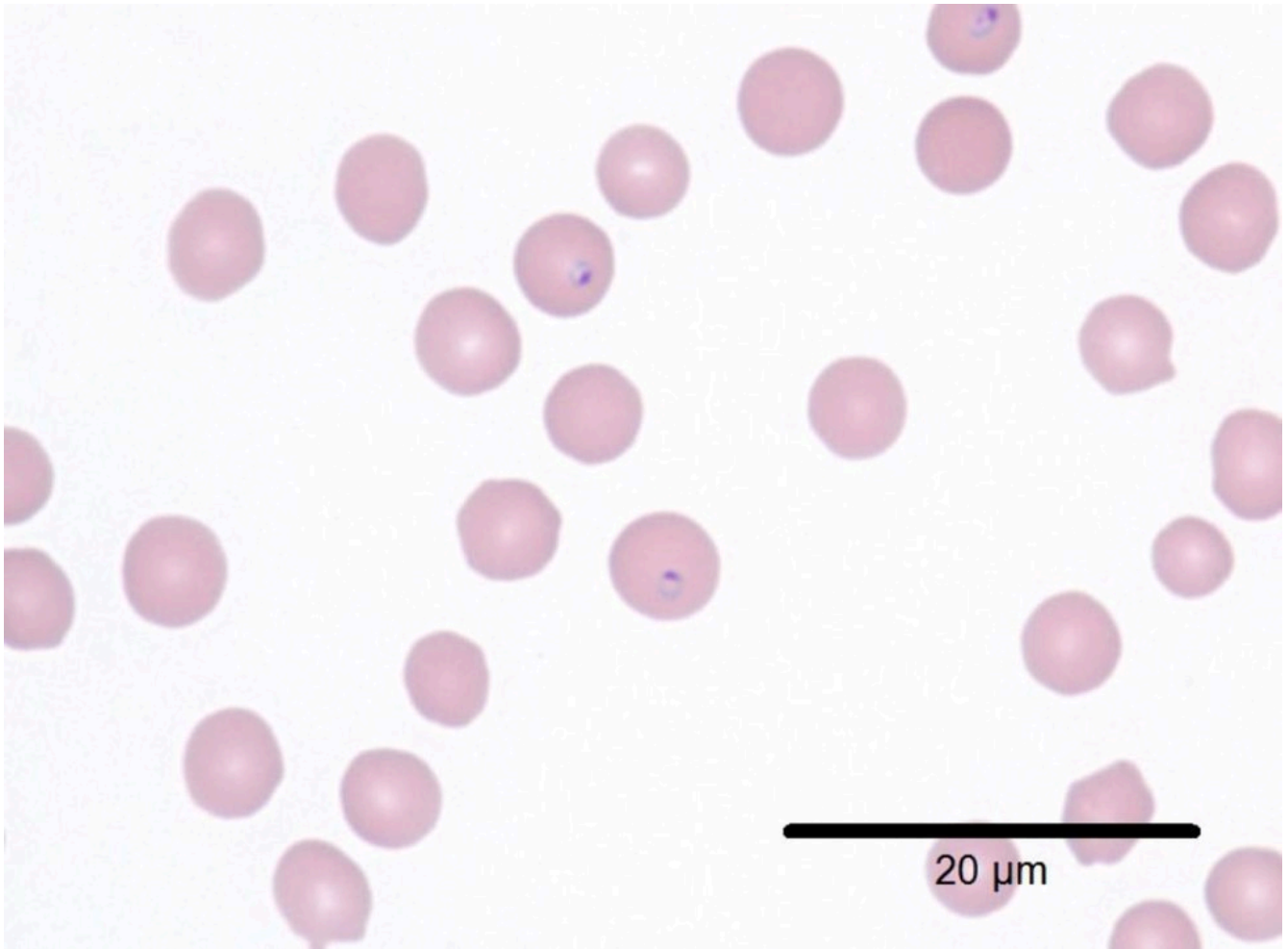
Here is a visual atlas of some of the organisms described in the table.



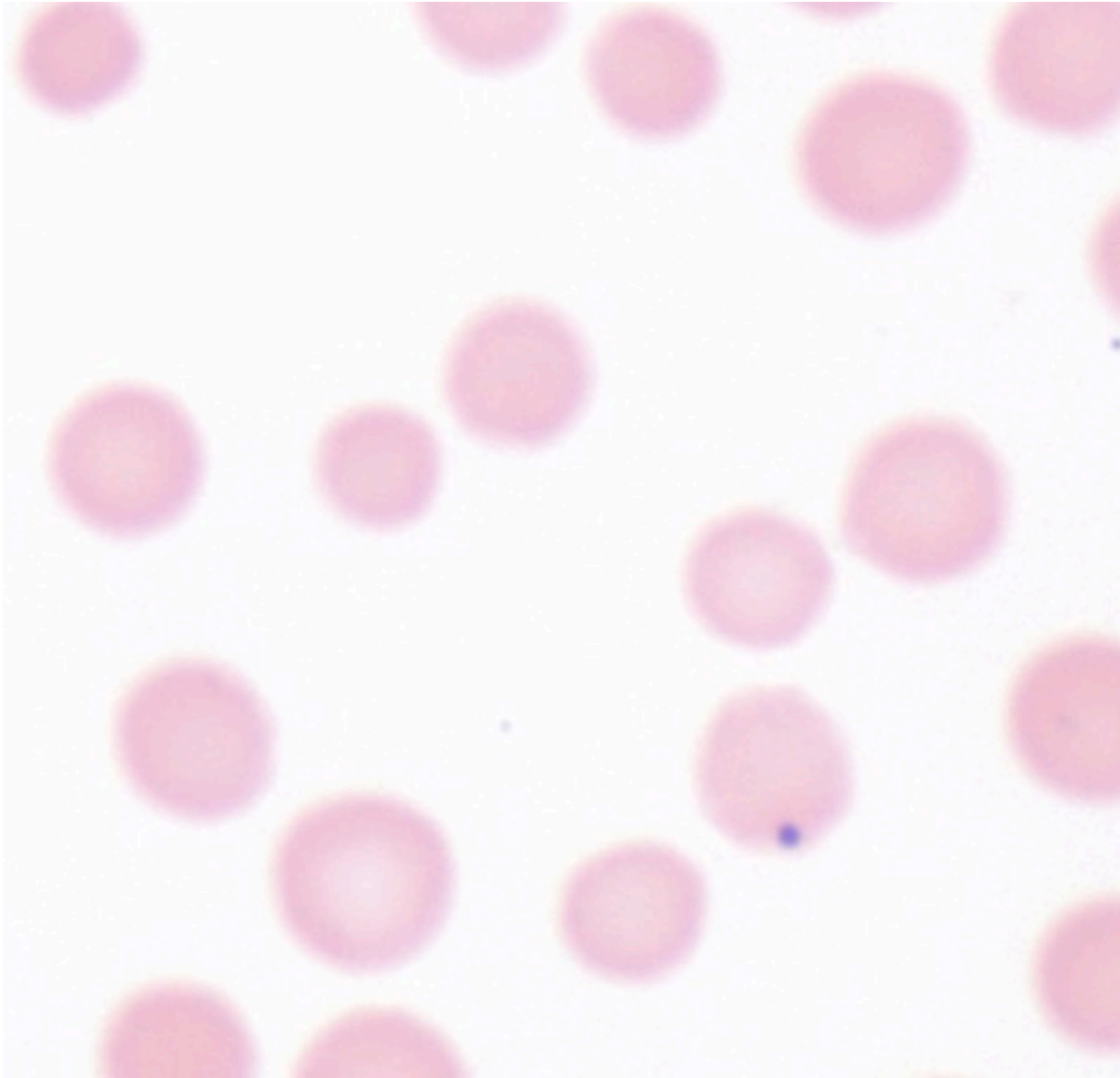
*Babesia* sp. in RBC of a canine blood smear (monolayer) using the 100x objective lens (oil).



*Histoplasma capsulatum* yeast within a monocyte on the feathered edge using the 100x objective lens (oil).



*Cytauxzoon felis* piroplasms within feline RBC's in the monolayer using a 100x objective lens (oil).



*Anaplasma marginale* morulae within an RBC of a bovine blood smear (monolayer) using a 100x objective lens.

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## Knowledge check



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## Key Takeaways

- Always begin by scanning the feathered edge at low power (10x objective) to identify large organisms (i.e. microfilariae, schizonts, macrophages with intracellular yeast)
- Organisms are largely predictable and have cells they prefer to invade and replicate in
- Small Intracellular pathogens are easiest to identify in the monolayer
- We use cytologic stains to screen for pathogens

You have now reached the end of Module 7. If you are enrolled in CVM 6925, please go to the Canvas page and take the quiz: “Module 7: Hematogenous infectious disease quiz.” There is an assignment that accompanies the in-person laboratory for this module.

## MODULE VIII

# MODULE 8: INTRODUCTION TO THE ROUTINE URINALYSIS

### Module Objectives

1. Correctly interpret urine dipstick and sedimentation results
2. Describe and memorize which dipstick pads are not routinely used in veterinary medicine
3. Compare and contrast collection methods
4. Summarize why a refractometer is preferred over dipstick for determination of USG



# MODULE 8.1: INTRODUCTION TO THE ROUTINE URINALYSIS

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## The routine urinalysis

The routine urinalysis is a quick and relatively inexpensive test that can be readily performed in a modest clinical laboratory or in a clinical setting. The results are useful in a variety of situations and are not limited to those directly involving the urinary tract.

Routine urinalysis is an essential part of the diagnostic evaluation of sick patients and the results should be interpreted along with the results of a chemistry panel. Ideally, urine should always be collected at the same time as blood for hematology and clinical chemistry and prior to any treatment (including intravenous fluids) or interventions. The complete interpretation of results of CBC/chemistry panels cannot be interpreted adequately without concurrent knowledge of the urinalysis, particularly abnormalities in renal (e.g. urea nitrogen and creatinine) or acid-base parameters. Similarly, interpretation of some abnormalities in urine (e.g. glucosuria, ketonuria) is facilitated by concurrent knowledge of chemistry results.

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## Knowledge check



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# MODULE 8.2: SAMPLE COLLECTION

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## Collection Methods

By far the most important step of the urinalysis is sample collection. As the method in which you collect (and store) the sample can affect the interpretation.

### 1. Off the floor:

This is the least optimal way of obtaining urine, but sometimes it is the only method available to clients (e.g. obtaining from kitty litter). This will have the highest number of contaminants, including the possibility of fecal elements.

### 2. Free catch:

A mid-stream catch is ideal for minimizing contaminating bacteria, but these still may be seen. May see contaminating bacteria, squamous cells from the distal urinary tract, genital tract, or skin.

### 3. Catheterized:

May slough off transitional epithelial cells so they are seen in higher quantities than normal. If traumatic or the urethra is irritated, hemorrhage may occur. This is done mostly in male dogs who are easier to catheterize than female dogs or other species. This sample should be collected as sterile as possible.

### 4. Cystocentesis:

This is the cleanest (most sterile) and the ideal method of obtaining urine especially when culture is likely needed. It is important to remember it is a traumatic method of collection and can result in microscopic hematuria (urine is still of normal color and is not red). It is not uncommon to see more than 100 RBC/HPF mimicking true hematuria. Increased protein is not usually seen with iatrogenic hematuria (but may not accompany true hematuria either).

# Sample storage

The time of collection relative to the time of analysis is important.

## Delay in analysis can result in:

1. Altered urine pH (increases)
2. Microbial proliferation (contaminants or pathogens)
3. Degradation of formed elements (cells and casts)
4. Degradation of chemical analytes (bilirubin, ketones)

If the analysis cannot be performed promptly (**<30 min**), the urine should be stored refrigerated to maximize cell preservation and minimize bacterial growth, but the analysis should still be performed within 12 hours. After 12 hours, cells will begin to lyse (usually by 24 hours, unless the urine contains a substantial amount of protein, which helps preserve cells), because urine is a “caustic” environment, and bacteria will proliferate. The addition of preservatives, e.g. formaldehyde, toluene, is not recommended as they all introduce artifacts in results (which vary, depending on the preservative). Before analysis, samples should be brought to room temperature. Note that artifacts are still seen with storage. Calcium oxalate and magnesium ammonium phosphate crystals all may develop over time with storage (i.e. were not present in a freshly examined sample). If the sample has been refrigerated, it is important to allow the urine to return to at least room temperature (ideally body temperature) prior to evaluation.

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## Knowledge check



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# MODULE 8.3: THE ROUTINE URINALYSIS

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## The routine urinalysis

For standardization, in veterinary medicine, the urine sediment exam is prepared with **3-5mL** of urine. This is important to help provide a semi-quantification of sedimentation results.

### **Routine urinalysis is comprised of these 4 steps:**

1. Assessment of visual urine attributes
2. Assessment of concentrating ability
3. Dipstick analysis
4. Sediment examination

The following sections will guide you through the urinalysis process.

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## Knowledge check



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## Assessment of visual urine attributes

The first step of any urinalysis is an assessment of visual attributes such as color and turbidity. Observations of **color** and **turbidity** are determined using a well-mixed urine specimen.

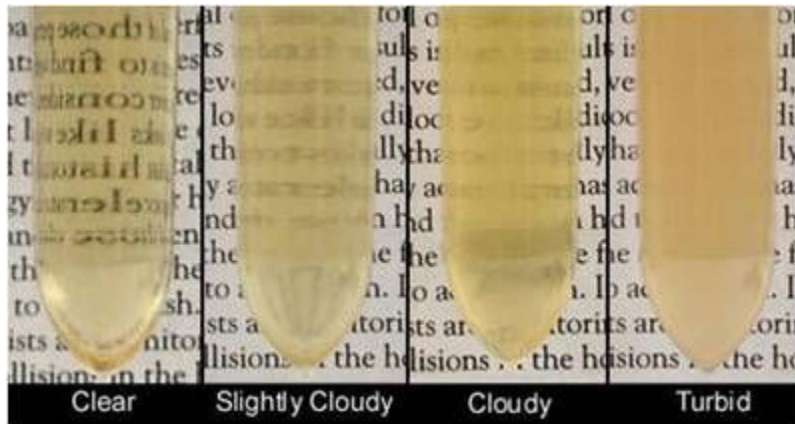
### Color?

- Color change, in most cases, is a common reason that patients will present to you as this is something the owners will visually observe
- **Color scale:** Light yellow, yellow, amber, red, and brown



### Turbidity?

- Evaluation of the clarity of the sample.
- For most species, we expect that the urine would be clear, but for some species (horse, rabbits) cloudy urine is normal. We use the ease with which an individual can read newspaper text behind the liquid to gauge clarity.
- **Turbidity scale:** Clear, slightly cloudy, cloudy, turbid



Urine Turbidity Scale

## Knowledge check



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## Assessment of concentrating ability

### Urine Specific Gravity (USG)

**Urine Specific Gravity** is a measure of the solute concentration in urine, and it is used to assess the ability of the renal tubules to concentrate or dilute the glomerular filtrate.

Two methods to determine USG:

1. Refractometer

- **Gold standard**

- Uses the refractive index as a measurement of the density of urine compared to pure water

## 2. Dipstick

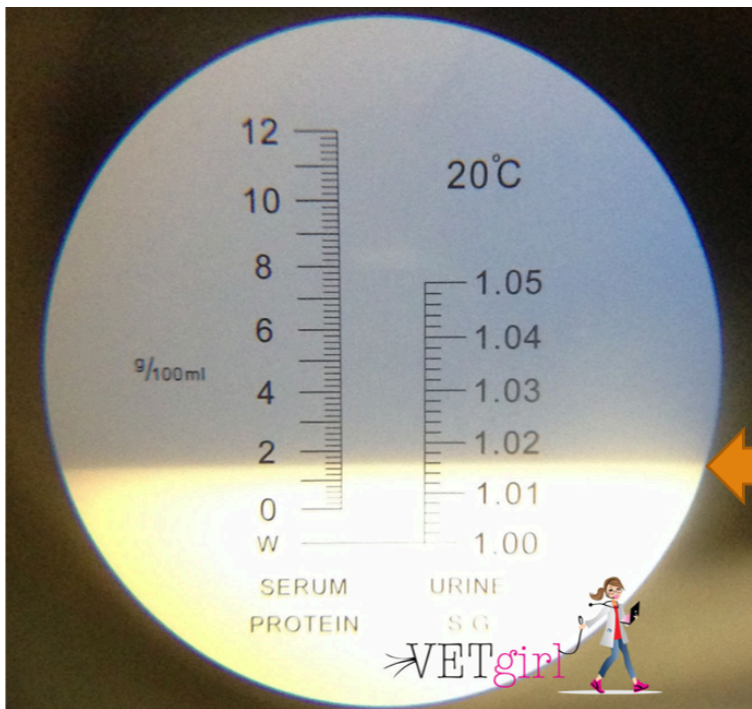
- Unreliable in veterinary medicine as we expect animals to concentrate their urine well beyond the range the dipstick is able to detect (1.000-1.030). When USG's get above 1.030 it is necessary to have a more quantitative value that cannot be achieved with the dipstick

## How do I read a refractometer?

Most refractometers are calibrated to measure both serum proteins and urine-specific gravity.

### **Steps:**

1. Place several drops of the sample liquid on the angled prism.
2. Seal the clear plate on top of it.
3. Look through the eyepiece while pointing the refractometer at a source of direct light.
4. Follow the line of refraction across the scale to determine the USG in your patient. (See image below)



The USG of this dog is about 1.016

Determining USG with a refractometer

## Knowledge check



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## Dipstick analysis

The dipstick examination is essentially the biochemical evaluation of urine using a urine dipstick pad. Dipsticks consist of various pads containing chemicals that provide a color change when a particular analyte is present in urine. This color change is converted to a semi-quantitative result for the analyte in question.

**The common chemical constituents measured in veterinary medicine are:**

- pH
- Glucose
- Ketones
- Bilirubin
- Protein
- Heme (Blood, Hemoglobin, and Myoglobin)
- The dipstick also detects leukocyte esterase, nitrites, urobilinogen; however, these 3 pads are unreliable in animals and we generally **ignore** their findings
- USG pad is also considered a fairly unreliable measurement of a patient's concentrating ability. Additionally, it only measures to 1.030 and our patients should be concentrating well above that value.

## Dipstick procedure

Please review the following video and handout on the dipstick procedure prior to the laboratory.



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Urinalysis procedure- See Dipstick Assignment Canvas Page

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## Knowledge check



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online here:

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## Sediment examination

The sediment examination is the microscopic evaluation of urine to identify cells, blood, crystals, bacteria, etc. of the urine that would not be detected using dipstick alone. The sediment exam is performed at low (10x obj.) and high (40x obj.) magnification. Subdued lighting is necessary to increase the refractivity of the unstained urine elements (lower the condenser and/or close down the substage iris diaphragm).

- Elements evaluated at 10x objective
  - Squamous and round/transitional epithelial cells
  - Crystals
  - +/- casts (sometimes easier to see at high mag.)
  - Parasite ova
- Elements evaluated at 40x objective
  - Bacteria
  - WBC
  - RBC
- The purpose of this lab is not to interpret sediment findings, but rather to become comfortable with the identification of common sediments. In your clinical pathology and medicine courses, you will discuss their significance.

The following pages will guide you through common structures observed on the sediment exam, beginning with those that can be seen on the 10x objective and moving towards those seen with the 40x objective.

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## Knowledge check



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# MODULE 8.4: COMMON STRUCTURES OBSERVED ON THE SEDIMENT EXAM

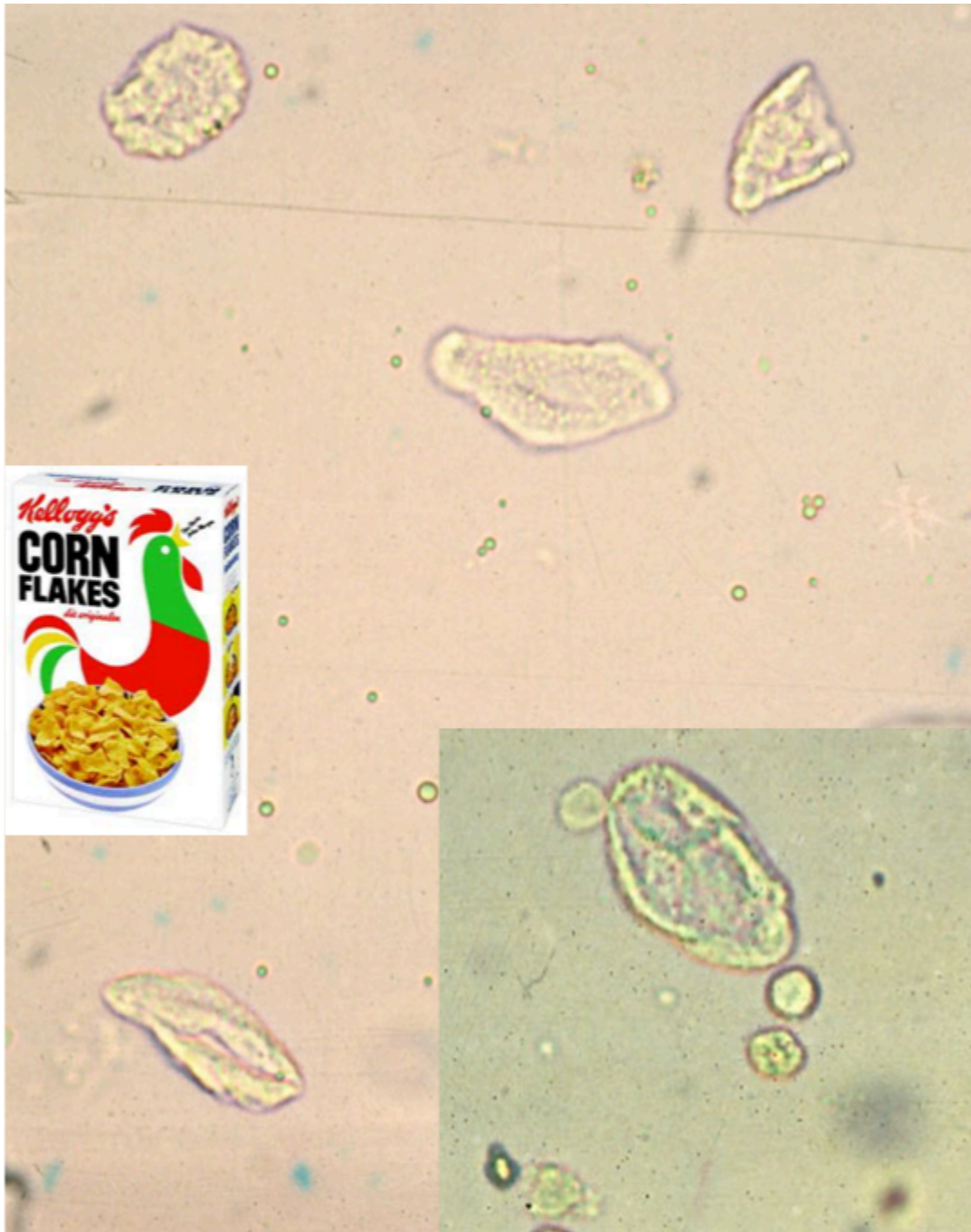
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## Epithelial cells

There are 2 types of epithelial cells that we can see in our urine sediment; transitional and squamous epithelial cells.

## Squamous epithelial cells

- The external urinary tract (skin, vulva, prepuce, etc.) is lined by squamous epithelial cells.
- These cells are anucleate, angular to polygonal, and vary greatly in size depending on how they exfoliated
  - Some describe as resembling “corn flakes”
- Common to see in voided and catheterized samples, not cystocentesis
- In the image below, you can see several squamous epithelial cells at 10x and 40x (inset) magnification. These cells have not been stained.
  - In the inset, you can also visualize 2 RBCs (smooth round structures directly adjacent to squamous epithelial cell) and 2 WBCs that are more irregular in shape and grainy appearance



Several squamous epithelial cells at 10x and 40x (inset) magnification. These cells have not been stained.

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## Knowledge check



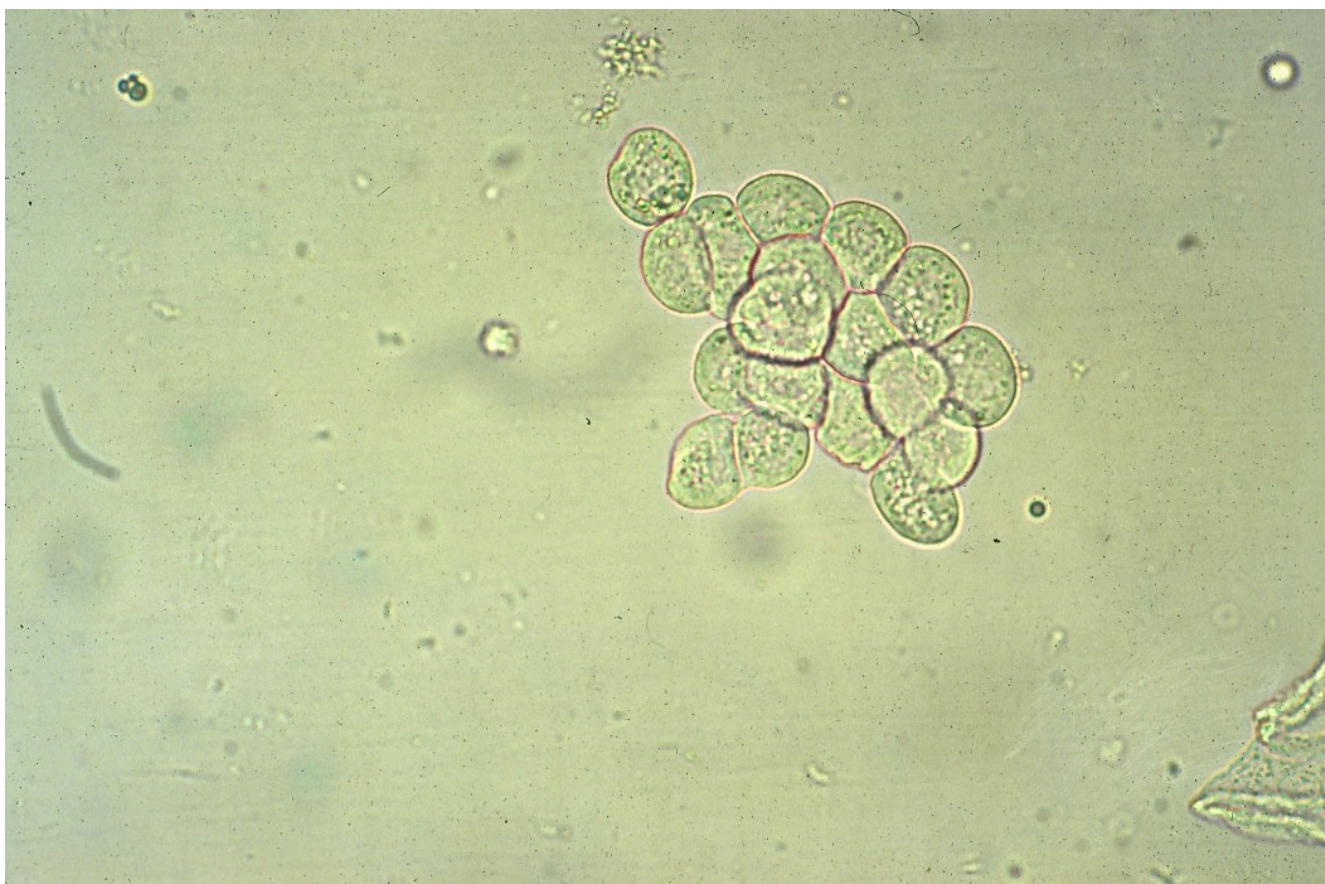
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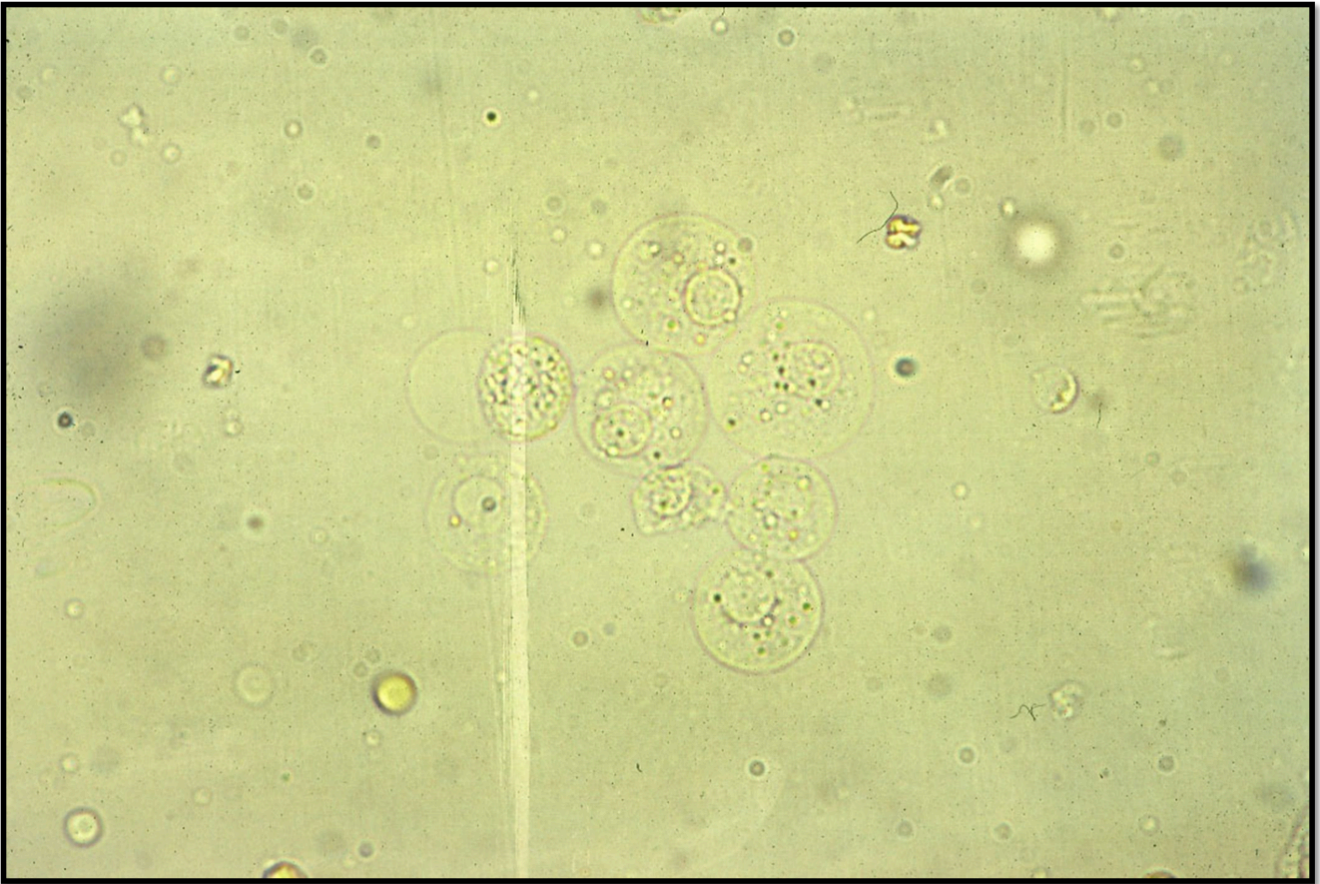
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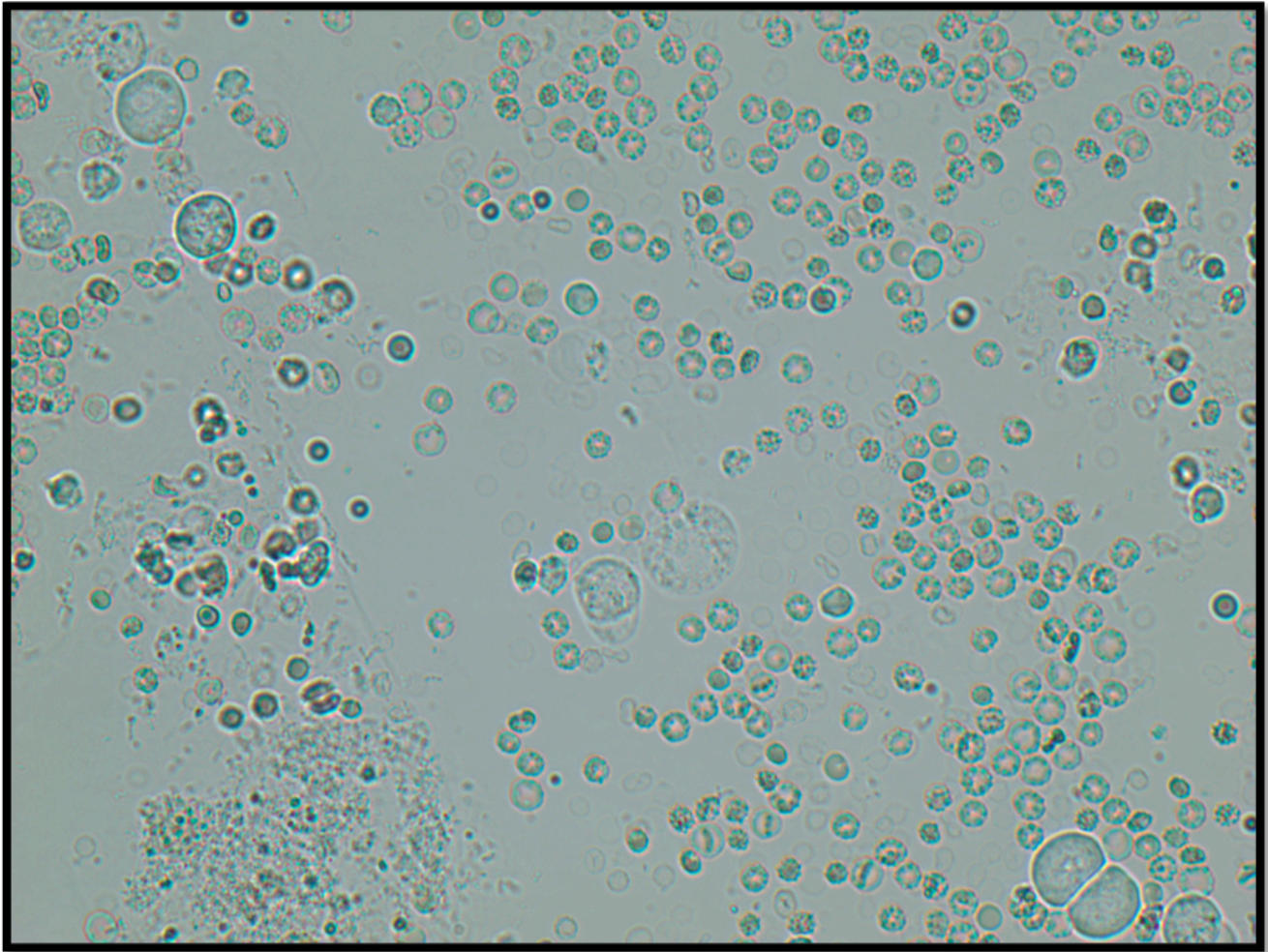
## Round (transitional) cell epithelium

- The internal urogenital tract is lined by transitional epithelium (also referred to as urothelial cells and round epithelial cells in some texts, all these terms are interchangeable).
- In cytology textbooks, when describing unstained **round in shape** epithelial cells in urine samples, you will see these cells referred to as **round or transitional epithelium**. We assume based on their shape and location that these epithelial cells are of transitional cell origin, but this needs to be confirmed using Romanowsky-type stains.
- These cells are normally shed into the bladder or along the urethra during normal cell turnover. Increased numbers in urine may indicate hyperplasia, inflammation, or neoplasia.
- These cells are typically 4-5x the diameter of a neutrophil with an eccentrically placed nucleus (see image). It is not unusual to see them individually or in small clusters.





Both images above have been taken using 10x objective (100x mag.). Especially visible in the image directly above is a smaller eccentrically placed circular structure within each individual larger cell. This smaller structure is the nucleus of the cell. Transitional (urothelium) epithelial cells are nucleated in comparison to anucleate squamous epithelial cells found on the external genitalia.



An example at 40x objective of several round cells with many RBCs and WBC's in the background for size comparison.

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## Knowledge check



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## Crystalluria

- The medical term for crystal formation in the urine is **crystalluria**.
- Not all crystals are pathogenic
- Knowing the pH of urine is important to help determine the type of crystal you are seeing as certain crystal tend to only form in either acidic or basic urine
- Crystals form in vivo and vitro

### Common causes for in vivo formation

- Diet
- UTI
- Abnormal urine pH
- High USG (very concentrated urine)

### Common causes for in vitro formation

- Temperature
  - Most commonly refrigeration
- Evaporation
- Urine pH

Please refer to [Cornell University's eClinPath](#) website for images and guidance of what types of crystals are precipitated out in what types of scenarios.

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## Knowledge check



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## Urinary casts

Casts are cylindrical structures that are composed primarily of **Tamm Horsfall Mucoprotein** (THP) in the renal tubules. THP is secreted by renal epithelial cells. These casts are “molds” of the renal tubules. Cellular constituents can become entwined in THP during specific pathologies and characterize the type of cast found. The most common casts seen in the urine of domestic animals are “hyaline casts”. These casts can be found in the urine of healthy animals as they are comprised almost entirely of THP. More information and pictures of casts can be found at [Cornell University’s eClinPath](#) website. Casts can be seen at both 10x and 40x objectives.

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## Knowledge check



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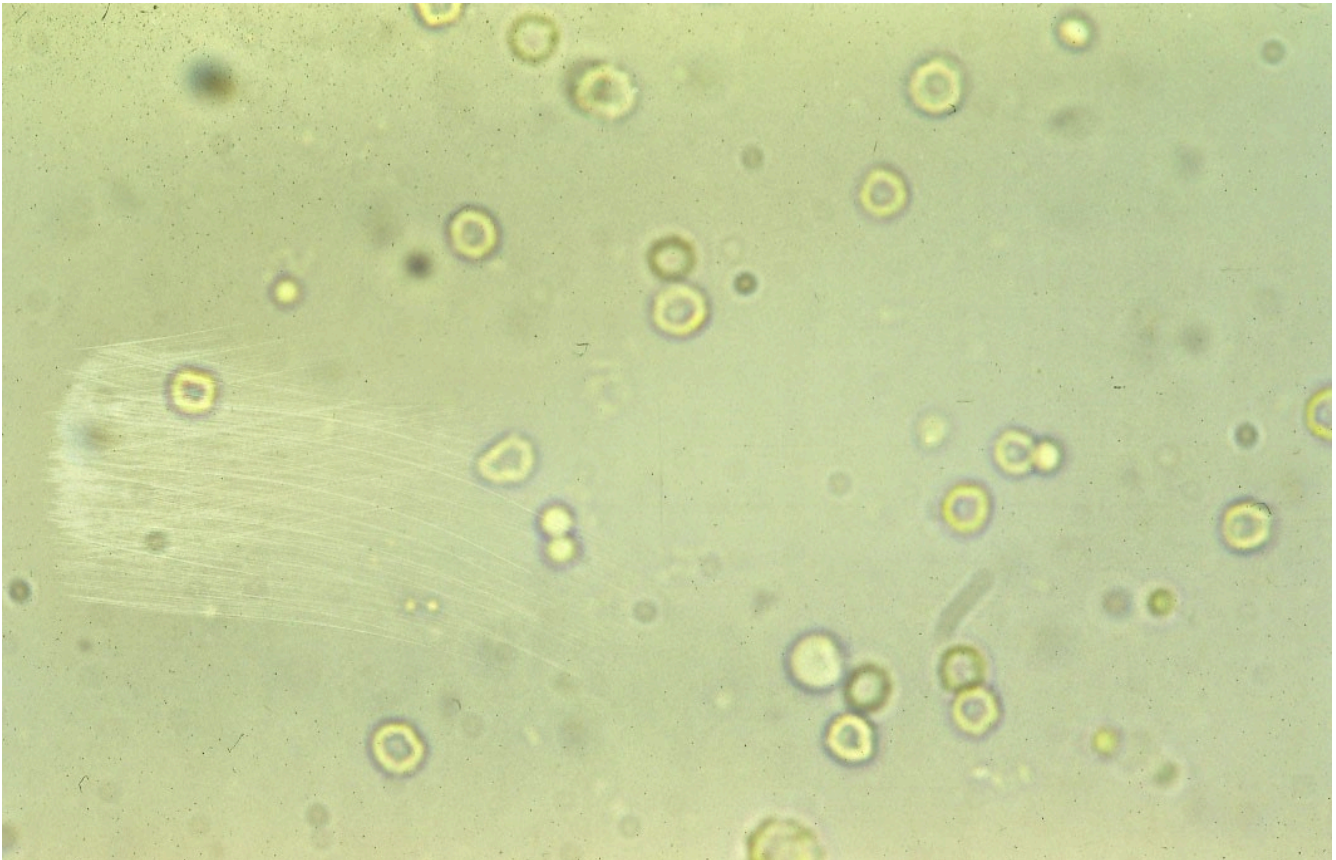
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## Red and white blood cells

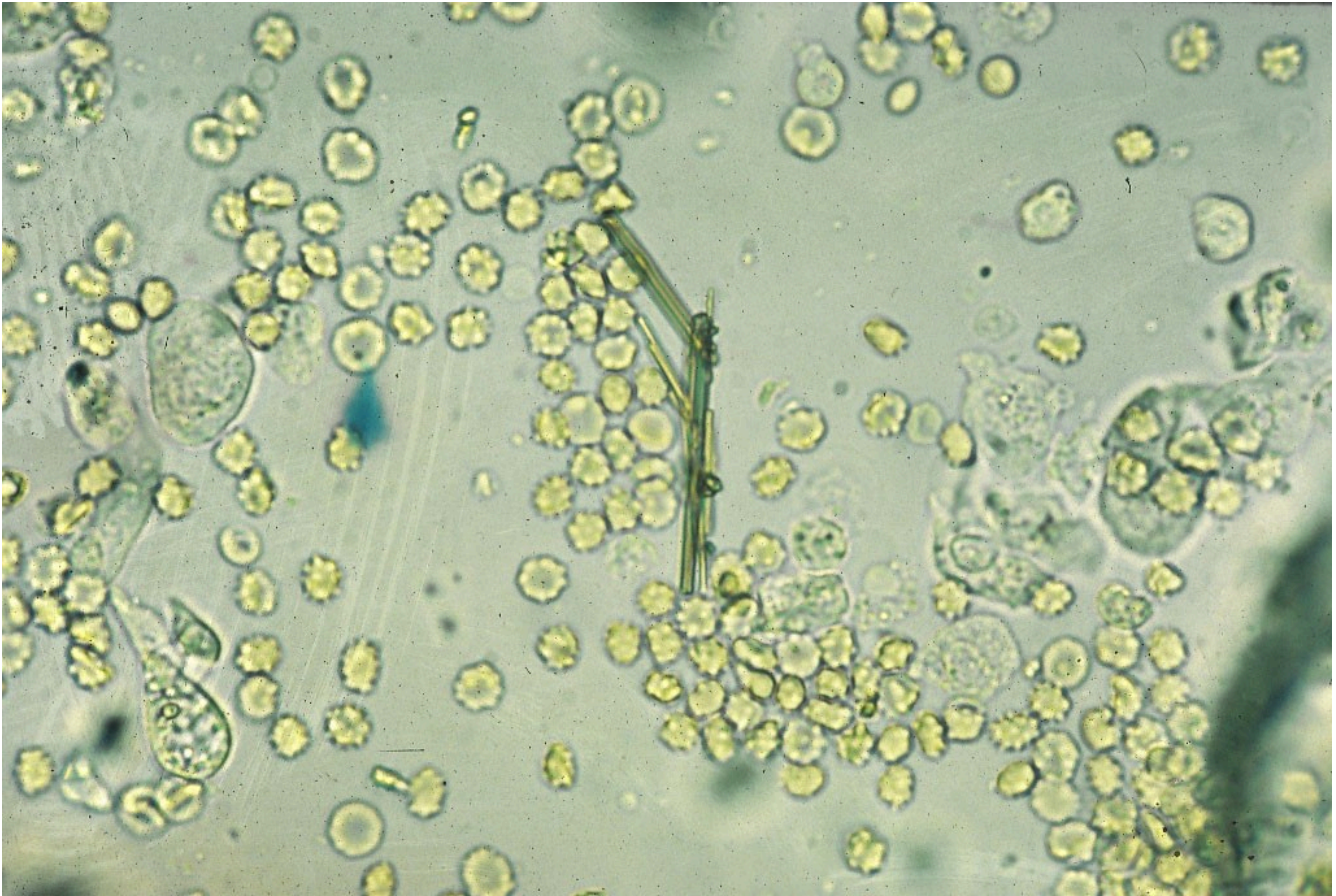
### Red blood cells

- Up to 5 RBC/hpf is generally considered acceptable for “normal” urine
  - Cystocentesis may result in larger numbers of RBCs in the sample
- Increased RBCs in the urine is called **hematuria**

- Can be secondary to hemorrhage, trauma, inflammation, necrosis, trauma, or neoplasia along the urinary tract
- The presence or absence of central pallor in the RBCs does not indicate the duration of time in the urine



Many RBCs that display central pallor at 40x objective



Many RBCs mixed with squamous epithelial cells. In the center of the photo are linear crystals that have precipitated off of the dipstick pad. 40x objective

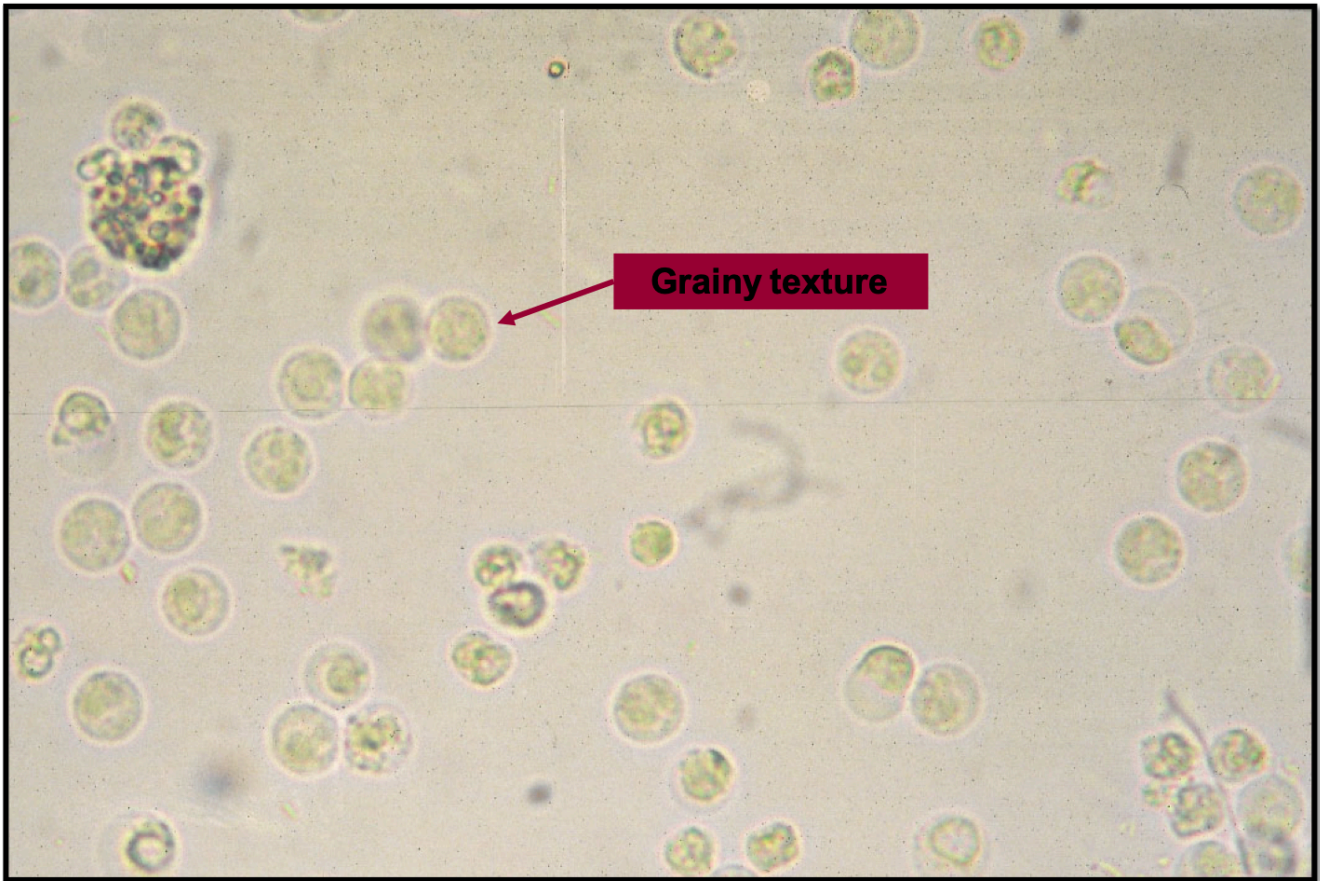
## White blood cells

- Less than 5 WBC/hpf is generally accepted as “normal” in voided samples
- Large numbers of WBCs in urine is called **pyuria**
- Pyuria generally indicates inflammation along the urinary tract

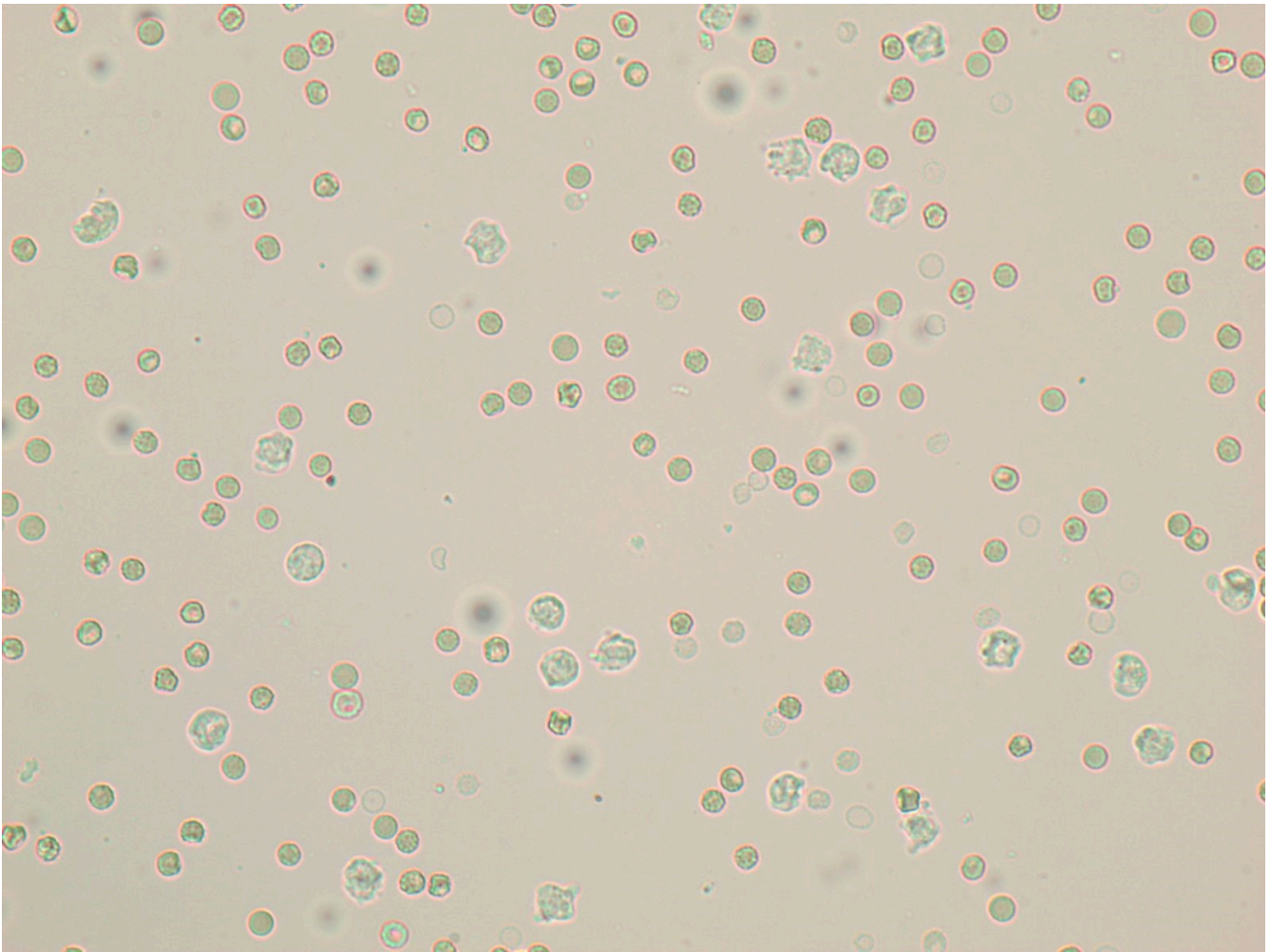
### Common causes of inflammation along the urinary tract include:

- Urinary tract infection (UTI)
- Neoplasia along the urinary tract
- Uroliths (bladder stones)

- Compared to RBCs, WBC's (15um) appear grainy and are slightly larger than an RBC (8 um). It is important to remember both RBCs and WBC's will flux in size depending on the osmotic concentration of the solution as the cell will take on or lose water.



40x magnification of WBCs in urine. There are also low numbers of RBCs in this image



40x objective of pyuria and hematuria. In this image, the RBCs appear deeper yellow (from Hb) and smaller than the lighter in color and grainy WBCs.

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## Knowledge check



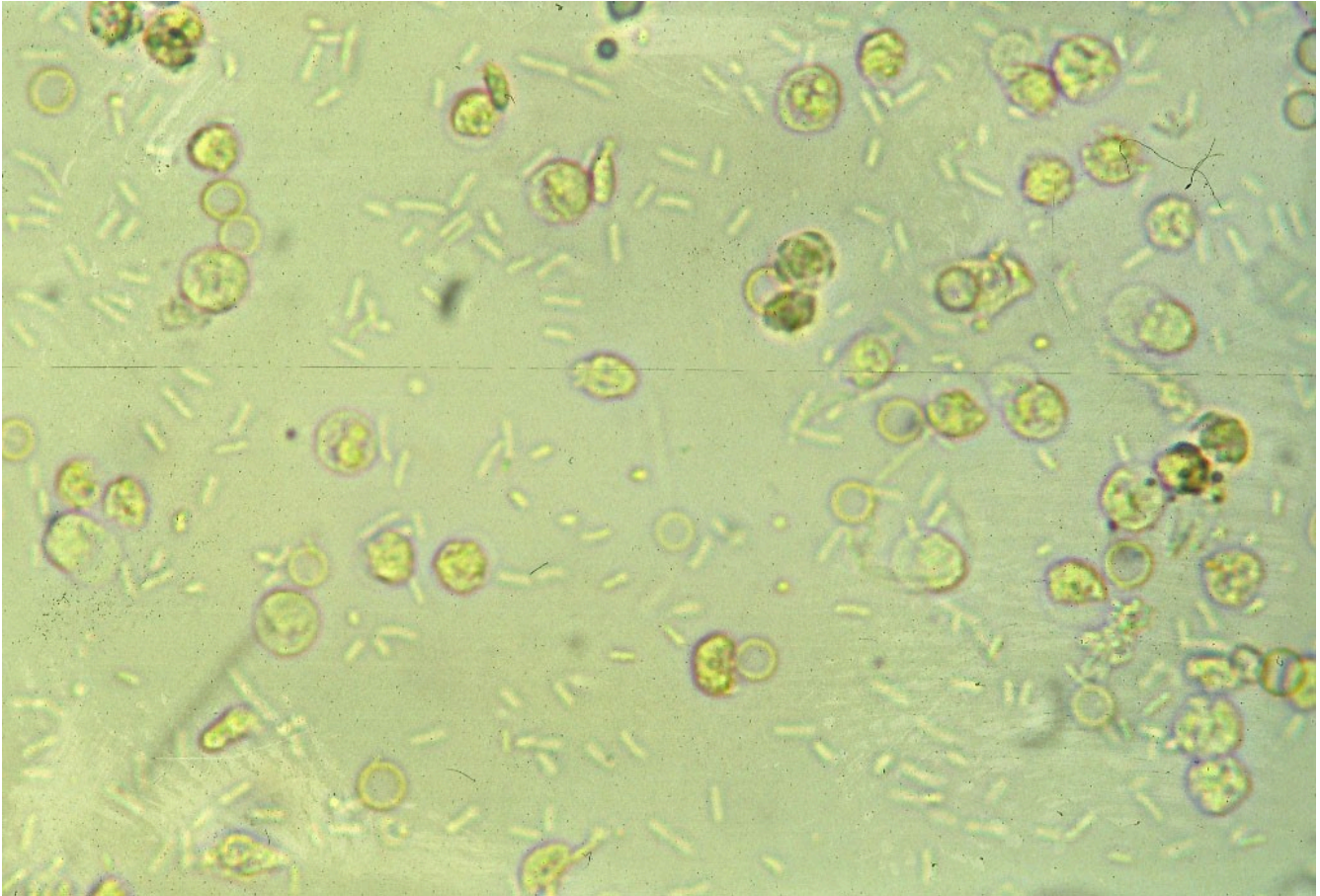
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## Bacteriuria

The urinary bladder is **not** sterile and has its own unique flora, just like the gastrointestinal tract. The overall biomass of bacteria in the bladder is minimal, thus it is unusual to see bacteria on routine urinalysis. When bacteria are observed in urine, it is important to determine if there is pyuria or hematuria present concurrently as that may indicate a urinary tract infection. The most common cause of urinary tract infections in dogs and cats is *E. coli*, a Gram-negative rod.



Urinary tract infection in a dog. Many rod-shaped bacteria (*E. coli* on culture) are seen free in the background with many WBCs and RBCs.

## Lab procedure

In the laboratory, you will be working through several case-based dipstick cases and performing several sediment exams. Please read the following procedure document in preparation for the lab.

[Urinalysis procedures](#)

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## Knowledge check



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### Key Takeaways

- The two major parts of the routine urinalysis is the dipstick and sedimentation exam
- We use a refractometer, not the dipstic pad, to determine USG
- Not all the dipstick pad results are useful in veterinary species
- Low magification is used to detect large cellular features in urine (i.e. epithelial cells, casts, crystals)
- High magnification is used to detect hematuria, pyuria, and bacteriuria

You have now reached the end of Module 8. If you are enrolled in CVM 6925, please go to the Canvas page and take the quiz: "Module 8: Urinalysis quiz"

## MODULE IX

# MODULE 9: URINE CULTURE AND SENSITIVITY

### Module Objectives

1. Using case data, correctly classify the type of UTI your patient has
2. Correctly interpret urine culture and sensitivity data
3. Recall the most common UTI pathogens in dogs
4. Provide reasonable treatment recommendations for the UTI cases provided



# MODULE 9.1: INTRODUCTION TO BACTERIAL URINARY TRACT INFECTION (UTI)

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## Urinary Tract Infection (UTI)

Bacterial urinary tract infection (UTI) is a common condition seen in both dogs and cats worldwide and considered one of the top reasons for antibiotic administration in small animal practices. In fact, 14% of all dogs (that's almost 1 in 5!) will have at least one UTI in their lifetime. As multiple drug-resistant pathogens (MDR), specifically bacteria, become more common increased diligence for targeted drug therapy based on diagnostic testing will become increasingly more common practice.

In dogs, the International Society for Companion Animal Infectious Disease (ISCAID) has developed [guidelines \(Links to an external site.\)](#) for antimicrobial use and stewardship to help decrease the number of MDR pathogens. In regards to UTI, they have broken the disease process up into 5 different groups.

### 1. Sporadic bacterial cystitis

Sporadic bacterial cystitis (sometimes referred to as 'simple uncomplicated UTI') is a sporadic bacterial infection of the urinary bladder with compatible lower urinary tract signs in dogs or cats.

### 2. Recurrent bacterial cystitis

Recurrent bacterial cystitis implies a diagnosis of three or more episodes of clinical bacterial cystitis in the preceding 12 months or two or more episodes in the preceding 6 months

### 3. Subclinical bacteriuria:

Subclinical bacteriuria is defined as the presence of bacteria in urine as determined by positive bacterial culture from a properly collected urine specimen, in the absence of clinical evidence of infectious urinary tract disease. Treatment may not be necessary as the urinary bladder is **NOT** sterile and has its own

unique microbiome. In humans, there is abundant support that antimicrobial treatment is not needed for asymptomatic bacteriuria (the human analogue of subclinical bacteriuria), even in most compromised patients

#### 4. Pyelonephritis:

**Pyelonephritis** is an infection of the renal parenchyma that can occur from ascending infection or **bacteremia**, with **Enterobacteriaceae** causing the majority of infection.

#### 5. Bacterial prostatitis:

Bacterial prostatitis is an uncommonly encountered problem in veterinary practices in some regions because of the high prevalence of castration in the canine population. However, it is second to benign prostatic hyperplasia/hypertrophy as a leading cause of prostatic disease

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## Knowledge check



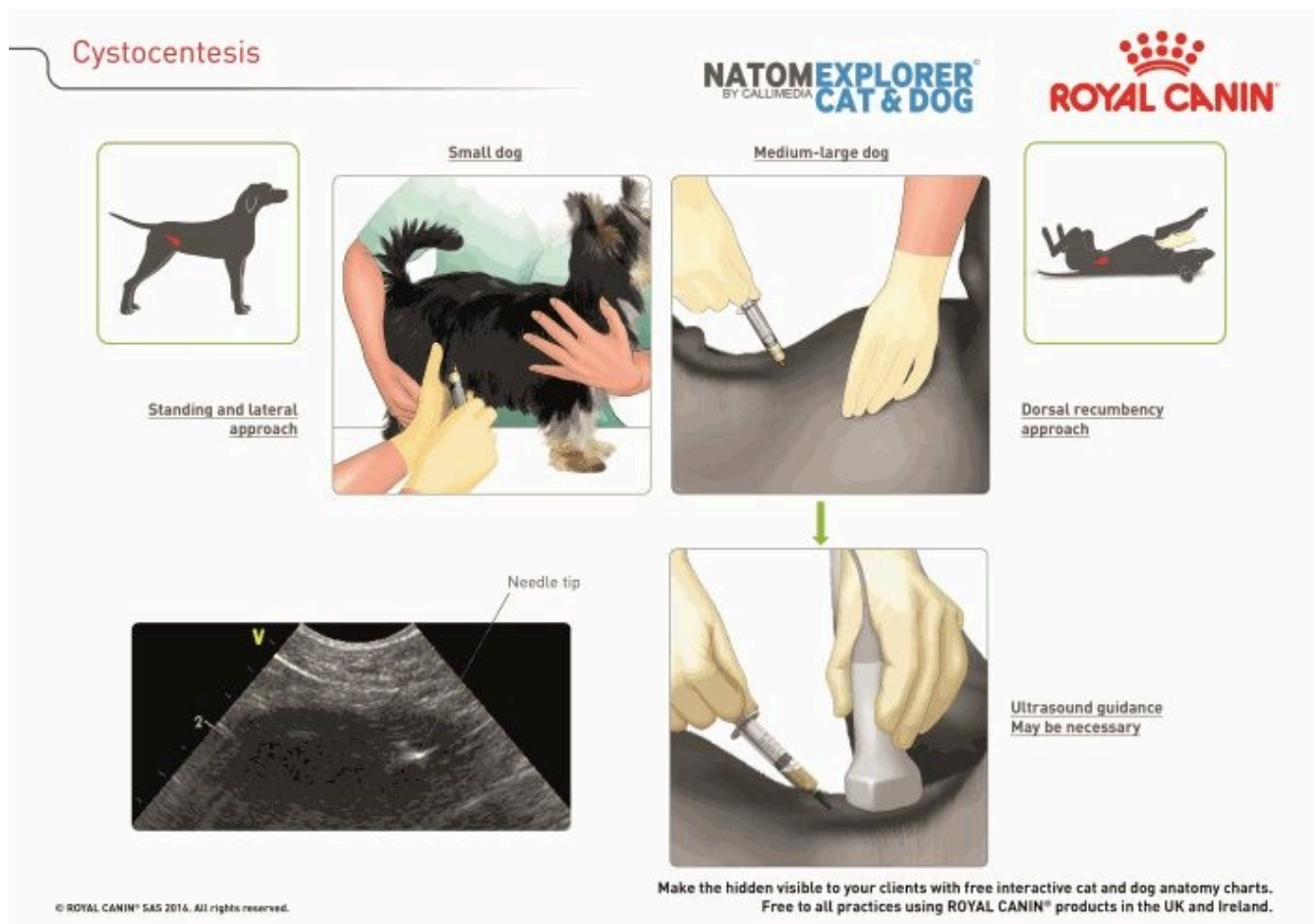
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# MODULE 9.2: COLLECTING A SAMPLE TO DIAGNOSE A UTI

## Cystocentesis

As stated before, the urinary bladder is not a sterile environment, so how do we define a UTI? If an animal is showing clinical signs of a UTI, the **gold standard** method for urine collection is via **cystocentesis** (direct extraction of urine from the bladder using a needle).



Approaches to cystocentesis

## Midstream urine catch



Sterile Urine Jar

For a myriad of reasons, cystocentesis may not be possible or feasible. In those cases, a **midstream urine catch** into a sterile urine jar is the next best. It is important that the collection be midstream to decrease the number of normal genital flora that ends up in the sample as the act of micturition releases build-ups of normal genital flora into the environment.

In most clinical veterinary settings, catching **5-10 mL of mid-stream**

**urine** into the small sterile cup is not easy nor feasible. In those cases, it is considered the accepted practice to use a clean (ideally sterile) soup ladle or small bowl for urine collection. It is good practice to document the collection method in case the results that you receive do not match your clinical suspicion and may indicate contamination.



A soup ladle can be used for urine collection



Collecting urine with a clean bowl

## What about timing?

In both people and animals, the best time to collect a urine sample for culture is the first thing in the morning.

### *Why do you think this would be?*

Overnight, while the animal is sleeping the bacteria continues to proliferate within the bladder, thus the first micturition of the morning is the best sample to culture for a UTI pathogen.

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## Knowledge check



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# MODULE 9.3: CULTURING URINE

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## Microbiology Culture Plates for UTI

In a laboratory setting, the urine is commonly cultured on 3 different types of plates:

1. Blood agar
2. MacConkey agar
3. HurBi plate (aka HardyCHROM Urine Biplate)

### Blood agar plate (BAP)

BAP is an agar plate enriched, differential media that contains mammalian blood, used to differentiate fastidious organisms and detect hemolytic activity. The three types of hemolytic activity include:

#### Alpha ( $\alpha$ ):

Partial lysis of RBCs (cell membrane remains) resulting in a green or brown discoloration around colony from the conversion of hemoglobin to methemoglobin

#### Beta ( $\beta$ ):

Lysis and complete digestion of RBCs surrounding the colony (I.E. *Streptococcus hemolyticum*)

#### Gamma ( $\gamma$ ):

AKA non-hemolytic is the term referred to as the lack of hemolytic activity.



Blood Agar Plate (BAP) with alpha, beta, and gamma hemolysis

## MacConkey agar (MAC)

MAC is a selective media plate that *selects* for Gram-negative bacteria. In addition to being Gram-selective, they are also indicator media as the colonies will turn “red” if the bacteria are able to ferment lactose. The most common UTI pathogens that are Gram-negative lactose fermenters are *E. coli*, *Enterobacter*, and *Klebsiella*.

*E. coli* colonies appear and smooth, round, and **bright pink colonies** on MAC because they are lactose fermentors (image below)



MAC plate with LF bacteria on the left and non-LF bacteria on the right




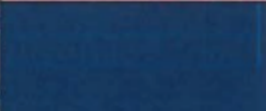

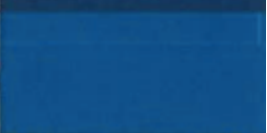



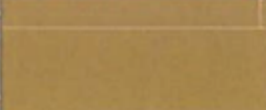
## HurBi plate

The HurBi plate is divided into two sides. These plates are both selective and differential.

One side has selective media to detect Gram+ bacteria and the other side for Gram – bacteria. In addition to the two different types of media, the media is enriched by chromographic substrates that are cleaved by enzymes specific to each bacteria resulting in the generation of unique identifying colored colonies.



HurBi Plate

<i>E. coli</i>	rose to magenta colonies with darker pink centers		
<i>Klebsiella, Enterobacter, and Serratia spp.</i>	deep blue or dark indigo colonies		
<i>Citrobacter spp.</i>	dark blue colonies often with a rose halo in the surrounding media		
<i>Proteus, Morganella, and Providencia spp.</i>	clear to light yellow colonies with golden-orange halo in the surrounding media (some <i>Proteus vulgaris</i> colonies will be blue-green)		
<i>Pseudomonas spp.</i>	colorless to light yellow-green colonies		

Enzymes specific to each bacteria cleave the chromographic substrates in the HurBi plate resulting in the generation of unique identifying colored colonies

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## Knowledge check



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# MODULE 9.4: ENUMERATION OF BACTERIA

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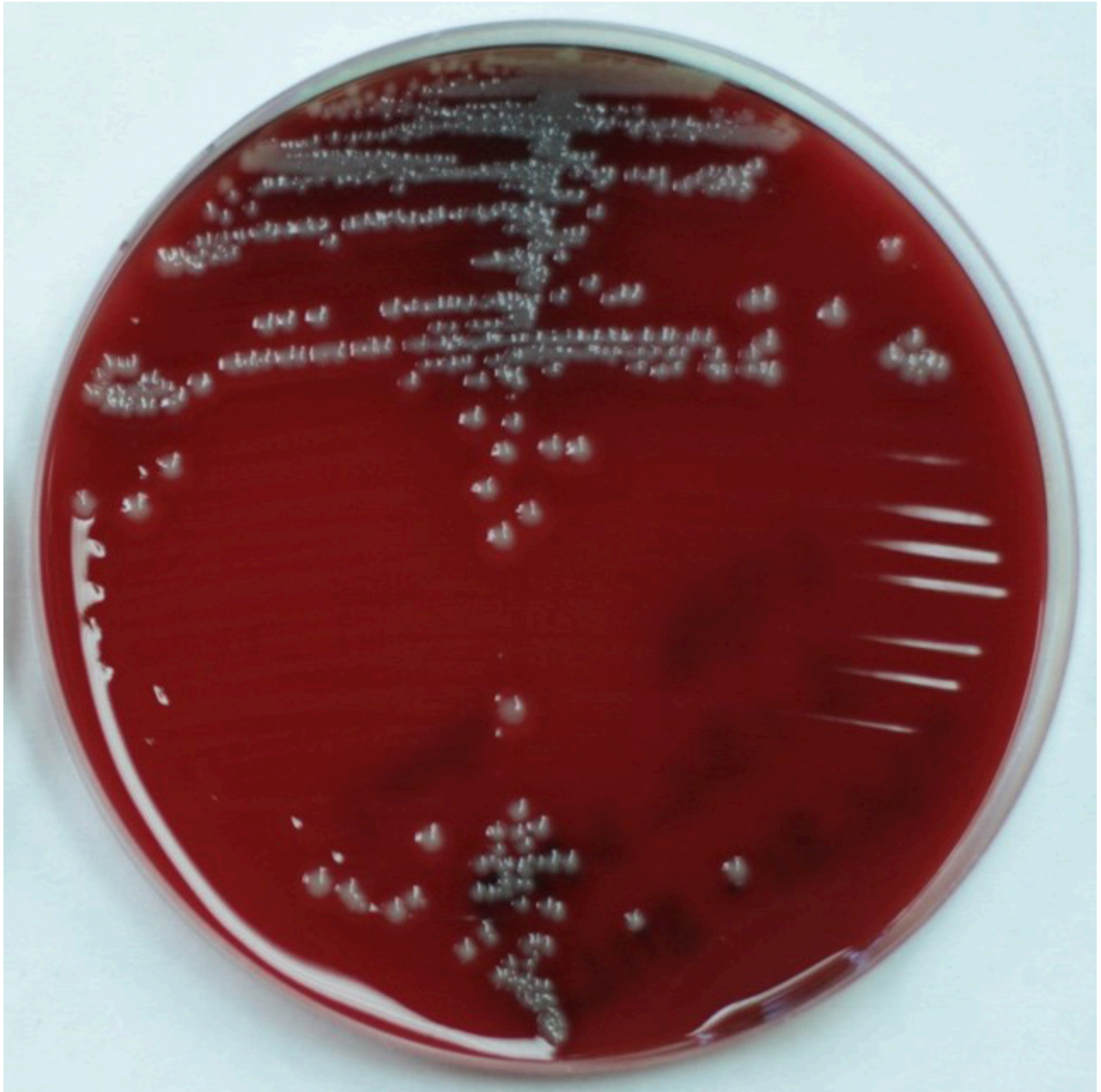
As we learned earlier, the bladder is not a sterile place, but it generally contains such low biomass of bacteria it is *generally* unculturable.

## *How can we determine the number of organisms cultured in the urine?*

For urine, we inoculate a **measured** amount of urine onto a non-selective (differential) plate such as the BAP, to determine the **colony-forming units (CFU)** present in the sample.

## *How do we calculate the CFU's in a sample?*

A **colony-forming unit (CFU)** is defined as a bacterial cell or cluster of cells that give rise to a colony on a plate.



BAP with many CFU's

On the entire BAP, each individual colony or cluster is recorded and then entered into the following equation. *You will be practicing this in the lab.*

CFU (per mL) Equation

$$CFU(per\ mL) = \frac{(\# \text{ colonies present on the plate})(\text{dilution factor})}{\text{volume plated (mL)}}$$

By counting the colonies, you can determine the concentration of bacteria in the original urine sample.

### Case Example Calculation

#### Things we know:

- The sample was collected via cystocentesis
- The loops that you will use in the lab are 1µl (volume plated)
- The number of colonies you counted on the plate was 50

#### Let's get started:

The first step is to convert µl to ml 1µl → is equal to 0.001 ml

Your urine was not diluted and applied directed from the sample collected from the animal, the dilution factor is 1.

#### So if we plug those values into the equation....

$$50 \times 10^3\ CFU\ per\ mL = \frac{(50\ colonies)(1)}{0.001\ mL}$$

Your patient has **50,000 CFU/ mL** of bacteria.

***Clinical question: Does this patient have a UTI from the CFU/mL calculated?***

## How can we determine if the number of organisms cultured is abnormal?

Based on the work of Edward Kass in the 1950's evaluating UTIs in women, we use the same cutoffs to define “too much bacteria” in the urine as the human medical profession.

The dividing line between disease and no disease is the following:

**Cystocentesis collection:  $\geq 10^3$  CFU/ml**

Catheterized specimens, counts  $\geq 10^4$  in males and  $\geq 10^5$  CFU/mL in females is significant.

\*Per [ISCAID](#), voided samples should never be used for culture. However if a voided sample is the only option ISCAID states, “Culture of voided samples should only be performed when cystocentesis is contraindicated because of the potential for both false positive and false negative cultures. Voided samples should only be cultured if they are refrigerated and processed by the diagnostic laboratory within a few hours or cultured in-house (Sørensen et al., 2016). The level of growth ( $\geq 100,000$  colony forming units (CFU)/mL), bacterial species (i.e. isolation of common uropathogens such as Enterobacteriaceae or coagulase-positive staphylococci) and whether pure growth is present are important factors to assess when evaluating culture results from voided samples, along with urine cytology and clinical signs. Laboratories should be informed whether urine samples are cystocentesis or voided samples, to ensure that quantitative culture is performed on voided samples.”

So for the patient above, culturing 50,000 CFU/ ml is too many. If the animal has clinical signs of UTI, then these results support the **clinical** diagnosis of UTI. The diagnosis of any disease using culture is should not be extrapolated from culture data alone. The bacterial culture data is used to **support your diagnosis**.

## Knowledge check



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# MODULE 9.5: DETERMINING ANTIBIOTIC SENSITIVITY OR SUSCEPTIBILITY AFTER ISOLATION OF THE PATHOGEN

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## Antibiotic sensitivity testing

Depending on the clinical signs and [ISCAID guidelines](#), you may opt to perform sensitivity (or susceptibility) testing on the bacterial isolate(s) to guide antimicrobial therapy for your patient. The antibiotic sensitivity testing procedure that you will be performing in the laboratory is the same across all testing scenarios whether it be fluid from a septic joint, milk in a cow that has mastitis, or a transtracheal wash from an animal with respiratory disease.

**After isolation of the pathogen, there are 4 major steps for the determination of susceptibility patterns for a specific pathogen.**

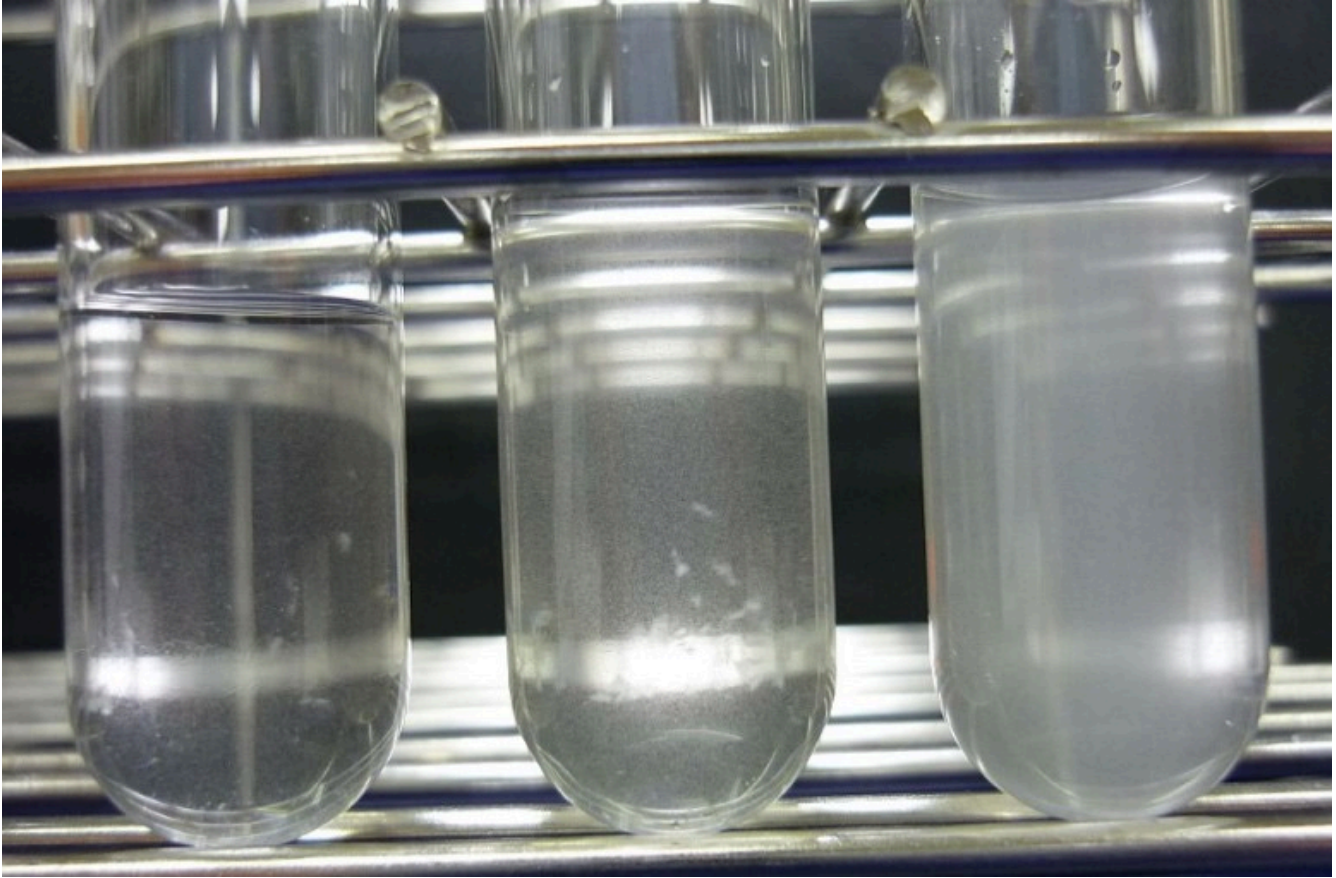
1. Using McFarland Standards to semi quantify the number of bacteria plated onto the Mueller-Hinton Antibiotic Sensitivity Plate
2. Inoculating the Mueller-Hinton plate with the bacterial suspension
3. Addition of the antibiotic discs (Kirby Bauer Disc Diffusion) to determine the zone of inhibition
4. Using the Mueller-Hinton Plate results to determine the minimum inhibitory concentration (MIC) of the drugs that the pathogen is sensitivity to using an E-test

Let's discuss these 4 steps in greater detail.

### 1. McFarland Standards

The McFarland Standards are used as a reference to adjust the bacterial concentration in a suspension using

turbidity to standardize microbial testing and data. If a suspension used is too heavy or too dilute, an erroneous result (either falsely resistant or falsely susceptible) for any given antimicrobial agent could occur.



From left to right, the McFarland Standards are 0.5, 1.0, and 2.0.

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## Knowledge check



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## 2. Antibiotic sensitivity testing

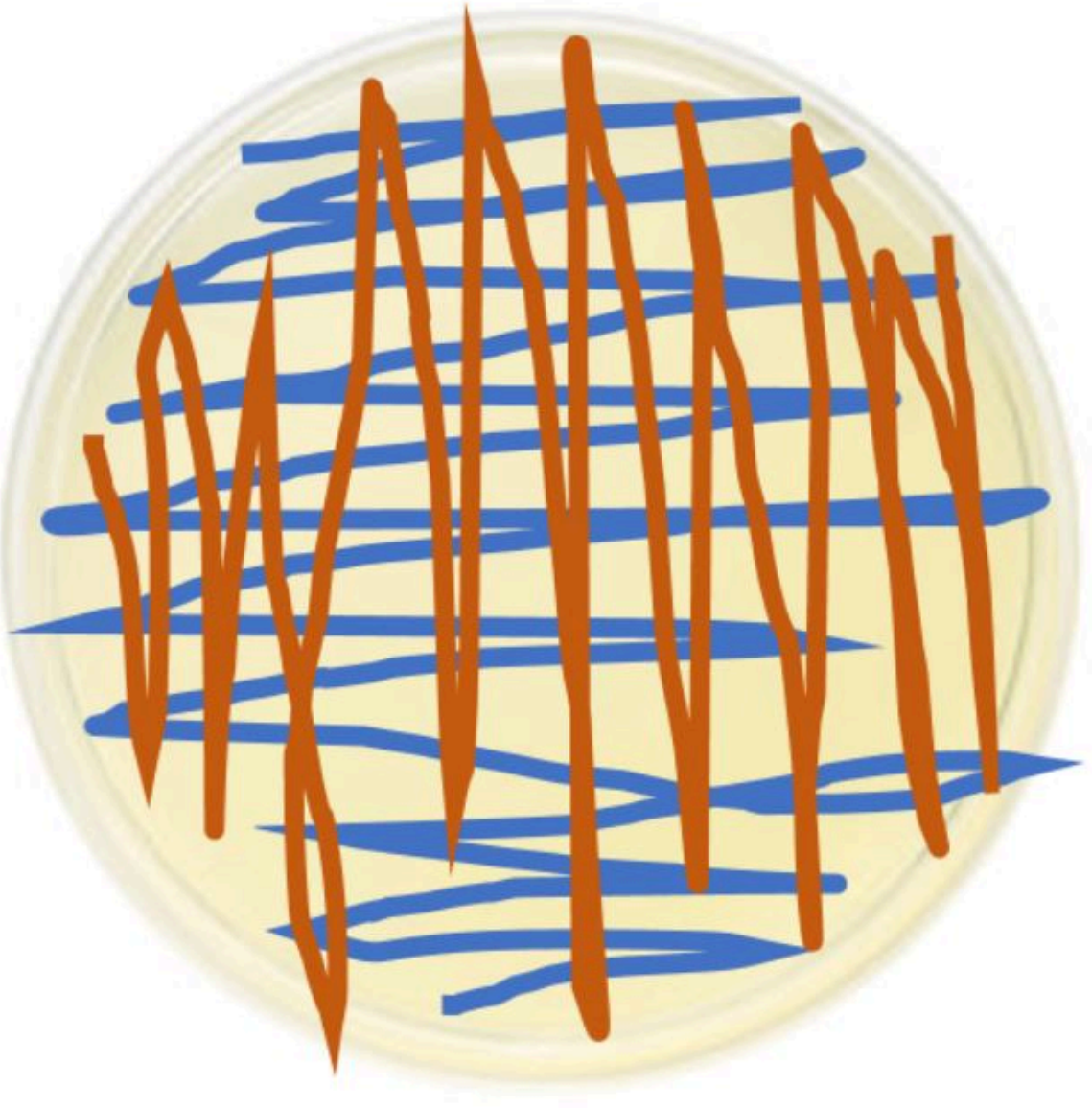
Once the bacterial suspension is prepared and **standardized it to 0.5** using the McFarland Standards, this suspension will need to be cultured WITH the specific antibiotics you are looking to use to target the infection in your patients. Generally, these antibiotics are selected by the reference laboratory and based on the original sample type.

### Preparing an antibiotic sensitivity plate

In this laboratory, your antibiotic plates, using the Mueller-Hinton plate, have been prepared for you and stamped with several discs that are impregnated with various antibiotics (Kirby Bauer Disc Diffusion Test)

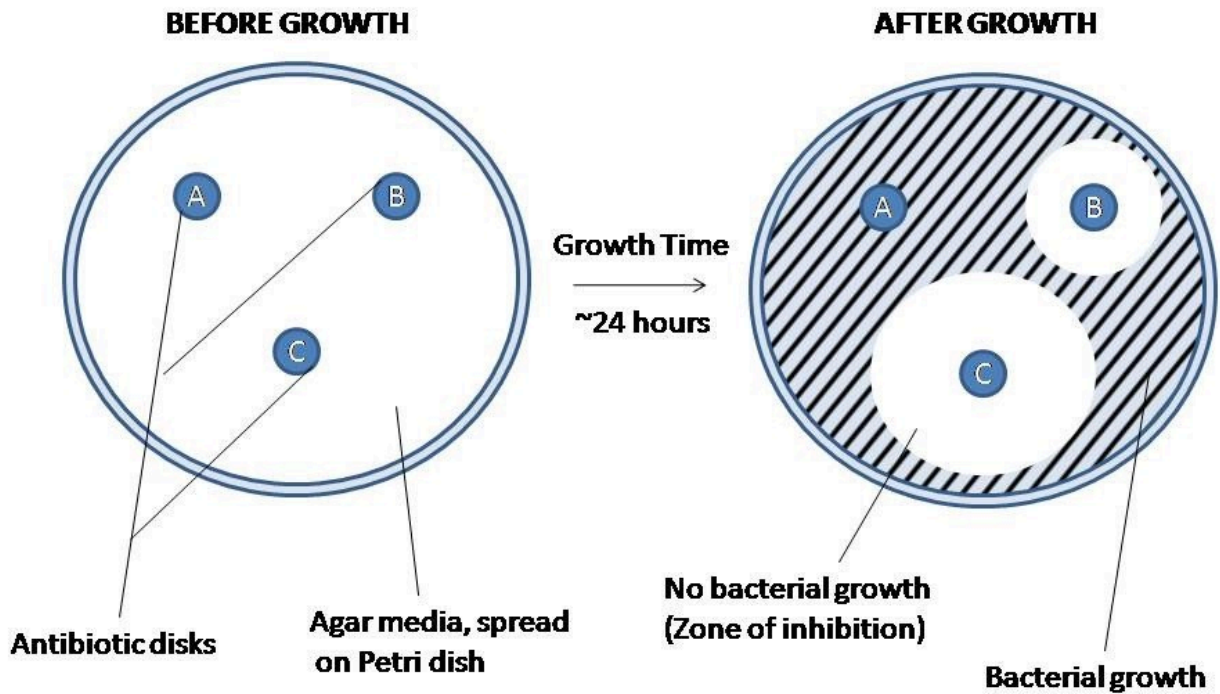
The following steps were taken to prepare your antibiotic sensitivity plate.

1. The Mueller-Hinton plate was inoculated by swabbing in three directions to evenly (and thin) coat the entire plate with the suspension. The plate was turned 45 degrees each time it was streaked. There should not be any areas on the plate that have not been entirely inoculated with bacterial broth.



Mueller-Hinton plate swabbed in three directions.

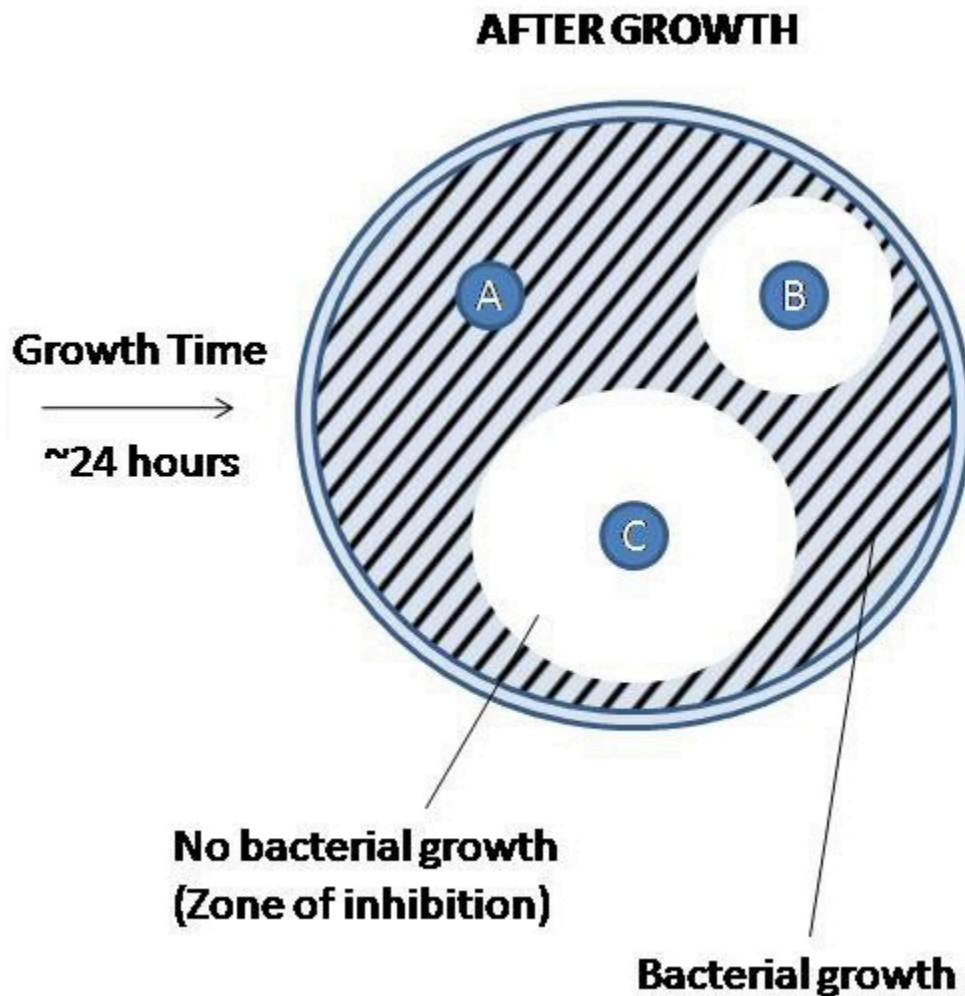
3. Antibiotic discs were stamped (using a stamping device) and incubated at 37 degrees F overnight.



Antibiotic disks are stamped onto the Mueller-Hinton plate and incubated for 24 hours.

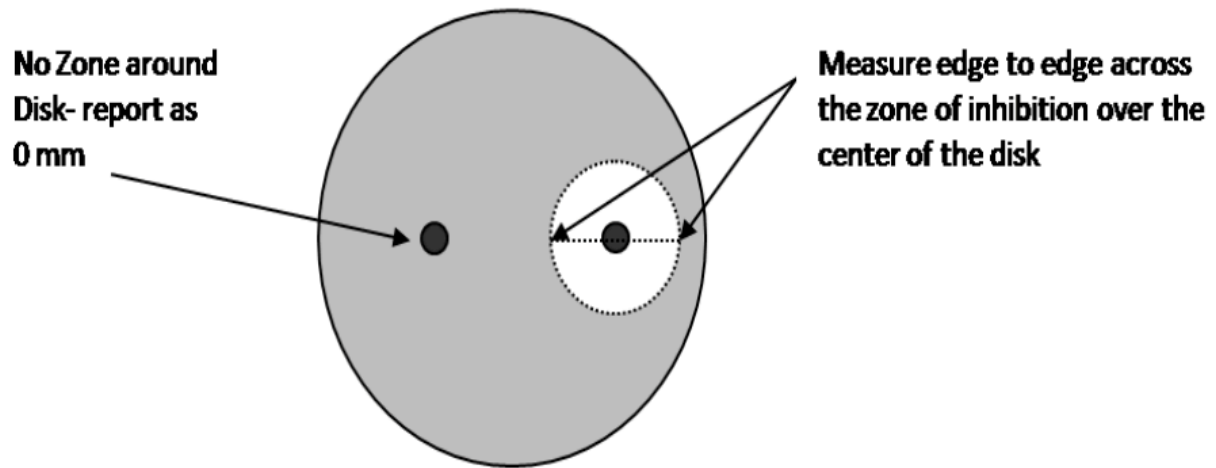
### 3. Interpreting your Kirby Bauer Disc Diffusion

After the isolate has been allowed to grow overnight, you should be able to observe the zone of inhibition (ZOI) surrounding the antibiotic discs.



Mueller-Hinton plate with zones of inhibition (ZOI) surrounding two of the three antibiotic discs

These zones are regions of the plate in which the antibiotic disc was able to diffuse across the agar and inhibit bacterial growth. The **diameter** of the zones is measured (millimeters) to determine the size of the zone of inhibition. The size of a normal zone of inhibition varies from drug to drug based on the properties of the drugs (predominately molecule size) as well as bacterial isolate. Larger molecules will not diffuse as far as smaller molecules. Based on the measurement of the ZOI, the drug is categorized as resistant, intermediate, and sensitive. It is important to remember that drugs being resistant, sensitive, or intermediate are based on behavior in an in vitro setting using a pure isolate and may not be predictive of biological behavior. This data is only used to help guide your decision based on the clinical presentation.



**ASM MicrobeLibrary © Hudzicki**

The table below is an example of the reference intervals for several drugs (bolded) and bacteria (white row) with each ZOI.

**Table 9.1: Reference intervals for several drugs and bacteria**

		Size of Zone of Inhibition (ZOI in mm)		
Antibiotic:	Disc	Resistant (R)	Intermediate (I)	Sensitive (S)
<b>Sulfamethoxazole-Trimethoprim</b>	<b>SXT</b>			
<i>Staphylococcus spp.</i>		≤10	11-15	≥16
<i>Enterobacteriaceae</i>		≤10	11-15	≥16
<i>Haemophilus influenzae</i>		≤10	11-15	≥16
<i>Streptococcus pneumoniae</i>		≤15	16-19	≥19
<b>Amoxicillin/Clavulanic acid</b>	<b>AMC-30</b>			
<i>Staphylococcus spp.</i>		≤19		≥20
Other organisms		≤13	14-17	≥18
<b>Ciprofloxacin</b>	<b>CIP-5</b>			
		≤15	16-20	≥21
<b>Nitrofurantoin</b>	<b>F/M-300</b>			
		≤14	15-16	≥17
<b>Ampicillin</b>	<b>AM-10</b>			
<i>Staphylococcus sp.</i>		≤28		≥29
<i>Enterobacteriaceae</i>		≤13	14-16	≥17
<i>Streptococcus (not S. pneumoniae)</i>		≤18	19-25	≥26
<b>Ceftriaxone</b>	<b>CRO-30</b>			

		≤13	14-20	≥21
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## Knowledge check



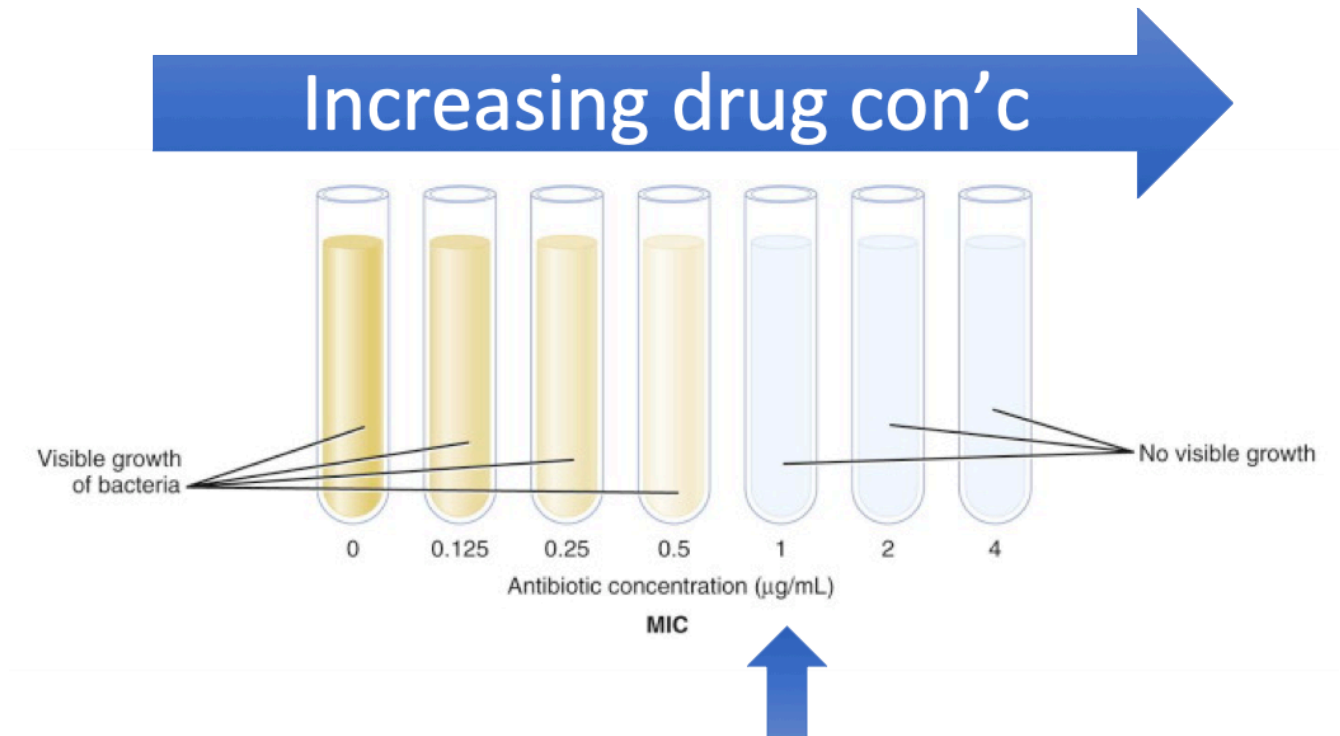
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## 4. Determining the minimum inhibitory concentration (MIC)

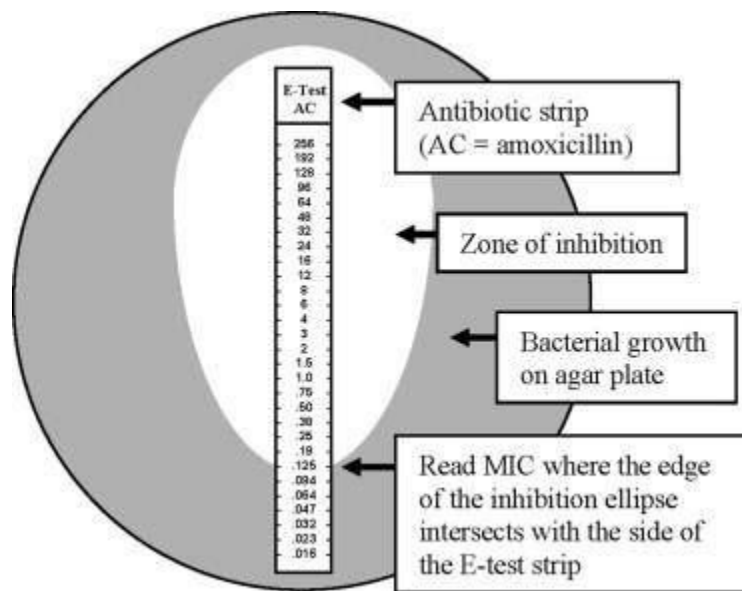
The minimum inhibitory concentration (MIC) is the lowest concentration of a chemical, usually a drug, which ***inhibits* visible bacterial in an *in vitro* setting**. This is **the bacteriostatic effect** of the drug. If we wanted to determine the bactericidal effect of the drug we would need to measure the minimum bactericidal concentration (MBC). This will not be performed in the lab.

For the image below (from Dr. Brown's lecture notes) you see that the tubes are arranged in increasing antibiotic concentration. Once the antibiotic concentration reaches 1 ug/mL the visible microbial growth can no longer be detected. Thus, the MIC for this isolate is 1 ug/mL.



Determining Minimum Inhibitory Concentration (MIC) from tubes of increasing drug concentration

In addition to using serial dilutions in test tubes, test strips that are impregnated with increasing concentration of a single drug called "**E-test**" can be applied directly to a Mueller-Hinton plate. The strip is ticked with various concentrations of the drug. The MIC is determined by reading where the edge of the inhibition ellipse intersects with the side of the E-test strip. In the example below the MIC is 0.125  $\mu\text{g/mL}$  for Amoxicillin (beta-lactam antibiotic).



E-test applied to a Mueller-Hinton Plate

Since E-tests are expensive, the Kirby Bauer Disc Diffusion test is performed first to evaluate which drugs a bacterial isolate is sensitive to before selecting which drugs to determine the MIC on. Due to time constraints, you will be applying an E-test directly to your Kirby Bauer test in the lab to determine the MIC of a certain drug (to be determined based on your isolates). The interpretation of specific cut-points for S, I, and R are determined by CSLI guidelines and vary for each drug and bacterial isolate.

## Knowledge check



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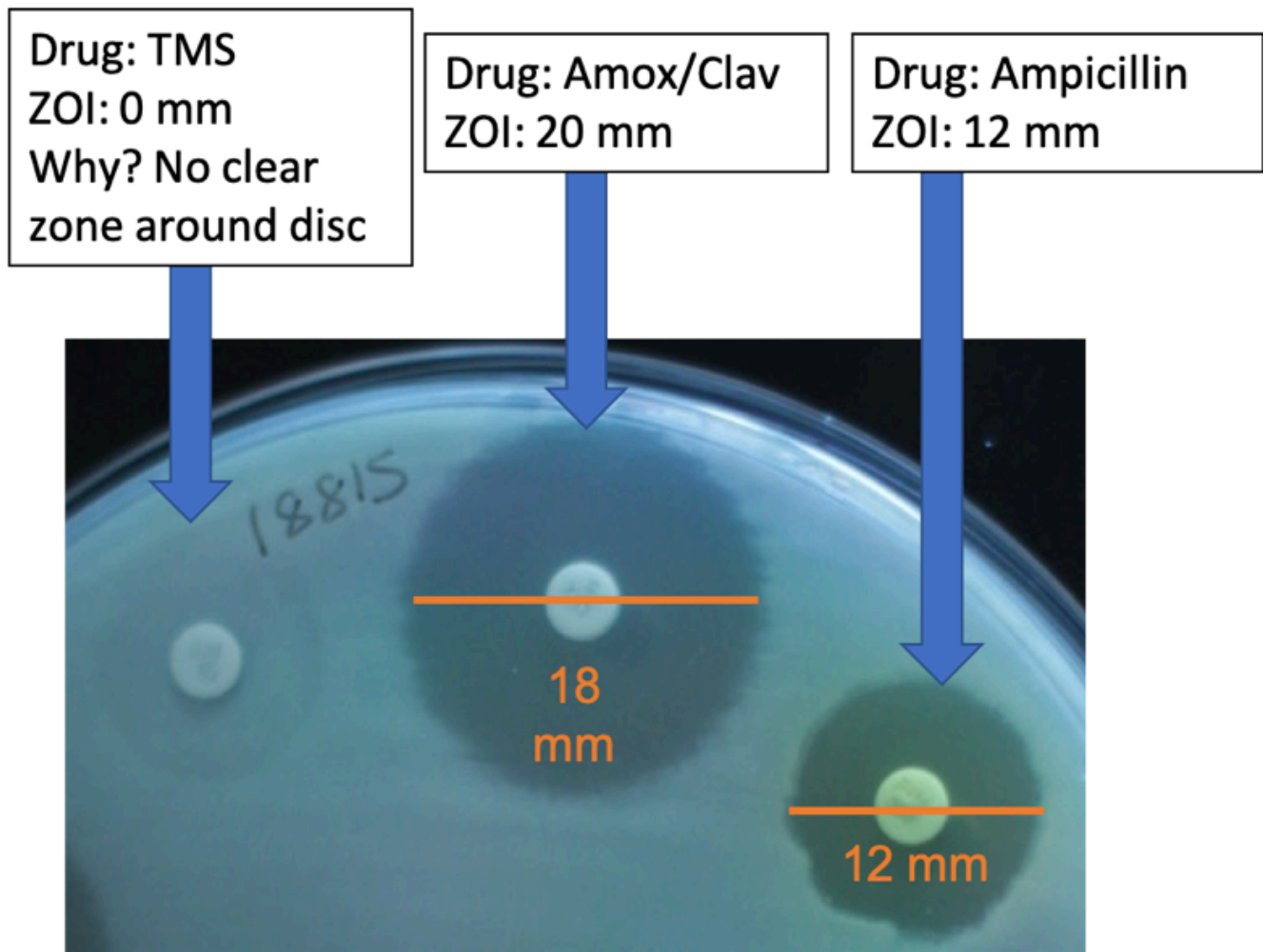
# MODULE 9.6: LET'S PRACTICE!

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## Practice example

You have isolated *E. coli* from the urine of a dog that has had multiple UTIs and been refractory to treatment. You decide to run antibiotic susceptibility testing on the animal.

The following is an image of the ZOI from your Kirby Bauer Disc Diffusion and the interpretation table for the various drugs.



**E. coli**

Zones of inhibition from Kirby Bauer Disc Diffusion

**Table 9.1: Reference intervals for several drugs and bacteria**

		Size of Zone of Inhibition (ZOI in mm)		
Antibiotic:	Disc	Resistant (R)	Intermediate (I)	Sensitive (S)
<b>Sulfamethoxazole-Trimethoprim</b>	<b>SXT</b>			
<i>Staphylococcus spp.</i>		≤10	11-15	≥16
<i>Enterobacteriaceae</i> *		≤10	11-15	≥16
<i>Haemophilus influenzae</i>		≤10	11-15	≥16
<i>Streptococcus pneumoniae</i>		≤15	16-19	≥19
<b>Amoxicillin/Clavulanic acid</b>	<b>AMC-30</b>			
<i>Staphylococcus spp.</i>		≤19		≥20
Other organisms		≤13	14-17	≥18
<b>Ciprofloxacin</b>	<b>CIP-5</b>			
		≤15	16-20	≥21
<b>Nitrofurantoin</b>	<b>F/M-300</b>			
		≤14	15-16	≥17
<b>Ampicillin</b>	<b>AM-10</b>			
<i>Staphylococcus sp.</i>		≤28		≥29
<i>Enterobacteriaceae</i>		≤13	14-16	≥17
<i>Streptococcus (not S. pneumoniae)</i>		≤18	19-25	≥26
<b>Ceftriaxone</b>	<b>CRO-30</b>			

		$\leq 13$	14-20	$\geq 21$
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**\*Remember *E. coli* is in the Family Enterobacteriaceae\***

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## Knowledge check



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### Key Takeaways

- Cystocentesis is the preferred collection method for culture when indicated
- The urinary bladder is not sterile and has its own and unique microbiome
- Antibiotic resistance and sensitivity profiles may change over time with continued antibiotic use
- The ISCAID guidelines are the gold stand for UTI management and treatment recommendations in veterinary medicine

You have now reached the end of Module 9. If you are enrolled in CVM 6925, please go to the Canvas page and take the quiz: "Module 9: Urine C&S quiz." There is an assignment that accompanies the in-person laboratory for this module.

## MODULE X

# MODULE 10: VETERINARY ECTOPARASITES

### Module Objectives

1. Group common veterinary parasites as insects or arachnids
2. Describe the pathognomonic features of common lice, ticks, mites, and fleas
3. Differentiate between a soft and a hard tick
4. Differentiate between a chewing and sucking louse
5. Correctly identify the common veterinary mites and ticks to the level of species (when asked)
6. Recall 2 diseases the 4 common ticks in the USA transmit

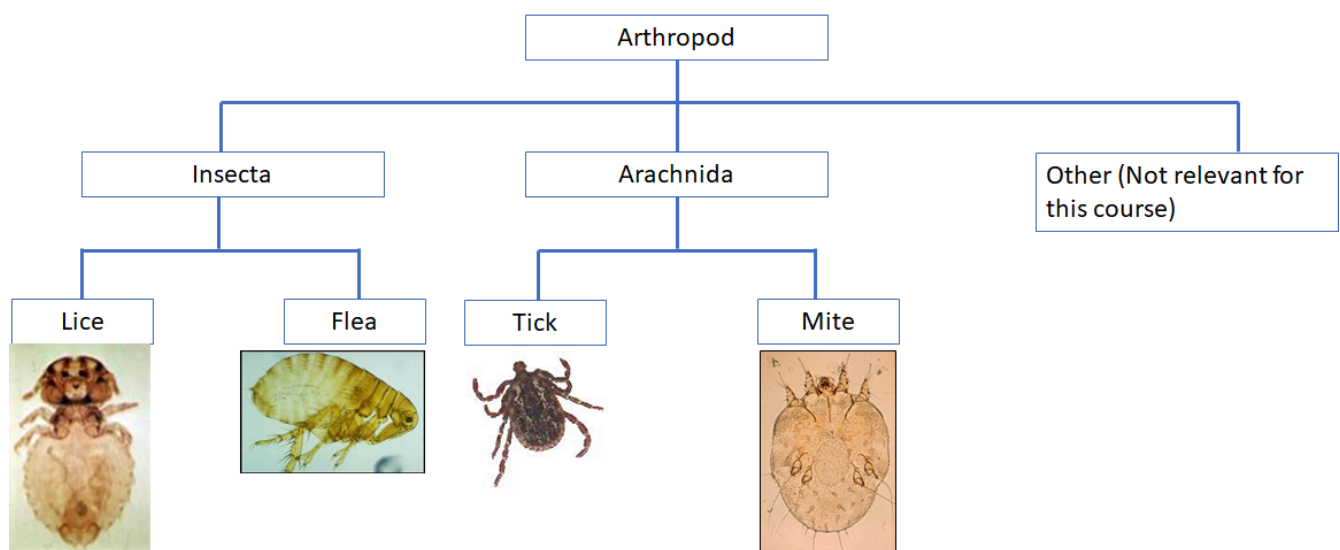


# MODULE 10.1: INTRODUCTION TO ECTOPARASITES

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## Ectoparasites

Ectoparasites are generally arthropods that live and feed on the exterior of the host, commonly the integument. In veterinary medicine, the most common ectoparasites that infest/infect our patients are ticks, mites, lice, and fleas. This module will guide you through the morphological characteristics that distinguish salient differences between ticks, mites, lice, and fleas as inevitably a client, family member, or member of the community will bring you a “bug in a jar” and want you to identify it. As a veterinarian, you are also on the frontlines to identify invasive or non-native parasites that have entered the country via the legal or illegal movement of animals.



Arthropod classification

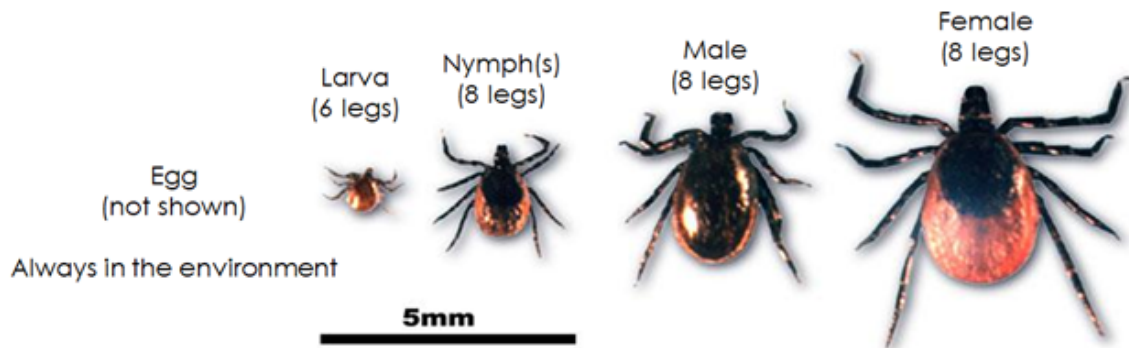
## General characteristics of insects and arachnids

### Arachnids

This class of ectoparasites includes ticks and mites. The major identifying feature of these arthropods in comparison to insects is **adult arachnids have 8 legs (4 pairs)**. The arachnids (ticks and mites)

undergo **gradual metamorphism** meaning that each stage of development can be challenging to distinguish from another as they all appear similar. The progression of development includes eggs that are laid in the environment molt into **6 legged larvae**, then molt into 8 legged nymphs, and then into 8 legged adults. Remembering that some stages of development have 6 legs is important especially with ticks since not all stages of development occur upon the same animal.

## Simple (= gradual) metamorphosis



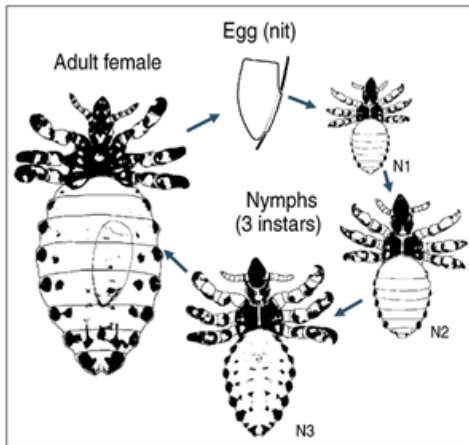
### Arachnid Metamorphism

## Insects

Important veterinary insects lice, fleas, and flies. In this laboratory, we will be focusing on the salient feature of just lice and fleas. Adult insects in comparison to arachnids are adults have 6 legs (**3 pairs**). Most insects (including fleas) undergo **complete metamorphosis** development which following hatching from an egg, the individual changes form radically between molts. In other words, unlike the mites and ticks, we can identify each separate stage of development. Insects such as lice undergo an **incomplete metamorphosis** which is when individuals of different ages are of similar form but live as progressively larger instars (larval stages) between molts. Incomplete metamorphosis is a similar process to gradual metamorphosis EXCEPT for that type of maturation occurs in ticks and mites.

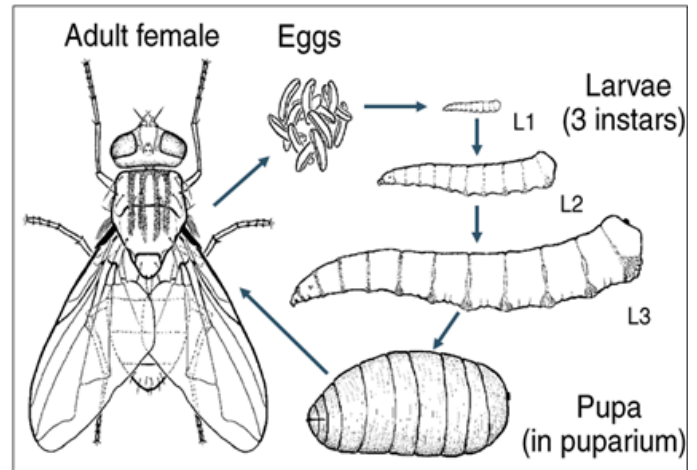
## Incomplete metamorphosis

Hog louse, *Haematopinus suis*



## Complete metamorphosis

House fly, *Musca domestica*



Incomplete vs Complete Metamorphosis

## Summary Table

Table 10.1: Ectoparasite Summary Table

Ectoparasite	Type of Arthropod	Pairs of Legs	Type of metamorphosis
<b>Ticks</b>	Arachnida	4 pairs of legs (8 legs)	Simple (gradual)
<b>Mite</b>	Arachnida	4 pairs of legs (8 legs)	Simple (gradual)
<b>Lice</b>	Insecta	3 pairs of legs (6 legs)	Incomplete
<b>Fleas</b>	Insecta	3 pairs of legs (6 legs)	Complete

## Knowledge check



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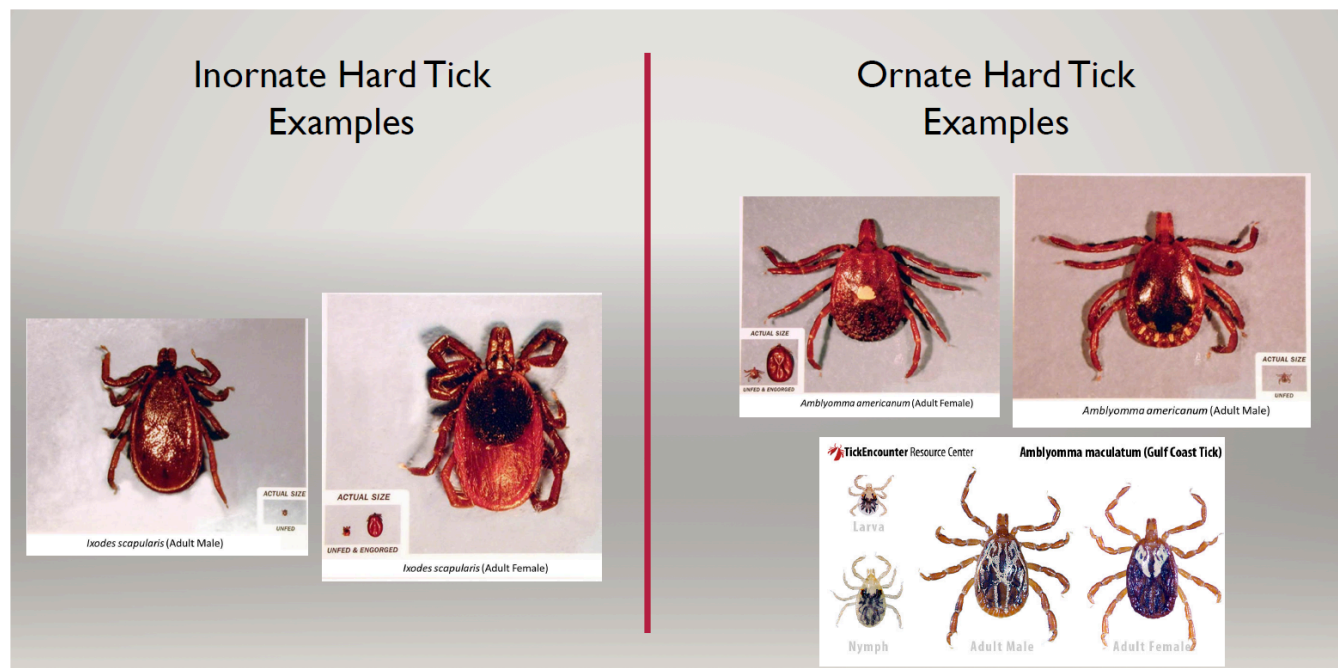
## Types of Ticks

Ticks can be divided into TWO major types: hard ticks and soft ticks based on the presence or absence of a scutum.

### Hard Ticks (Ixodidae)

This is the most common veterinary species in North America. The dorsal surface of all stages has a **scutum** (see diagram above of female tick). In male ticks, the scutum covers the entire dorsal surface, wherein female ticks, nymphs, and larvae have a scutum that covers only the anterior half. The capitulum (mouthparts) arises from the anterior of the body in each stage.

There are two subclasses of hard ticks based on the pattern on the scutum: **inornate** (no pattern) or **ornate** (white or iridescent pattern).



Inornate and Ornate Hard Ticks

## Soft Ticks (Argasidae)

There are very few species of soft ticks of veterinary importance in North America. In comparison to the hard ticks, they do not have a hard plate (scutum). Their mouthparts (capitulum) are tucked up underneath their bodies ventrally. Soft ticks live in habitats such as heavily wooded areas or under rocks as the lack of a hard scutum requires them to be adapted to sheltered/protected niches.

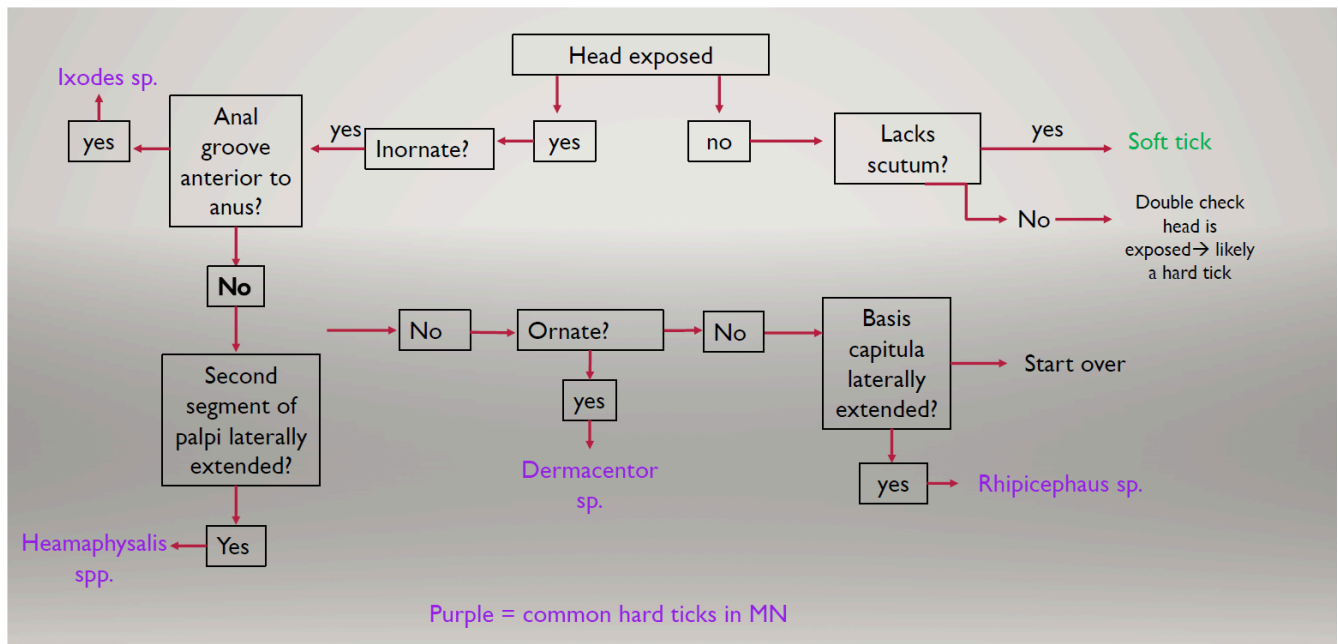


Soft tick

## Summary table and charts

Table 10.2: Tick Summary Table

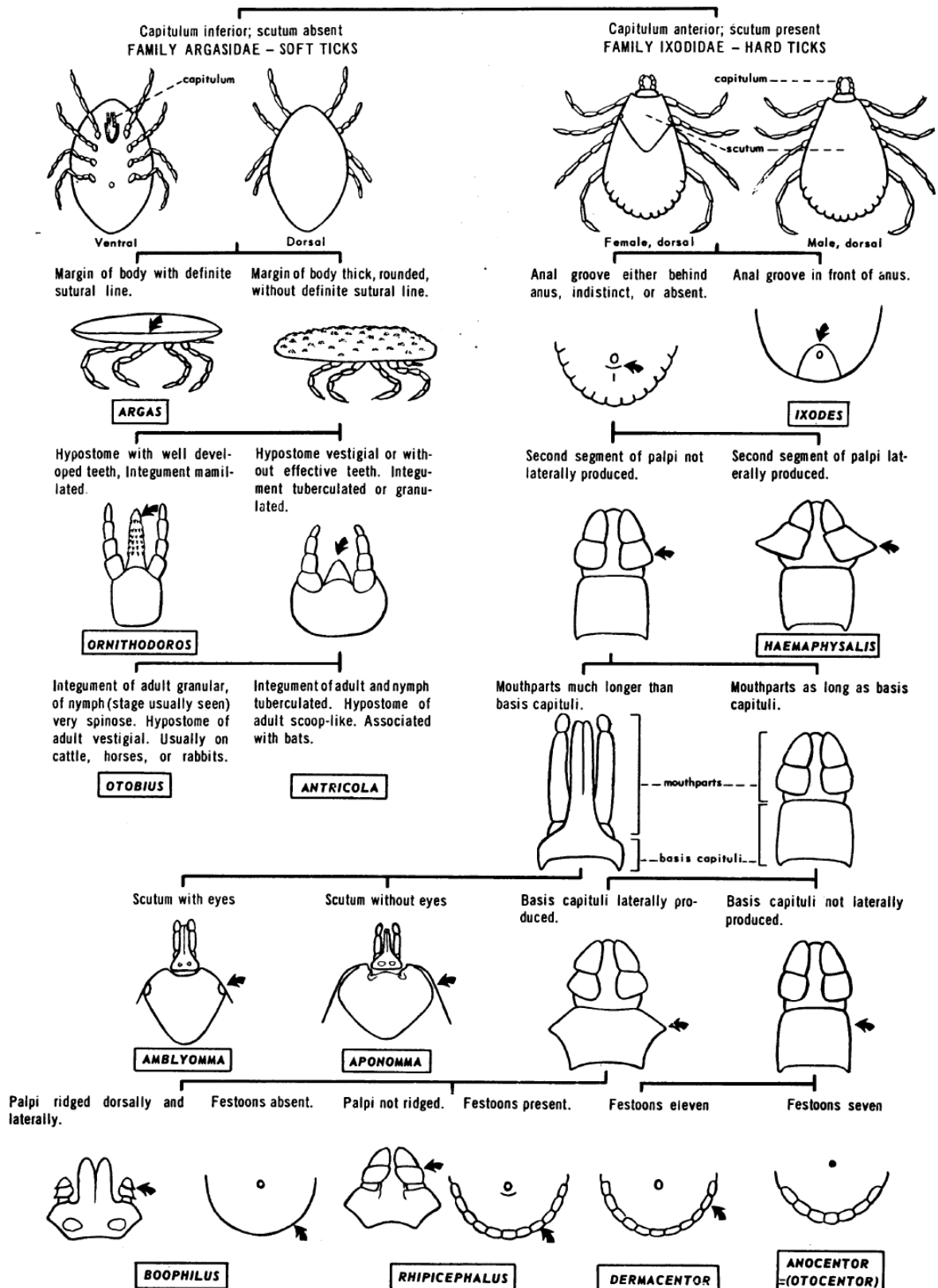
Tick Type	Scutum	Capitulum (mouthparts) location	Habitat
Hard Tick	yes	Anterior of the body	Wide diversity
Soft Tick	no	Tucked underneath the body ventrally	Woods or rocks- need protection



Tick identification flow chart

**TICKS: KEY TO GENERA IN UNITED STATES**

Harry D. Pratt



This flow chart can be found in your parasite laboratory manual and will be used to guide you through the laboratory exercise.

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## Knowledge check



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# MODULE 10.3: HARD TICKS

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## Common hard ticks

The next few sections will guide you through some of the common hard ticks found in the United States.

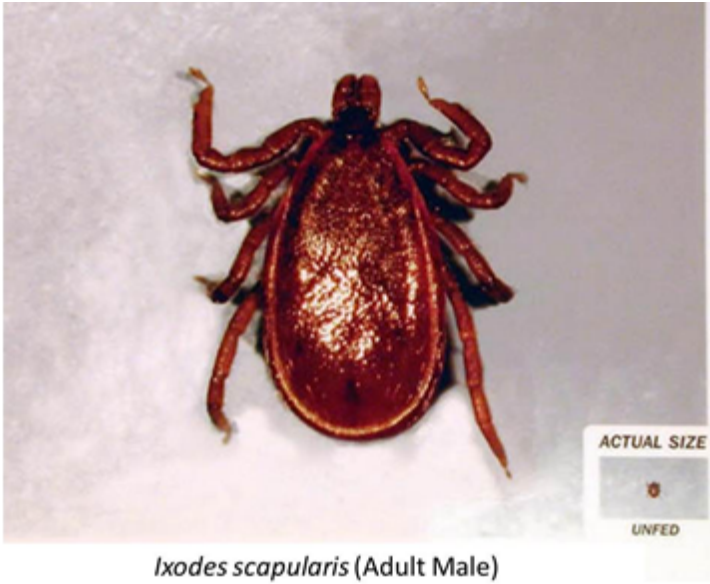
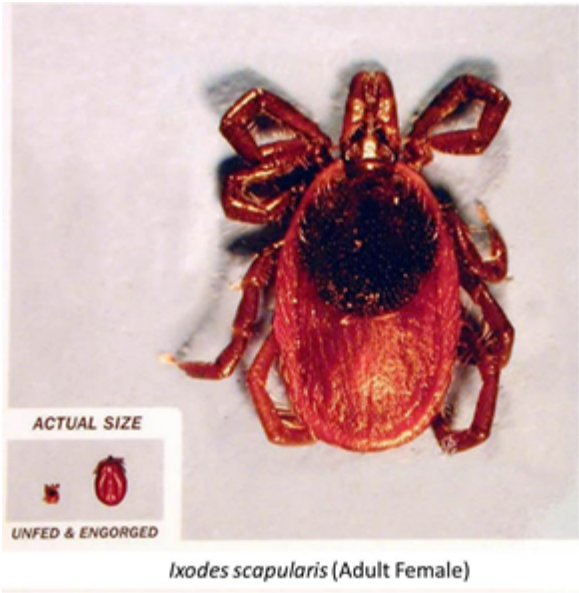


Common Hard Ticks

### *Ixodes spp.*

In the United States, there are two *Ixodes* spp. of veterinary and public health importance. These include *Ixodes scapularis* (deer tick or black-legged tick) and *Ixodes pacificus* (Western black-legged tick). These ticks use 3 hosts (require 3 separate hosts to take a blood meal for maturation) for their life cycle and prefer to live in wooded areas. *Ixodes spp.* are hard ticks that are inornate (no pattern on scutum).

**What differentiates *Ixodes* ticks from other hard ticks are their long palps and the prominent anal groove on the ventrum. The adult ticks are also the smallest of the common ticks we find in the United States. The body of an unengorged (not full of blood) is approximately the size of a 1/4 carat diamond stud. Larvae are the size of a poppy seed!**



Ixodes scapularis (deer tick or black-legged tick) and Ixodes pacificus (Western black-legged tick)

## Disease Transmitted by Ixodes spp. Ticks

Ixodes ticks are important vectors for disease transmission in animals and humans. Here are a few examples.

**Table 10.3: Disease transmitted by *Ixodes spp.* ticks**

Disease	Species affected	Type of disease transmitted
Lyme Disease ( <i>Borrelia burgdorferi</i> )	dogs and humans	bacteria
Human and Canine Granulocytic Anaplasmosis ( <i>Anaplasma phagocytophilum</i> )	dogs, humans, horses	bacteria

## Knowledge check



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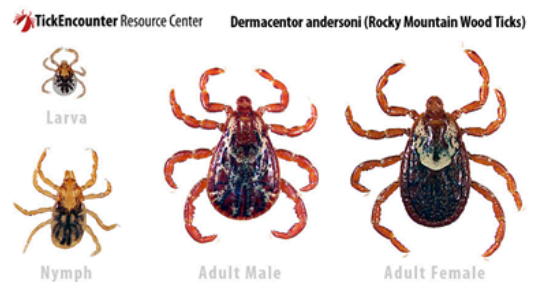
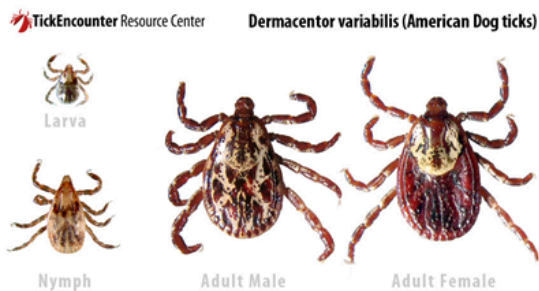
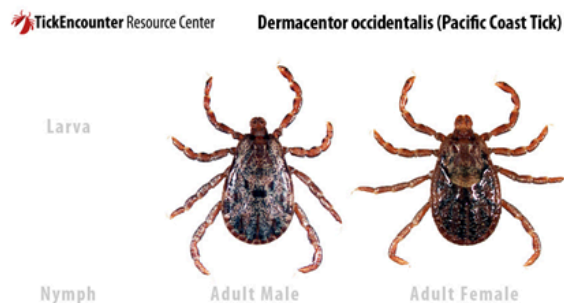
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## *Dermacentor spp.*

There are several species of *Dermacentor* of veterinary importance. These ticks vary on the number of hosts required to complete their life cycle. Overall, *Dermacentor spp.* tend to prefer to live in tall grassy areas or grassy wooded areas. These ticks are hard ticks that are ornate (white or iridescent pattern on scutum). Species of veterinary importance are *D. variabilis* (American dog tick or Wood tick), *D. andersoni* (Rocky Mountain Wood Tick), and *D. albipictus* (Winter tick).

While there are several species in this genus one of their most distinctive traits is the incredibly ornate pattern easily visualized on their scutum, specifically the males. In comparison to *Ixodes scapularis* (Deer tick), aside from being ornate, their mouthparts, specifically the palps, are much shorter than the Deer tick. In Minnesota, *D. variabilis*, *I. scapularis*, and *Rhiphacephalus sanguines* are the most common hard ticks observed on people and domestic animals.



*D. variabilis* (American dog tick or Wood tick), *D. andersoni* (Rocky Mountain Wood Tick), and *D. albipictus* (Winter tick)

## Disease Transmitted by *Dermacentor spp.* Ticks

*Dermacentor* spp. are important vectors for disease in people and animals. Here are a few of the common diseases transmitted.

**Table 10.4: Disease transmitted by *Dermacentor spp.* ticks**

Disease	Species affected	Type of disease transmitted
Tularemia ( <i>Francisella tularensis</i> )	humans, cats, small mammals	bacteria
Rocky Mountain Spotted Fever ( <i>Rickettsia rickettsii</i> )	Dogs and humans	bacteria

## Knowledge check



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## *Amblyomma americanum* (Lone Star Tick)

Of the ticks found in North America, *Amblyomma americanum* ticks have the greatest impact on disease transmission in both people and domestic animals. These ticks are aggressive **questers**, have a wide host range they will take blood meals from (not picky eaters), and transmit the majority of fatal diseases of veterinary and public health importance. In general, these ticks require 3 hosts to complete their life cycle, but the host range is quite diverse.

Similar to *Dermacentor*, Lone star ticks are ornate hard ticks. They live in deep leaves and foliage mainly in

the Southeastern USA, with the heaviest concentration in Missouri and Oklahoma, but also found as far north as southern Iowa. Most recently, these ticks have been found in Austin, MN so they are moving northward as a result of global warming. The female tick displays a single or “lone” star on their scutum, while the males have small, white ticked lines on the festoons (the ridges on the “skirt” of the scutum).



*Amblyomma americanum* (Adult Female)



*Amblyomma americanum* (Adult Male)

*Amblyomma americanum* (Lone Star Tick) Male and Female

## Disease Transmitted by *Amblyomma americanum* Ticks

**Table 10.5: Disease transmitted by *Amblyomma americanum* ticks**

Disease	Species affected	Type of disease transmitted
<i>Granulocytic ehrlichiosis</i> ( <i>Ehrlichia chaffeensis</i> , <i>E. ewingii</i> )	humans, dogs, cats	bacteria
<i>Tularemia</i> ( <i>Francisella tularensis</i> )	humans, cats, small mammals	bacteria
<i>Cyathuxzoon felis</i>	wild and domestic cats	protozoan

## Knowledge check



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### *Rhipicephalus sanguineus* – “Brown dog tick” or “kennel tick”

The brown dog tick is the most common tick in Minnesota, United States, and worldwide. This is a cosmopolitan tick and prefers **to live indoors** on dogs and humans. *Rhipicephalus sanguineus* requires 3 hosts to complete its life cycle. Different than the other hard ticks, each of these “hosts” is usually a dog. It can be the same dog, but following a blood meal, the tick will fall off the dog, molt in the environment, and quest for a new meal, that may be the same dog or a different dog (or human!).

Similar to the *Ixodes* ticks, these ticks are **inornate** (no pattern on scutum). However, it is fairly easy to tell the difference between *Ixodes* and *Rhipicephalus*, as the capitulum of the *Rhipicephalus* is hexagonal with short stumpy palps. If you remember, *Ixodes* has a rectangular capitulum and long palps. Lastly, the size can be a giveaway. Adult *Rhipicephalus* are generally larger than adult *Ixodes* ticks. Unengorged *Rhipicephalus sanguineus* are approximately the same size as a shucked sunflower seed.



*Rhipicephalus sanguineus* (Brown dog tick)

## Disease Transmitted by *Rhipicephalus sanguineus* Ticks

**Table 10.6: Disease transmitted by *Rhipicephalus sanguineus* ticks**

Disease	Species affected	Type of disease transmitted
Canine monocytic ehrlichiosis ( <i>Ehrlichia canis</i> )	Dogs	bacteria
Rocky Mountain Spotted Fever ( <i>Rickettsia rickettsii</i> )	Dogs and humans	bacteria

## Knowledge check



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<https://pressbooks.umn.edu/cvdl/?p=371#h5p-68>

# MODULE 10.4: SOFT TICKS

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## *Otobius megnini* (Soft Tick)

The most important **soft tick** in North America is *Otobius megnini* (spinose ear tick) that infects all domestic animals but is considered of a particular nuisance to livestock, especially to horse and alpaca owners. The spinose ear tick is a one host tick. The larval and nymph stages are parasitic and live deep in the ear canal development. The nymphs fall off the host to molt to adults that live and mate in the environment. This tick does not have a hard scutum and is considered a soft tick despite being armed with thousands of small spines throughout its integument.



*Otobius megnini* (Nymph)

<https://capovet.org/guidelines/ticks/>



<http://csu-cvmb.colostate.edu/vdl/Pages/spinose-ear-tick.aspx>

*Otobius megnini* (spinose ear tick)

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## Knowledge check





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# MODULE 10.5: MITES, LICE, AND FLEAS

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## Mites

Mites are divided into families based on where you find them within or on the integument (skin). The 4 families of veterinary importance are Sarcoptidae, Psoroptidae, Cheyletidae, and Demodecidae.

**Table 10.7: Mites of Veterinary Importance**

Family	Location in host integument	Common Species
<b>Sarcoptidae</b>	Burrow into the skin	<i>Sarcoptes scabiei</i>
<b>Psoroptidae</b>	Reside on skin	<i>Octodectes cynotis</i>
<b>Cheyletidae</b>	Reside on skin	<i>Cheyletiella spp.</i>
<b>Demodecidae</b>	Reside in hair follicles	<i>Demodex spp.</i>

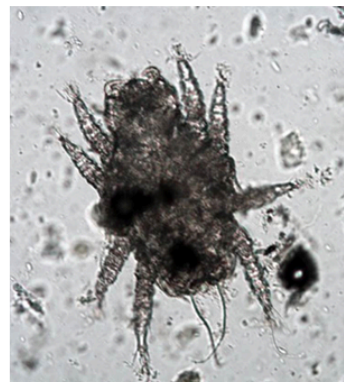
Similar to ticks, these arachnids undergo **gradual metamorphosis** and have 4 pairs (**8 legs**). Different than adult ticks, the majority of mites are **microscopic** and difficult to see with the naked eye. Therefore, the microscopic evaluation of skin scrapings (or sometimes scotch tape preps) is the first diagnostic test we reach for with suspected mite infestation.

## Mite identification table

Below is a table of the common mites of veterinary importance. We will discuss these mites in more detail in Agents of Disease; however, their salient morphological features are listed below and will help guide you through the identification of the mites in the laboratory exercises.

**Table 10.8: Morphological Features of Common Mites**

Species of Mite	Family of Mite	Location on host	Transmission	Host	Identifying characteristics
<i>Sarcoptes scabiei</i> (Itch mite)	Sarcoptidae	Burrow into the epidermis	Direct contact	Dogs, pigs, cattle, horses, sheep, humans	Globose in shape, 3 <sup>rd</sup> and 4 <sup>th</sup> pairs of legs are short
<i>Octodectes cynotis</i> (ear mites)	Psoroptidae	Reside on the skin or within the ear	Direct contact	Cat and dog	Males have two suckers ventrally, females have long hairs attached to 3 <sup>rd</sup> and 4 <sup>th</sup> pair of legs
<i>Cheyletiella</i> spp. (walking dander)	Cheyletidae	Reside on skin	Direct contact- species specific	Cat, dog, rabbit, humans	Large claws
<i>Demodex</i> spp.	Demodecidae	On hair follicle	Skin to skin contact- normal inhabitant	Multiple forms on dog, cat, cow, pig, human	Cigar-shaped, 8 legs toward the head

*Sarcoptes scabiei**Octodectes cynotis**Cheyletiella blakei**Demodex* spp.

## Common Mite Species

# Knowledge check



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## Lice

Lice are **host-specific** and transmitted by direct contact between animals. Animals are usually infected in autumn and with significant infestation occurring over winter. The two suborders of lice are Mallophaga (chewing lice) and Anoplura (sucking). Adult lice are seen easily with the naked eye and approximately the same size as a sesame seed.

In this laboratory, it will not be as important to identify lice to the specific genus or species, but **I do want you to be able to identify a chewing versus sucking louse**. It is important to be able to distinguish between the two as prevention and control and clinical signs differ between the two major types.

Table 10.9: Chewing Versus Sucking Louse

Suborder	Identifying characteristics	Movement
Mallophaga (biting/ chewing lice)	<ul style="list-style-type: none"><li>• Wide mandible, chewing parts</li><li>• 3mm long</li><li>• Yellow bodies</li><li>• Not bloodsuckers</li></ul>	Rapid/fast
Anoplura (bloodsucking lice)	<ul style="list-style-type: none"><li>• Pointed nose</li><li>• Large, 3-5mm</li><li>• Dark blue, depending on the amount of blood ingested</li><li>• Large claws to grasp hair</li></ul>	Slow

## Suborder: Mallophaga

“Chewing”



*Trichodectes canis*

## Suborder: Anoplura

“Sucking”



*Linognathus setosus*

The two suborders of lice: Mallophaga (chewing lice) and Anoplura (sucking lice)

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## Knowledge check



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## Fleas

Fleas are of the order Siphonaptera. Depending on the species of flea these parasites prefer to live on the

host (dog or cat) or live in a nest (prairie dog fleas). The most common flea in small animals (dogs/cats) is *Ctenocephalides felis*. While these fleas prefer to feed on dogs and cats, they will feed opportunistically on humans in a pinch.

Compared to lice, these insects are similar in size to sucking lice (sesame seed) but are rapidly moving. Under the microscope, the legs get larger posteriorly, with powerful posterior legs that are used for jumping from host to host. Fleas also have a pronotal and genial (it looks like a mustache!) comb that entomologists use to help speciate.

In the laboratory, **you will not be asked to speciate fleas**, but you will be required to distinguish a flea from other ectoparasites microscopically.

Table 10.10: Flea Species

Flea Species	Identifying characteristics	Transmission	Species commonly infected
<i>Ctenocephalides felis</i>	<ul style="list-style-type: none"><li>– eggs: white, oval with rounded ends</li><li>– larvae: found in the environment are maggot-like</li><li>– pupae: white-colored</li><li>– adults: eyes are present, comb located on the head with spines pointed horizontal, 6 legs</li><li>– flea dirt: reddish-black pellets of dried blood excreted by adult fleas</li></ul>	Contact with infected environment	Dog, cat



*Ctenocephalides felis* female



*Ctenocephalides felis* male

*Ctenocephalides felis* male and female

## Disease Transmitted by *Ctenocephalides felis flea*

Fleas have been transmitting terrible diseases to both humans and animals for thousands of years (anyone remember the Black Plague?) Below is a list of some diseases transmitted by fleas in general.

**Table 10.11: Disease Transmitted by *Ctenocephalides felis flea***

Disease	Species affected	Type of disease transmitted
<i>Bartonella henselae</i>	cats, humans	bacteria
<i>Dipylidium caninum</i>	Dog, cat, humans	tapeworm (vector)

## Knowledge check



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### Key Takeaways

- Lice, mites, ticks, and fleas are some of the most common ectoparasites of veterinary species
- Mites and ticks as nymphs and adults have 8 legs, but 6 when they are larvae
- Lice and fleas have 6 legs as adults
- Adult ticks, fleas, and lice can be seen with the naked eye
- Mites often require a microscope to be observed

You have now reached the end of Module 10. If you are enrolled in CVM 6925, please go to the Canvas page and take the quiz: "Module 10: Ectoparasites quiz." There is an assignment that accompanies the in-person laboratory for this module.

## MODULE XI

# MODULE 11: RAPID POINT OF CARE (POC) TESTING

### Module Objectives

1. Explain the principle of how a lateral flow ELISA test works
2. Memorize which tests detect antibodies or antigens for 4Dx Plus, Foal IgG, K9 Parvovirus, and K9 Giardia tests
3. Memorize what type of sample is required for each test
4. Paraphrase the procedures for each of the tests listed above



# MODULE 11.1: POINT OF CARE DIAGNOSTIC TESTING

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## Introduction to point of care diagnostic testing

While there are many point-of-care diagnostic tests available on the market for both companion and production animals, we are going to focus on the screening tests that use plasma, serum, feces, and/or whole blood to generate a laboratory result. There are many examples of these tests available to veterinarians from several different pharmaceutical companies and new ones are always coming on the market. In the virtual laboratory, we are going to be using lateral flow assays (LFA), the most common point of care testing, on patient samples, and case studies. Other common names for these type of tests include the following:

- Lateral flow test (LFT)
- Lateral flow device (LFD)
- Lateral flow assay (LFA)
- Lateral flow immunoassay (LFIA)
- Lateral flow immunochromatographic assays
- Dipstick
- Pen-side test
- Quick test
- Rapid test
- Test strip

Below are images of common LFA used in everyday practice in the medical community. Examples in the images include a SNAP Test, a human pregnancy test, a rapid human Strep test, and a general heartworm test.



SNAP test



Human Pregnancy Test



Rapid Human Strep Test



General Heartworm Test

## Methods of lateral flow testing

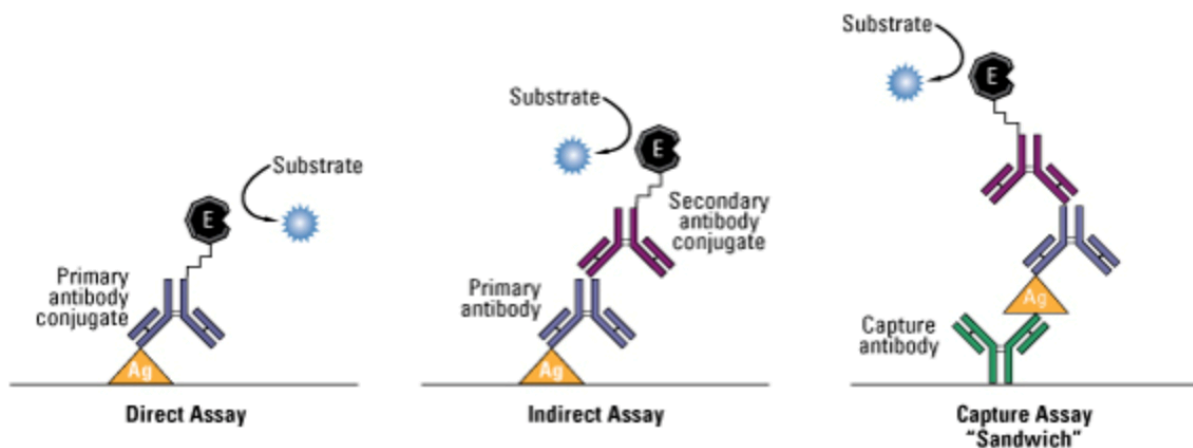
These tests use ELISA technology in which labeled antibodies react with a patient antigen or antibody that results in a visible color change at the test line or dot position.

**There are 3 main types of ELISA's:**

- Direct Assay

- Indirect Assay
- Sandwich Assay

The most common type of assay used in POC LFA is the **Capture or Sandwich ELISA** as the other two techniques often require a pure sample not easily achieved in a busy clinical setting.



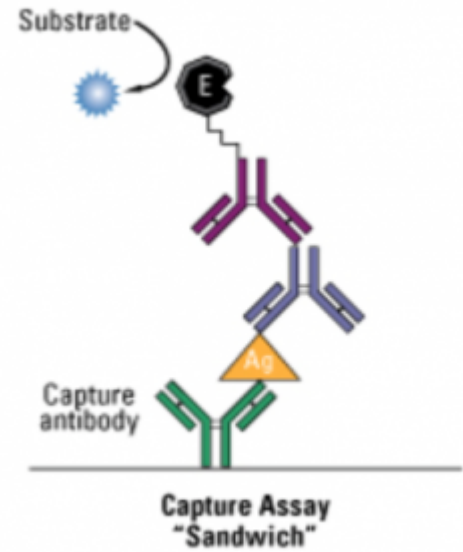
Direct Array, Indirect Array, and Capture or Sandwich Assay

## How does this technology work?

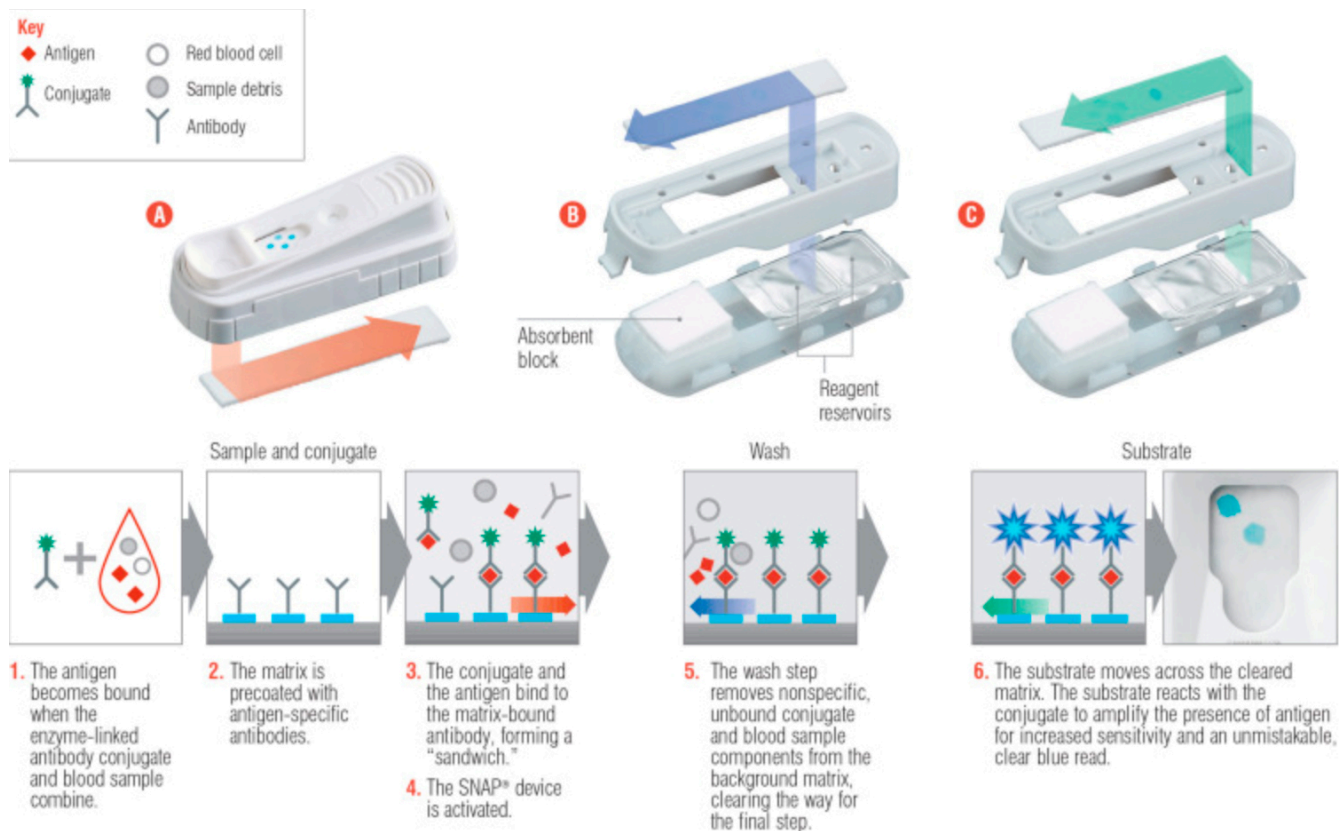
- The figure to the right demonstrates the reaction within the test
  - This sandwich ELISA is detecting the patient's **blue antibody**
  - The capture antibody has an antigen attached to it that "captures" the patient's blue antibody
  - The magenta (purple) antibody is the secondary antibody conjugate that changes color following attachment to the patient's antibodies.
- The description that follows is how the SNAP ELISA works
  - The sample is dropped into the well of the test (below image "A"). The sample pad acts as the first stage of the absorption process, and in some cases contains a filter, to ensure the accurate and controlled flow of the sample.
  - The conjugate pad, which stores the conjugated labels and antibodies, will receive the sample. If the target is present, the immobilized conjugated antibodies and labels will bind to the target and continue to migrate along with the test.
  - As the sample moves along the device the binding reagents situated on the nitrocellulose

membrane will bind to the capture antibodies at the test line or dot. A colored line or dot will form.

- The sample will continue passing through the nitrocellulose membrane into the absorbent pad. The absorbent pad will absorb the excess sample. The specification of the absorbent pad will have an impact on the volume of the sample the test can incorporate.
- If either a positive or negative sample does not result in activation of the positive control line or dot, then the test needs to be re-run



Capture or Sandwich Assay



How the SNAP ELISA works

Here is a video explaining how a lateral flow assay works:



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ELISA tests, in general, make great **screening tests** as they are often **very sensitive**, but not always **very specific**. This makes sense if we think about it from a strictly biological standpoint. In our bodies, antibodies are created by our B-cells in response to a myriad of causes for antigenic stimulation. In some cases, antibodies created to detect a certain protein or substrate secreted or on the surface of a pathogen or drug will have a similar molecular structure to that of a protein, peptide, receptor, etc. on one of our cells (aka molecular mimicry). This cross-reaction results in our cells being attacked and destroyed by immune cells. An example is this rheumatic fever in people with Strep A infections as the antibodies produced to attack the bacteria cross-react with proteins found in the synovium, myocytes, and heart valves resulting in immune-mediated polyarthropathy (IMPA) and heart failure.

While the antibodies that are used in these tests have been produced in laboratory animals and then purified to increase specificity, they are still not perfect. Thus it is recommended that all point of care testing be confirmed with a secondary test. This confirmation test will vary depending on the positive result.

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## Knowledge check



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# MODULE 11.2: TYPES OF POINT OF CARE DIAGNOSTIC TESTS

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## Point of Care Testing Laboratory Exercises

In the virtual laboratory, you will have to evaluate 4 different point of care diagnostic tests. The next few sections will guide you through the 4 different diagnostic tests that you will be using in the laboratory.

### **4 Different POC Diagnostic Tests:**

1. Canine SNAP® parvovirus testing
2. SNAP® Giardia Test
3. Heartworm Test
4. SNAP® Foal IgG Test



4Dx Plus SNAP Test

# MODULE 11.3: CANINE SNAP® PARVOVIRUS TESTING

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## Canine SNAP® parvovirus testing

Canine parvovirus is a highly contagious and severe viral diarrheal disease commonly seen in puppies and unvaccinated dogs throughout the world. For animals that do not receive aggressive supportive care during the disease, the course will commonly succumb to this disease. While the exact origin of this virus is unknown, it is believed to originate from the feline panleukopenia virus, thus, the Canine SNAP® Parvovirus test can also be used off label to screen for Feline panleukopenia virus.

## Procedure

1. Bring test to room temperature if it has been refrigerated
2. Obtain a sampling swab and a SNAP device for each sample to be tested. Pull and twist the tube covering the swab tip to remove the tube from the swab/reagent bulb assembly. Using the swab, coat the swab tip with **fecal material**. Then, return the swab to the tube. NOTE: Only a thin coat of fecal material on the swab is required; do not coat the swab with excess feces.
3. Break the purple valve stem inside the bulb assembly by bending the assembly at the narrow neck, re-bending the opposite way may be helpful. Squeeze the reagent bulb three times to pass the blue solution through the swab tip and mix it with the sample.



#### Canine SNAP® parvovirus test

4. Place the SNAP device on a horizontal surface. Using the swab as a pipette, dispense **5 drops of the fluid** into the sample well, being careful not to splash contents outside of the sample well. The sample will flow across the result window, reaching the activation circle in **30–60 seconds**. Some samples may remain in the sample well.

Fun fact: The word **FECES** has 5 letters in it and the Parvo test required 5 drops of the **brown** reagent/fecal solution

5. When color **FIRST** appears in the activation circle, push the activator firmly until it is flush with the device body
6. Some samples may not flow to the activation circle within 60 seconds, and, therefore, the circle may not turn color. In this case, press the activator after the sample has flowed across the result window.
7. Read the test result at **8 minutes**.

## Interpretation



**SNAP® Parvo**—Test dogs that present with sudden onset of lethargy, vomiting, fever, and diarrhea.

### Interpreting a SNAP® parvovirus test

If the positive control does not display or the negative control appears positive then the test should be re-run. The positive color change of the negative control likely signifies test interference with another protein or antigen and test results cannot be trusted.

To answer the questions in your laboratory activity. Please refer to the [IDEXX website](https://www.idexx.com).

## Knowledge check



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# MODULE 11.4: SNAP® GIARDIA TEST

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## SNAP® Giardia Test

*Giardia* is a protozoan parasite that causes small intestinal disease and diarrhea in a wide variety of animals, including people. In 2018, the state of Minnesota, 6.47% of dogs and 4.13% of cats tested were positive for this disease. Because of *Giardia*'s worldwide distribution and potential zoonotic potential, many diagnostic tests including point of care testing are available for the detection of *Giardia*. These tests are summarized in the following table.

**Table 1. Summary of different methods for diagnosis of *Giardia* infection in dogs**

	Method	Target detected	Specificity	Sensitivity	Test information
Performed at veterinary clinic	Direct smear	Cysts, trophozoites	Low	Low	Qualitative
	Microscopy after flotation	Cysts	Low	Low	Qualitative
	Point-of-care test (e.g., immunochromatographic tests)	Antigen	High	High	Qualitative
Performed at parasitology laboratory	Immunofluorescence microscopy	Cysts	High	High	Qualitative and quantitative
	Polymerase chain reaction <sup>a</sup>	DNA	High	High	Qualitative (quantitative for qPCR)

<sup>a</sup>PCR performed with primers for different loci often gives varying results indicating that a multilocus genotyping approach is recommended.

### Giardia Diagnostic Tests

The Giardia SNAP® Test we will be using in the laboratory is similar to the Canine parvovirus test in that it is also used to detect the free-floating antigen dispersed in the feces. This refers to an antigen that is not associated with a *Giardia* cyst wall or trophozoite.

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## Knowledge check



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## Patient selection for Giardia testing

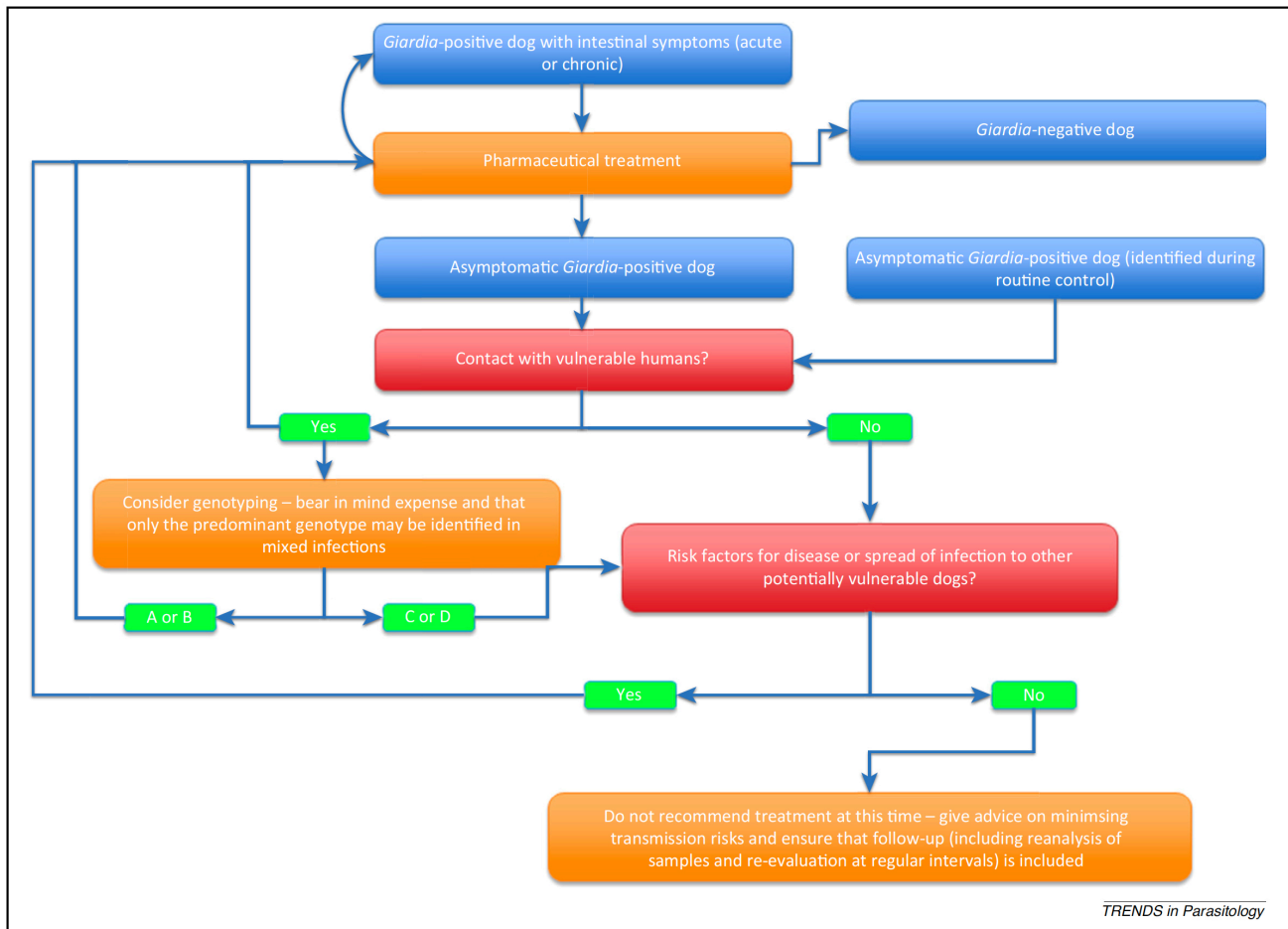
The role of *Giardia* in the development of intestinal disease is still largely unknown in dogs as we can detect Giardia cysts and antigens in dogs and cats that are asymptomatic and having diarrhea. (See AOD1 notes for more specifics on the life cycle and pathogenesis)

### A few hypotheses for asymptomatic animals and detection of the protozoan include:

- Does *Giardia*, similar to other diseases such as Coronavirus infections, develop a carrier state?
- Are these protozoans commensals? Are increases in shedding indicative of a state overall of microbial dysbiosis?
  - This theory stems from the finding of commensal protozoans in other species including ruminants and humans that are beneficial to intestinal health

Since we can detect Giardia in both asymptomatic and symptomatic dogs, CAPC recommends that only **symptomatic** dogs be tested for Giardiasis using point of care testing including the Giardia SNAP Test. Additionally, for those dogs that are symptomatic and have completed the appropriate therapy, CAPC recommends the following, “Follow-up testing may be done 24-48 hours after the completion of therapy if clinical signs have not resolved. It is recommended to perform a fecal flotation with centrifugation primarily for the detection of cysts in solid or semisolid stools. **‘ELISA tests may remain positive even after treatment for variable periods of time and should not be used as a guide to determine reinfection or failure of treatment.’**” Thus a positive SNAP test should always be followed up with a fecal flotation to identify active disease in cases that may be refractive to treatment.

While we will not be discussing treatment specifically in this course, the following is a decision tree that may aid in answering the conundrum of “to treat or not to treat” in asymptomatic animals that Giardia is detected on fecal flotation.



**Figure 1.** Decision tree for treatment of canine giardiasis. When treating *Giardia* infections, especially asymptomatic cases, in dogs, a decision tree of this type may be useful. By using a stepwise approach such as that described by this algorithm, a clinical veterinarian may be able to reach an evidence-based decision on the most appropriate treatment option for a particular dog in specific circumstances (Box 1). As we acquire more and better data on canine giardiasis the decision tree may be modified or expanded to provide a more useful tool.

Decision tree on whether to treat asymptomatic animals for whom *Giardia* is detected on fecal flotation

## Knowledge check



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## Giardia SNAP test procedure & interpretation

In the lab, you will be using the *Giardia* SNAP test to answer questions on your case. The following are guided instructions on how to perform the *Giardia* SNAP test followed by interpretation guidelines. You should notice that the interpretation guidelines are always in light of the fecal flotation findings.

1. Bring test to room temperature if it has been refrigerated
2. Obtain a sampling swab and a SNAP device for each sample to be tested. Pull and twist the tube covering the swab tip to remove the tube from the swab/reagent bulb assembly. Using the swab, coat the swab tip with **fecal material**. Then, return the swab to the tube. NOTE: Only a thin coat of fecal material on the swab is required; do not coat the swab with excess feces.
3. Break the purple valve stem inside the bulb assembly by bending the assembly at the narrow neck, re-bending the opposite way may be helpful. Squeeze the reagent bulb three times to pass the blue solution through the swab tip and mix it with the sample.
4. Place the SNAP device on a horizontal surface. Using the swab as a pipette, dispense **5 drops of the fluid** into the sample well, being careful not to splash contents outside of the sample well. The sample will flow across the result window, reaching the activation circle in **30–60 seconds**. Some samples may remain in the sample well.

**Fun fact:** The word **FECES** has 5 letters in it and the Parvo test required 5 drops of the brown reagent/fecal solution

5. When color **FIRST** appears in the activation circle, push the activator firmly until it is flush with the device body
6. Some samples may not flow to the activation circle within 60 seconds, and, therefore, the circle may not turn color. In this case, press the activator after the sample has flowed across the result window.
7. Read the test result at **8 minutes**.



## Interpretation of Giardiasis testing

The following information below was taken directly from the [Cornell University Animal Health Diagnostic Center Website](#).

### ***Giardia* ELISA Positive/Flotation Positive**

The animal is infected with *Giardia*.

### ***Giardia* ELISA Positive/Flotation Negative**

The animal may be infected with *Giardia* but is shedding cysts below the limits of detection by flotation. Alternatively, the ELISA is a false positive that may be seen most frequently when the results are in the low positive range. To resolve the issue, collect a second sample for analysis.

### ***Giardia* ELISA Negative/Flotation Positive**

The animal may be infected with *Giardia* but is producing antigen below the limits of detection by ELISA. Alternatively, the ELISA is a false negative. To resolve the issue, collect a second sample for analysis.

### ***Giardia* ELISA Negative/Flotation Negative**

To answer the questions in your laboratory activity. Please refer to the [IDEXX website](#).

## Knowledge check



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online here:

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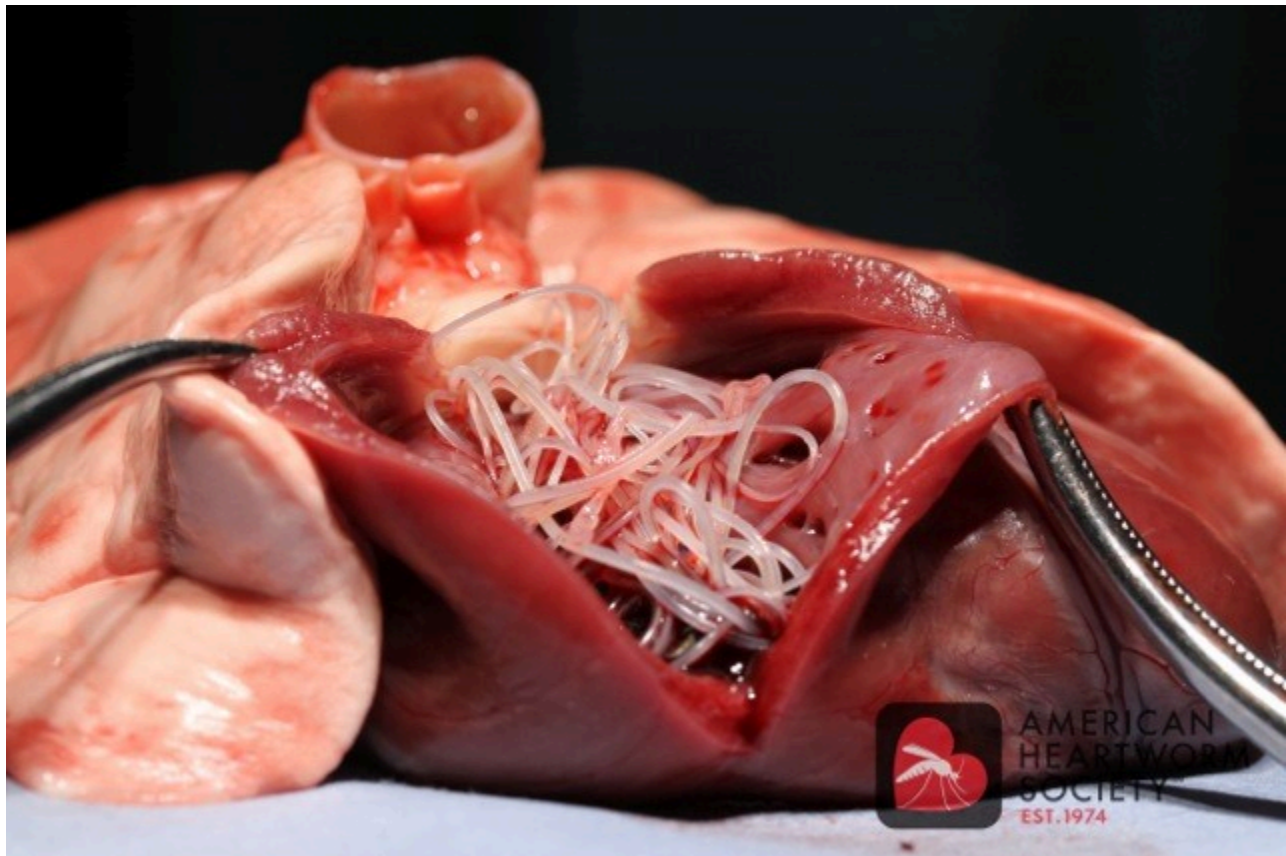
# MODULE 11.5: HEARTWORM TESTING

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## Heartworm testing

Heartworm disease (HWD) is a mosquito-borne filarial disease that infects dogs and cats worldwide caused by the nematode *Dirofilaria immitis*. (See Agents of Disease 2 notes for more specifics on the disease and disease presentation) As a result of climate change and the increased movement of both dogs and cats from regions of the United States (and the world) that have a high prevalence of HWD to regions of the country that have traditionally had a low prevalence, HWD has become more widespread. In fact, CAPC has reported an overall 20% increase in HWD prevalence between 2013-2017 for the United States overall, but especially in parts of the country that were considered to have low prevalence.

As HWD becomes more prevalent throughout the United States and worldwide, the need for routine HWD (and other vector-borne diseases) testing will become more common in clinical practice.



Heartworm Disease (HWD)

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## Knowledge check



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<https://pressbooks.umn.edu/cvdl/?p=411#h5p-80>

## Heartworm ELISA (LFA) testing

In today's laboratory, you will be using the IDEXX SNAP 4Dx Plus results to answer the questions in your case study. However, there are several other tests that are available on the market for heartworm screening.

The following chart summarizes the 3 most common LFA tests available on the market for in-clinic testing.

**Table 11.1: The 3 Most Common LFA Tests for In-Clinic Testing**

HW test	Heska SOLO®	IDEXX SNAP® 4DX Plus	Zoetis Witness®
<b>What sample (s) can you use to run this test (blood, serum, saliva, etc.)</b>	Plasma, serum, anticoagulated whole blood	Plasma, serum, anticoagulated whole blood	Plasma, serum, anticoagulated whole blood
<b>What does this test specifically detect?</b>	Antigen secreted by the adult worm	Antigen secreted by the adult worm	Antigen secreted by the adult worm
<b>Does this test detect any other diseases?</b>	No	<i>Anaplasma phagocytophilum</i> (Ab), <i>Anaplasma platys</i> (Ab), <i>Ehrlichia canis</i> (Ab), <i>Ehrlichia ewingii</i> (Antibody), <i>Borrelia burgdorferi</i> (Ab), <i>Dirofilaria immitis</i>	No

## Knowledge check



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## 4Dx Plus Procedure

1. If stored in a refrigerator, allow all components to equilibrate at room temperature (18–25°C) for 30 minutes before use. Do not heat.
2. Using the pipette provided, dispense **3 drops** of the patient sample into a new sample tube.
3. Holding the bottle vertical, add **4 drops of the conjugate** to the sample tube.
4. Cap the sample tube and mix it thoroughly by inverting it 3–5 times.
5. Place the device on a horizontal surface. Add the entire contents of the sample tube to the sample well, being careful not to splash the contents outside of the sample well. The sample will flow across the result window, reaching the activation circle in **30–60 seconds**. Some samples may remain in the sample well.
6. When color **FIRST** appears in the activation circle, push the activator firmly until it is flush with the device's body.

**Note:** Some samples may not flow to the activation circle within 60 seconds, and therefore, the circle may not turn color. In this case, press the activator after the sample has flowed across the result window.

7. Read the test result at **8 minutes**.

**Note:** The positive control may develop sooner, but results are not complete until 8 minutes.

**Fun fact:** The SNAP test conjugate is BLUE, the word blue has 4 letters and you also use 4 drops of the conjugate to run this test. Blood is RED and the word red has 3 letters in it therefore you only need 3 drops of blood product (serum, plasma, or whole blood) to run the

test.

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## Knowledge check



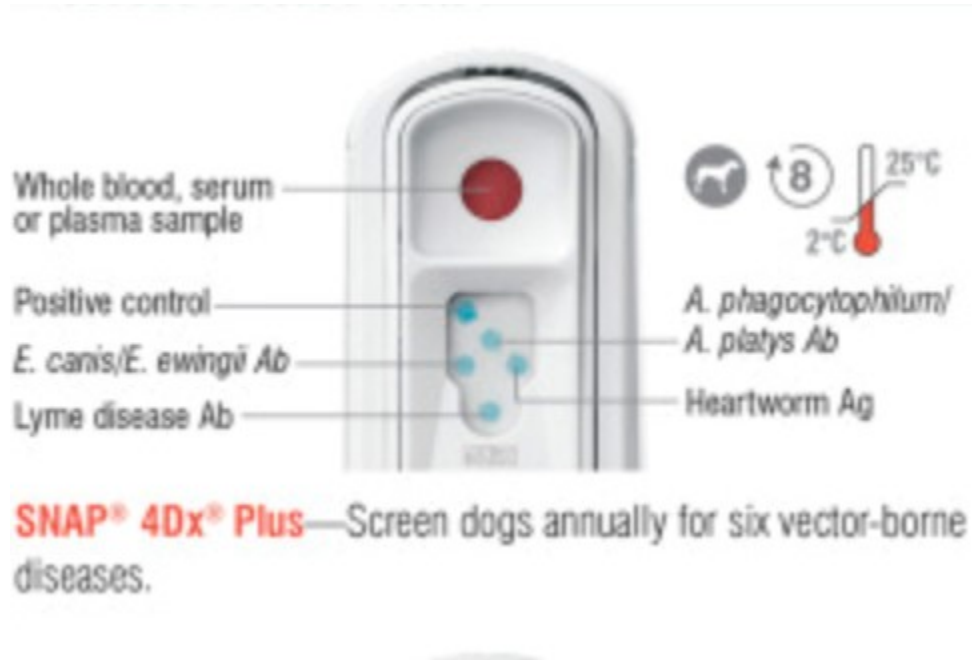
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## Interpretation of 4Dx Plus

The SNAP 4Dx Plus detects 4 pathogens including *D. immitis*, *Borrelia burgdorferi*, *Anaplasma* sp., and *Ehrlichia* sp



#### SNAP 4Dx Plus Interpretation

For dogs that test positive for HWD using an LFA, prior to administering adulticidal therapy a secondary confirmatory test needs to be performed to rule out a false positive.

A few secondary tests available includes:

- Modified Knott's test
  - This test looks for living larvae in blood
  - Concentrates and allow speciation of microfilariae in regions of the country where other microfilariae species aside from *D. immitis* are present. (i.e. *Acanthocheilonema reconditum*)
- ELISA testing performed in a reference laboratory

#### Other diagnostic aids

- Thoracic radiography
- Echocardiogram to detect adult worms

To complete the question in your laboratory case, please refer to the [American Heartworm Society's guidelines](#) and [website](#).

## Knowledge check



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# MODULE 11.6: SNAP® FOAL IGG TEST

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## Equine failure of passive transfer testing

In your large animal medicine courses, you will learn about the failure of passive (FPT) transfer in more clinical detail than what will be covered in this course. Depending on the placental structure, gravid animals may or may not have the ability to transfer antibodies to their offspring in utero. For animals such as horses and cows, antibodies are not transferred to their offspring in utero during pregnancy making the ingestion of colostrum, which is rich in maternal immunoglobulins, immediately after birth essential to survival. This transfer of **humoral immunity** from the dam to the offspring is called **passive transfer**.

**Failure of passive transfer** (FPT; agammaglobulinemia or hypogammaglobulinemia) occurs when the neonatal foal fails to ingest and/or absorb an adequate amount of immunoglobulin from colostrum

There are both foal and dam factors that may result in FPT. A few of those include:

### Dam factors (failure to produce or deliver adequate colostrum to foal)

- Inadequate amount of immunoglobulins transferred into colostrum
- Loss of colostrum prior to parturition (*g.* premature lactation)
- Behavioral factors (unwillingness to accept foal or allow it to nurse)
- Dam death (orphan foal)

### Foal factors (failure to suckle or absorb adequate colostrum)

- Congenital abnormalities that prevent the foal from standing or nursing
- Illnesses or injuries that prevent the foal from standing or nursing
- Inability to absorb immunoglobulins from the gut

To answer the questions in your laboratory activity. Please refer to the [IDEXX website](#).

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# Knowledge check



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<https://pressbooks.umn.edu/cvdl/?p=416#h5p-84>

## SNAP® Foal IgG Test

There are several in-clinic diagnostic tests that can be used to screen for FPT in foals (and other species). These tests use serum or whole blood is taken from the foal to detect IgG levels. Unlike the other SNAP tests that you will run in this laboratory, the SNAP Foal IgG test is semi-quantitative, as the intensity of color change compared to the calibration spot is used to semi-quantify IgG levels in the foal's blood.

## Procedure

1. Remove the cap and dropper tip from the sample diluent bottle.
2. Gently mix whole-blood samples by inverting in the EDTA tube
3. Using the plastic sample loops provided, carefully immerse **ONLY** the loop tip into the blood or serum sample. Visually confirm that the loop is filled.

\*For whole-blood samples, we recommend immersing the loop tip in the sample that remains in the cap of the sample collection container. **Immerse the loop tip only. Do not immerse the loop handle in the sample.**

\*For serum and plasma, use one loop. **For whole blood, use two separate loops.**

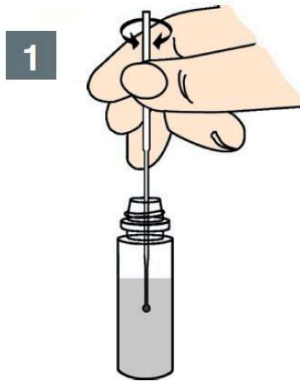
4. Transfer the filled loop by immersing and twirling the loop tip in the bottle of sample diluent.
5. Firmly seat the dropper tip on the sample diluent bottle. Mix thoroughly by inverting five times. Hold the sample diluent bottle vertically and **dispose of the first 5–10 drops** from the bottle.
6. Place the SNAP device on a flat surface. With the bottle tip ½ to 1 inch directly above the SNAP device, carefully apply one drop of the **diluted sample directly onto the sample spot in the result window**. Visually confirm that the drop of the diluted sample has wetted the sample spot completely. **If the drop of the diluted sample has not wetted the sample spot completely, repeat the sample application**

**using a new SNAP device.**

7. Remove the cap from the conjugate bottle and pour its contents into the sample well of the SNAP device. (Some of the contents will remain in the conjugate bottle.) The sample will flow across the result window, reaching the activation circle in 30 to 90 seconds. (Some conjugate will remain in the sample well at activation.) Watch the device carefully for color in the activation circle. When color **FIRST** appears in the activation circle, push the activator firmly until it is flush with the device's body. Keep the device horizontal to ensure accurate results.
8. Wait **7 minutes**. Visually read the test result.

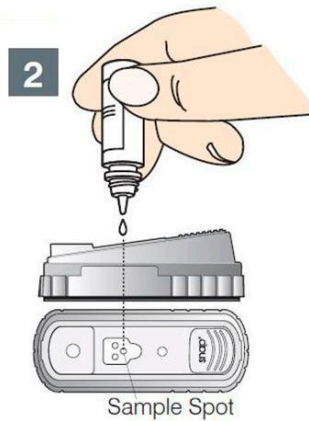
Results in less than 10 minutes

Step 1



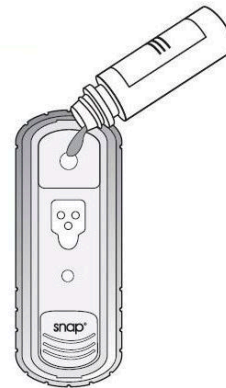
Dilute sample. Mix thoroughly and dispose of first 5–10 drops from the bottle.

Step 2



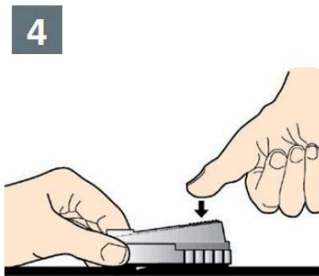
Apply one drop of sample over sample spot.

Step 3



Pour conjugate into the sample well.

Step 4



Snap down activator until it is flush with the base.

Step 5



Wait seven minutes.

## SNAP Foal IgG Procedure

## Knowledge check

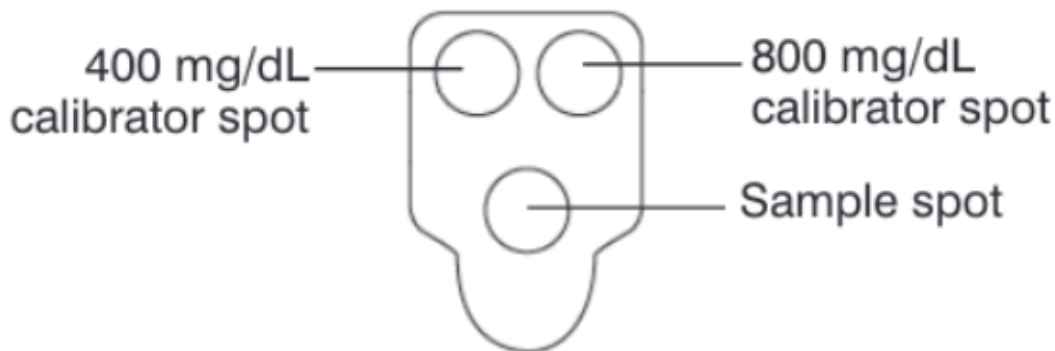


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<https://pressbooks.umn.edu/cvdl/?p=416#h5p-85>

## Interpretation of Foal IgG

The window of your SNAP test should have three **BLUE** dots following testing. These dots include the 400 mg/dL spot, 800 mg/dL spot, and our patient sample spot.



SNAP test window with calibrator spots and sample spot

Depending on the intensity of your patient spot in comparison to the calibration spots is used to determine the blood levels of IgG in the foal. The sensitivity of this test is high but the specificity for FPT is lower meaning this test is only used on animals with a **high index of clinical suspicion for FPT**.

For example, if the patient sample spot is lighter blue than the 400 mg/dL and 800 mg/dL spots, then the foal is estimated to have <400 mg/dL of IgG in his/her bloodstream. This would be interpreted as the following: Since the sensitivity of this test is high, but the specificity is low, in a patient with a high clinical index of suspicious for FPT, following additional testing (CBC/ Chem), this patient is considered to likely be suffering from FPT.

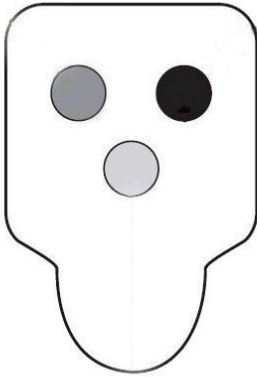
On the reverse end of the testing, if the patient color spot is stronger blue than the 800 mg/dL spot, then the clinical signs observed in your patient are unlikely to be the result of FPT.

## Quick and dirty interpretation

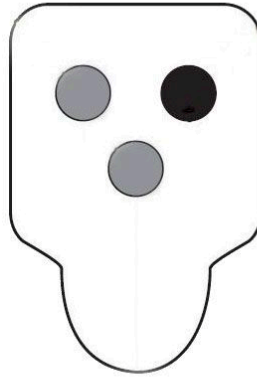
**IgG > 800 mg/dL** → adequate passive transfer (good/normal immune protection)

**IgG between 400 – 800 mg/dL** → partial failure of passive transfer (partial protection)

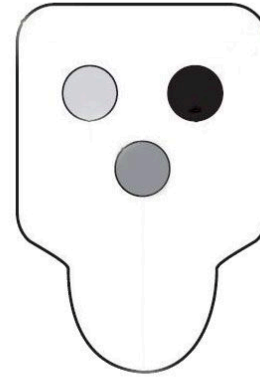
**IgG < 400 mg/dL** → complete failure of passive transfer (very susceptible to infection)

**Less than 400 mg/dL IgG**

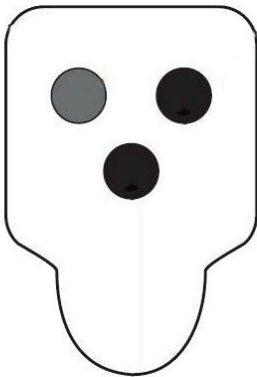
The color intensity of the sample spot is lighter than the 400 mg/dL calibrator spot

**Approximately 400 mg/dL IgG**

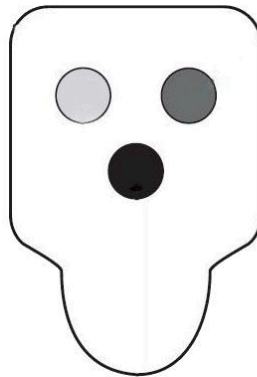
The color intensity of the sample spot is the same as the 400 mg/dL calibrator spot

**400 to 800 mg/dL IgG**

The color intensity of the sample spot is darker than the 400 mg/dL calibrator spot but lighter than the 800 mg/dL calibrator spot

**Approximately 800 mg/dL IgG**

The color intensity of the sample spot is the same as the 800 mg/dL calibrator spot

**Greater than 800 mg/dL IgG**

The color intensity of the sample spot is darker than the 800 mg/dL calibrator spot

## Interpreting the Foal IgG test

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## Knowledge check



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online here:

<https://pressbooks.umn.edu/cvdl/?p=416#h5p-86>

### Key Takeaways

- Rapid point of care tests commonly used ELISA techniques
- These tests can be used to detect a wide variety of things including antibodies, antigens, proteins, chemicals, etc.
- Most rapid point of care tests are used as screening tools

You have now reached the end of Module 11. If you are enrolled in CVM 6925, please go to the Canvas page and take the quiz: "Module 11: Point of care testing quiz." There is an assignment that accompanies the in-person laboratory for this module.

## MODULE XII

# MODULE 12: INTRO TO MASTITIS TESTING

### Module Objectives

1. Describe which tests are used on the individual cow and herd levels
2. Indicate when it is appropriate to use a California Mastitis Test
3. Interpret bulk tank culture results
4. Interpret culture plate results.



# MODULE 12.1: INTRODUCTION TO MASTITIS

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## Introduction to mastitis

Mastitis is inflammation of the mammary gland. While mastitis can occur in any species, it is of particular economic importance to dairy herds. In dairy cows, 30-50% of cows will experience clinical mastitis at least once per lactation. While mastitis takes an obvious toll on individual animal health, the economic losses from decreased milk quality to decreased production to general farm economics are high. It is estimated that a single case of mastitis costs approximately \$100-400. Additionally, mastitis treatment and prevention and control account for the majority of antibiotic use on dairies. With the push for decreasing antibiotic use in food animals, early detection and conservative antibiotic usage are becoming more important to dairy herd management.

# MODULE 12.2: DETECTING SUBCLINICAL MASTITIS

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## Detecting subclinical mastitis

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### California Mastitis Test (CMT)

The California Mastitis Test (CMT) is a patient-side, rapid test for detecting **subclinical** mastitis in individual animals. **We do not use the CMT on animals with clinical mastitis.** Other tests such as the Portachek Somatic Cell Counts (SCC) and IDEXX SCC are also available, but the CMT is still the most common test used by the producer because it is easy, fast, and inexpensive. The CMT uses a specific reagent that lyses the white blood cells (WBC) present in the milk. When WBC counts reach above 200,000 cells per milliliter the lysing of the WBCs and the reagent begin to form a gel.

### Equipment

California Mastitis Paddle

CMT Reagent

2cc of milk from each quarter

### Procedure

1. Take about 1 teaspoon (2 cc) milk from each quarter. This is the amount of milk that would be left in the cups if the CMT Paddle were held nearly vertical – the milk should come up to the outside line on the bottom of the cup when held nearly vertical.
2. Add an equal amount of CMT solution to each cup in the paddle. (roughly 2cc reagent)
3. Rotate the CMT Paddle in a circular motion to thoroughly mix the contents. Do not mix more than 10 seconds.
4. Read the test quickly. The visible reaction disintegrates after about 20 seconds. The reaction is scored visually. The more gel formation, the higher the score.

5. Rinse the CMT paddle after each test.

## CMT Procedure Video



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## Interpretation

The table below describes the CMT scoring system. Each quarter is scored using the following scale. These scores correlate to an average SCC range for each quarter.

**Table 12.1: CMT Scoring System**

CMT Score	Description	SCC Range
N (negative)	No thickening of the mixture	0 – 200,000
T (trace)	Slight thickening of the mixture. Trace reaction seems to disappear with the continued rotation of the paddle.	200,000 – 400,000
1	Distinct thickening of the mixture, but no tendency to form a gel. If the CMT paddle is rotated for more than 20 seconds, thickening may disappear.	400,000 – 1,200,000
2	Immediate thickening of the mixture, with a slight gel formation. As the mixture is swirled, it moves toward the center of the cup, exposing the bottom of the outer edge. When the motion stops, the mixture levels out and covers the bottom of the cup.	1,200,000 – 5,000,000
3	Gel is formed and the surface of the mixture becomes elevated (like a fried egg). The central peak remains projected even after the CMT paddle rotation is stopped.	Over 5,000,000

## Knowledge check



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# MODULE 12.3: INDIVIDUAL COW MASTITIS TESTING

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## Determining the type of pathogen that is causing subclinical mastitis in a single cow

Using the results that you have gained from the CMT to determine which cow has subclinical mastitis, in the laboratory you will culture that cow to determine which mastitis bacterial pathogen is causing her mastitis. As you learned in the lecture, there are two major categories of mastitis pathogens, **environmental** and **contagious**. It is important to determine the specific pathogen as each pathogen has different treatment, control, prevention, and herd management strategies.

	Gram-Positive	Gram-Negative	Other
Contagious	Staph aureus Strep agalactiae		Mycoplasma sp. Prototheca?
Environmental	Strep uberis, Strep dysgalactiae  Entero/Aero/Lactococcus sp.  Coagulase-negative Staphs (>50 spp)  Bacillus, T. Pyogenes, Clostridium, etc.	Coliforms: E. coli, Klebsiella, Enterobacter  Non-coliforms: Serratia, Pseudomonas, Proteus, etc.	Yeast Prototheca?

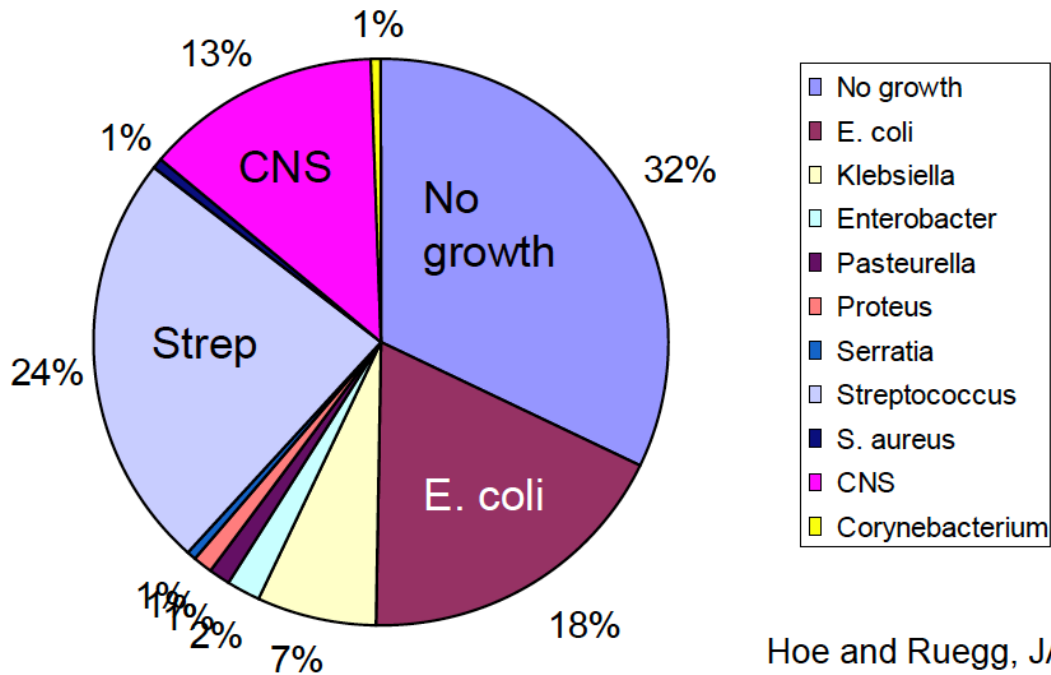
Common Contagious and Environmental Mastitis Pathogens

## Distribution of Mastitis Etiology

What pathogens are most commonly implicated in mastitis?

- 15-70% Gram-positive
- 8-44% Gram-negative
- 20-40% No growth

From these distributions, the 3 most common cultured pathogens are *Streptococcus* (24%), *E. coli* (18%), and Coagulase-negative *Staphylococcus* (13%).



Hoe and Ruegg, JAVMA, 2005

Distribution of Mastitis Pathogens

## Negative culture results

If a cow has subclinical or clinical mastitis, then why are 25-40% of the milk samples negative on routine culture (i.e. will not grow bacteria)?

- False negatives (rare):
  - Possibly not sensitive enough to detect at low shedding pathogens
  - Bacteria present do not grow or do not grow quickly enough, in culture media or the incubation environment (*Mycoplasma*, etc.)
- True negatives (most):
  - By the time the sample was taken the cow had already cleared the bacteria (possible/likely for Gram-negatives)
  - Not a real infection

## Knowledge check



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## Milk Culture

Milk culture is considered the gold standard test for diagnosis of mastitis and identification of the pathogen causing infection. Milk culture may be performed at several levels, and each level has value and limitations which must be acknowledged:

- **Low-level**

Typically rely on a combination of selective culture media to categorize culture results, such as Growth/No Growth, Gram-positive/Gram-negative; Rapid, inexpensive, and accuracy limited to broad categories; Performed on-farm, or in vet clinics.

- **Mid-level**

Utilize some additional microbiological techniques to identify some pathogens at the genus or species level, for example, coagulase test for *Staph aureus* or microscopic wet mount for *Prototheca*; Performed in vet clinics or other service laboratories.

- **High-level/Diagnostic lab**

Technicians with advanced training and advanced diagnostic equipment allows accurate pathogen identification to the species level in most cases. Most expensive, may have delayed turnaround compared to low- and mid-level labs, but will be most accurate with a wider range of testing options.

## Minnesota Easy® Culture system

In this laboratory, we will utilize the Minnesota Easy® Culture system – a rapid culture system designed to be used on-farm or in a veterinary clinic lab setting. The Minnesota Easy® Culture system has two plate options – a bi-plate and tri-plate – which utilize the following selective culture media:

- **Factor®** media selects for Gram-positive bacteria such as Staphylococci, Streptococci, Bacillus, and Corynebacteria.
- **MacConkey** media selects for Gram-negative organisms. The most common Gram-negative mastitis organisms belong to a group of organisms known as Coliforms. Examples are E. coli and Klebsiella.
- **Focus®** (formerly MTKT) media select for Streptococcus and Streptococcus-like bacteria, such as Enterococcus and Aerococcus species (Tri-plate only).

## Individual milk culture procedure

### Supplies

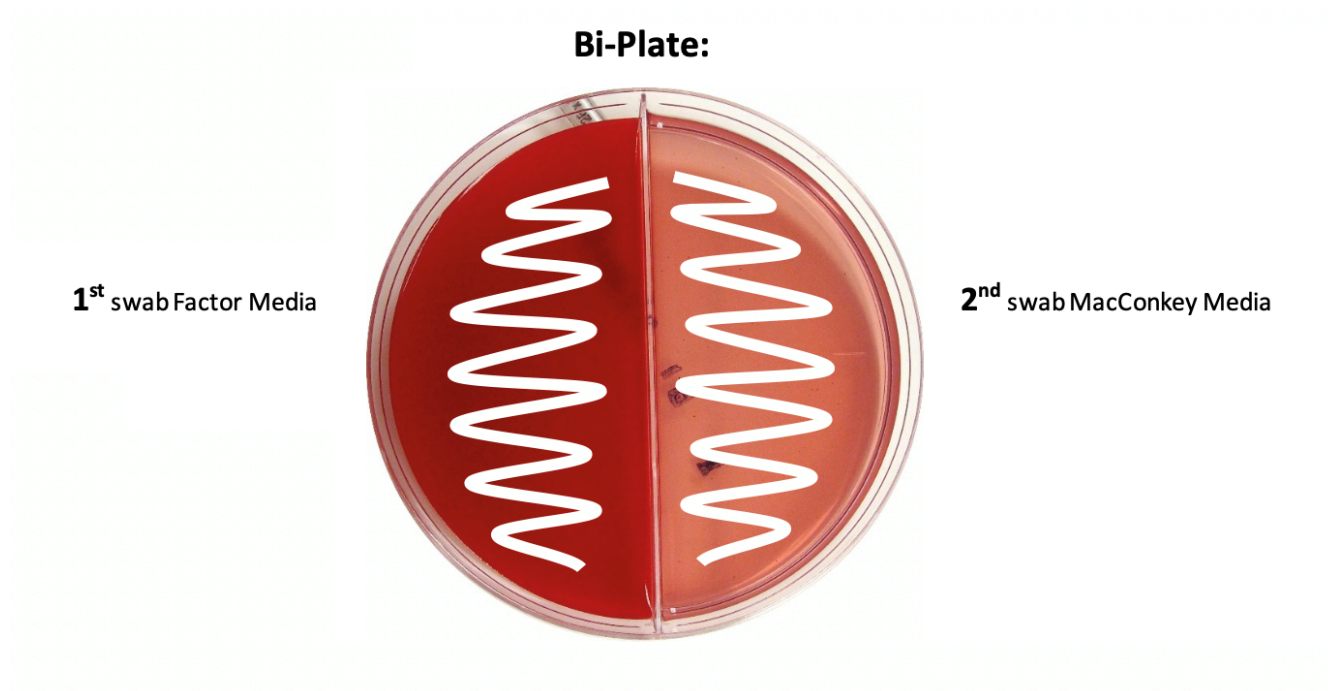
- Milk sample from cow with suspected mastitis
- Triplate
- Cotton Swab
- Sharpie
- Disposable Gloves

### Procedure

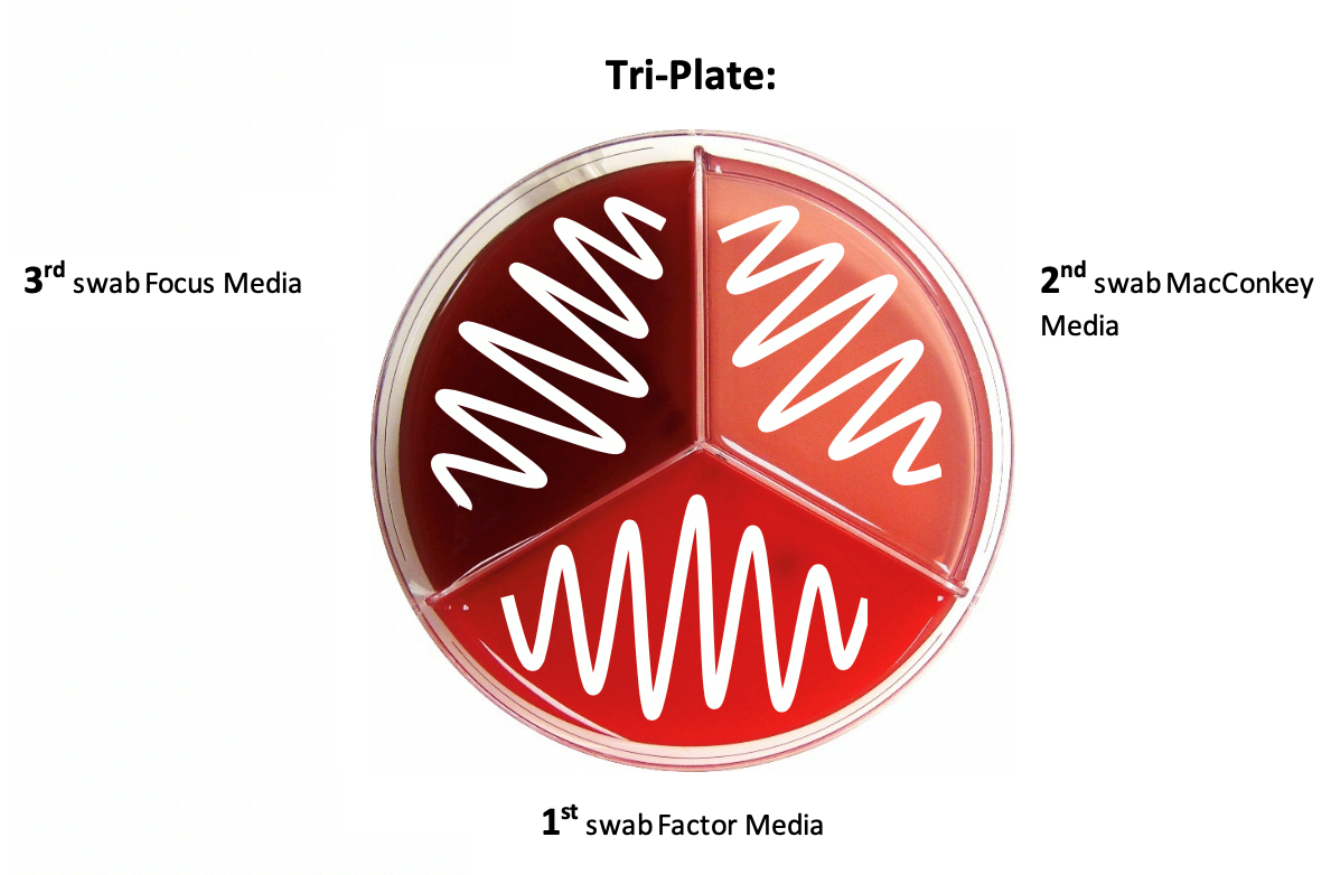
1. Wear clean disposable gloves.
2. Mix the sample vial well by gently inverting the sample approximately 15 times.
3. Turn the plate upside down and label the bottom of the plate with the group ID, sample ID, and date. (normally this would be the cow and quarter ID)
4. Use a new sterile cotton swab.
5. Avoid touching the plate or cotton end of the sterile swab with your fingers or to any surface as this will result in contamination. (Open cotton swab packages such that the cotton end remains covered and only the stick is exposed.)
6. Place a sterile cotton swab end in the milk sample, rolling the non-cotton end of the swab between index finger and thumb, for approximately 5 seconds, or until the swab becomes completely saturated with milk. It will help to bump the swab on the bottom of the tube to break up the

cotton fibers.

7. Try to avoid plating clumped milk. You can roll the swab on the side of the sample vial to knock milk clumps off the cotton tip. If milk clumps happen to get onto the plate use a marker on the bottom of the plate to note where they are so as not to confuse them with bacterial growth.
8. Swab the sample onto each of the Minnesota Easy™ Culture System media. To do this, the media plate should be lid down on the counter, pick up the media side of the plate in the palm of your non-dominant hand. Take your cotton swab that you saturated with the milk sample and streak the milk over the entire surface of the plate section. Take care not to gouge the surface of the media with your swab. Swab each section in the pattern and order indicated below.
9. ***Re-dip the swab in milk between each section of the culture plate.***
10. Once the plate has been swabbed, place the lid back on the plate and immediately re-seal the lid on the milk sample.
11. **Place the plate in the incubator upside down** (media facing down or place the plate on its lid) so that any condensation on the lid will not drip onto the plated sample.
12. The plates should remain in a 37° C incubator for up to 48 hours.



Bi-Plate Procedure



Tri-Plate Procedure

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## Knowledge check



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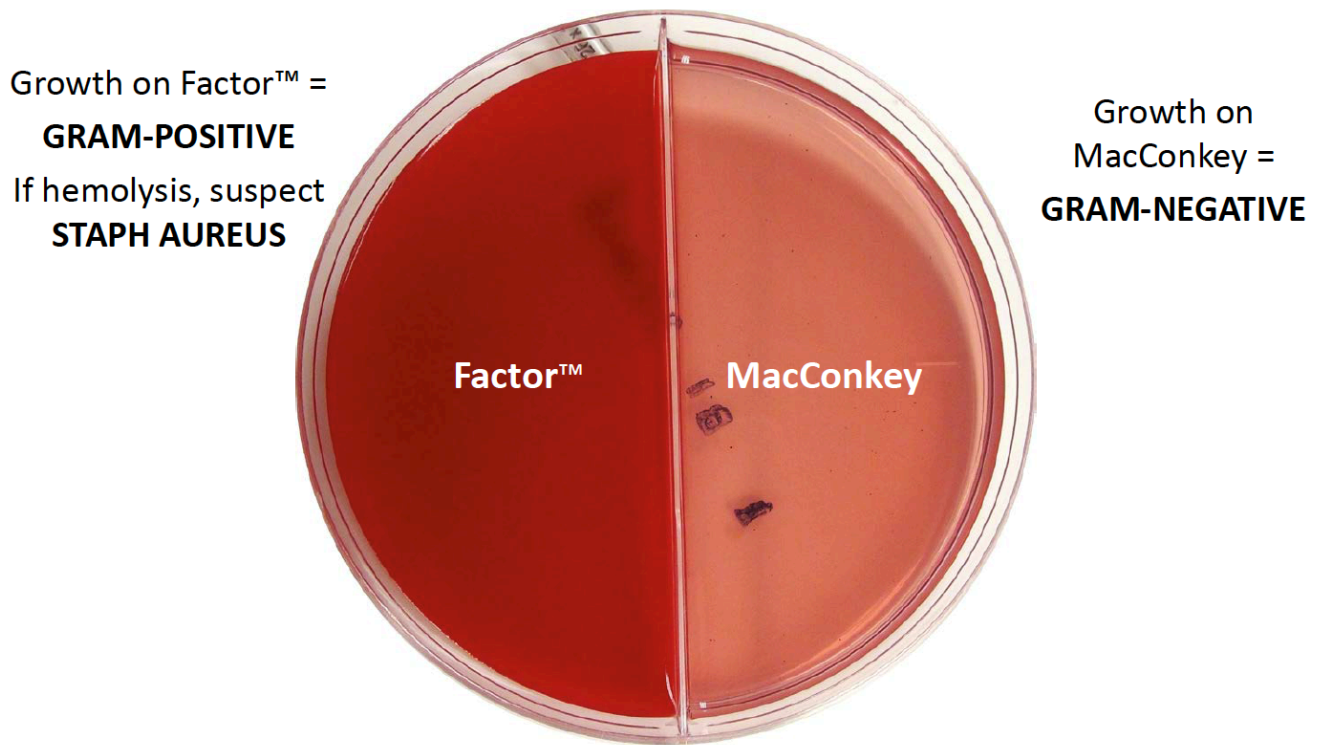
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## Interpretation

The Minnesota Easy® Culture Bi-plate is used to differentiate between Gram-positive and Gram-negative

bacteria. One side (bright red) has a Factor<sup>®</sup> media (a type of Blood Agar Plate) to detect Gram-positive bacteria and on the other side MacConkey media to grow Gram-negative bacteria. Of the types of Gram-positive bacteria that result in mastitis, *S. aureus* is one of the only ones that will result in hemolysis on the Factor<sup>®</sup> media. However, we use the Tri-Plate and additional biochemical tests for confirmation.

## Minnesota Easy<sup>™</sup> Culture Bi-Plate Interpretation

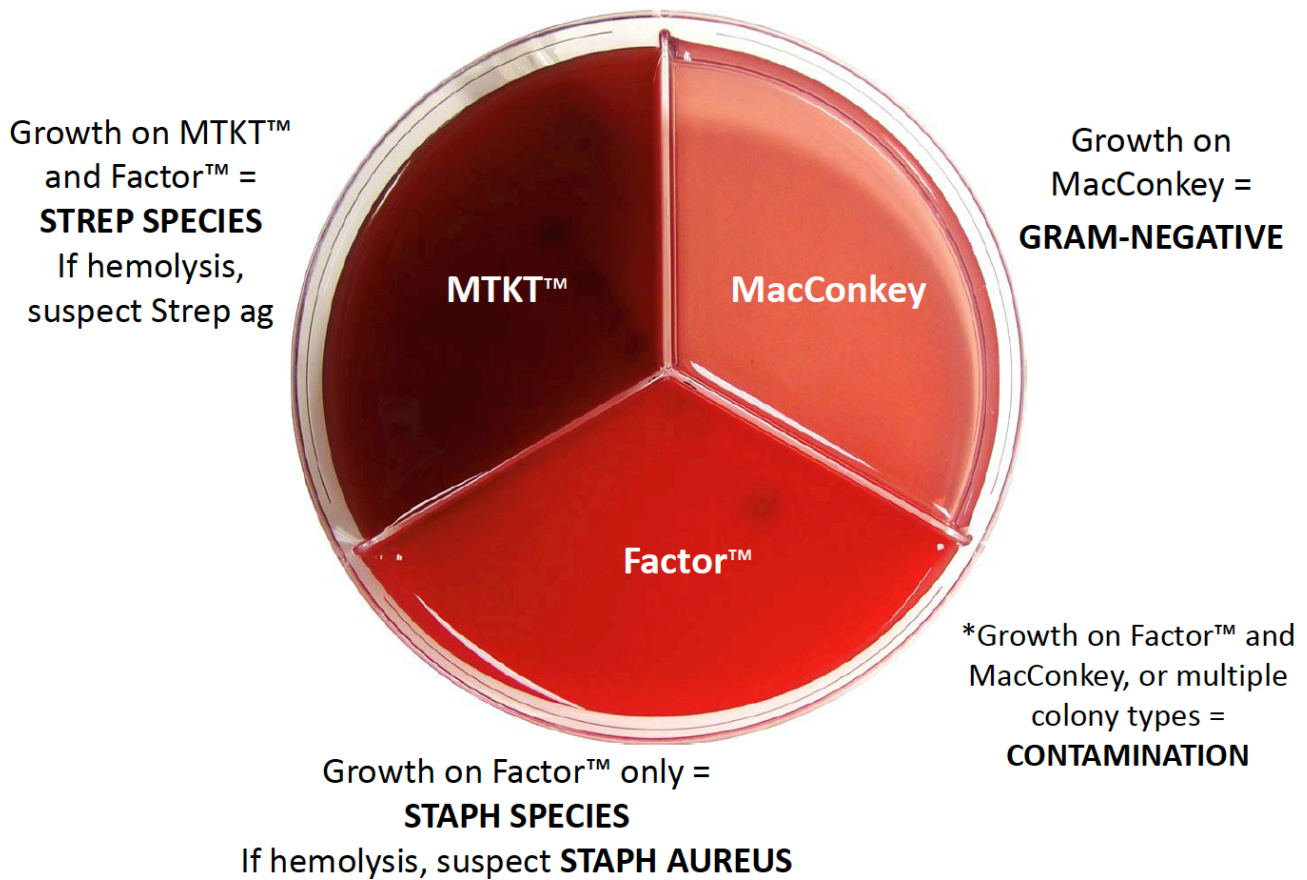


\*Growth on both sides, or multiple colony types = **CONTAMINATION**

### Bi-Plate Interpretation

The Minnesota Easy<sup>®</sup> Culture Tri-plate is used to further differentiate between *Staphylococcus* and *Streptococcus* sp. The plate is divided into 3 wells; Focus<sup>®</sup> (formerly MTKT, maroon/deep red), MacConkey (pink), and Factor<sup>®</sup> (bright red) media.

## Minnesota Easy™ Culture Tri-Plate Interpretation



### Tri-Plate Interpretation

## Limitations of selective culture medium

- *Prototheca* (algae) can grow on all media, but not always
- *Bacillus* (Gram-positive) grows on Factor, and will occasionally grow on MacConkey media
- Yeast grows on Factor™ media
- *Mycoplasma* will not grow on any media in Minnesota Easy® Culture System (requires special growth conditions)

**Clinical Pearl:** Culture plates are able to tell us if the mastitis pathogen is Staphylococcus, Streptococcus, or Gram-negative pathogens. We need to do additional biochemical tests to determine the specific pathogen resulting in mastitis. Let's talk about some of those tests.

## Knowledge check



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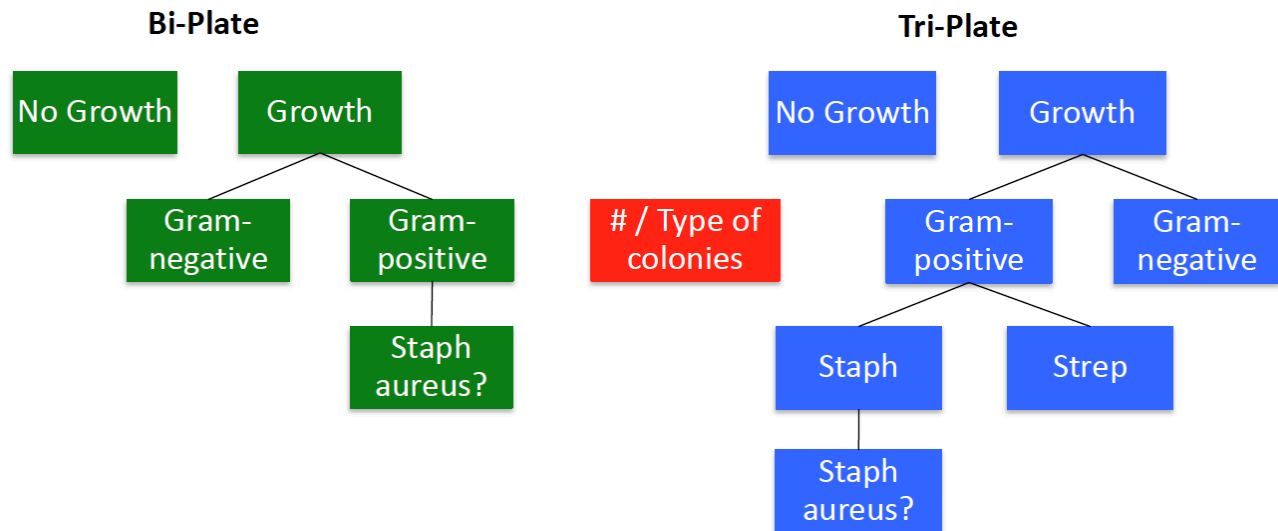
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# MODULE 12.4: SPECIAL BIOCHEMICAL TESTS FOR FURTHER DIFFERENTIATION OF MASTITIS PATHOGENS

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## Further Differentiation of Mastitis Pathogens

Once we have the mastitis pathogen narrowed down to *Strep* sp., *Staph*. sp., or a Gram-negative pathogen using the Bi-plate and Tri-plate culture plates, it is important to further determine their identity if possible.

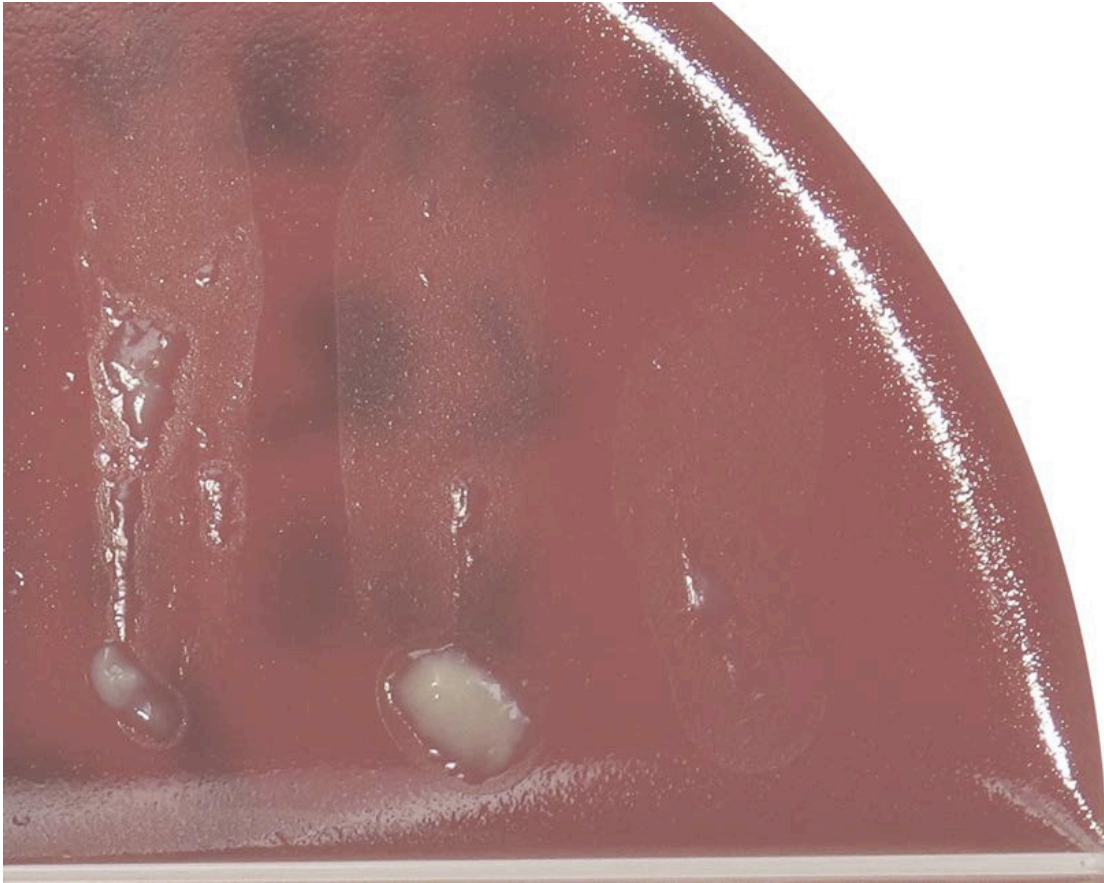


Flow chart for further differentiation of mastitis pathogens

The following sections will guide you through the steps for further characterization of mastitis pathogens.

## Step 1: Growth or no growth

The first step to any bacterial culture is to determine if there is actual growth on your plates. It is especially important to not interpret milk globs or agar clumps from aggressive streaking as growth on your plates.



Example of a milk glob

## What if there is growth?

- Count the number of colonies and type of colonies (Gram +/-)
- If there are 3 or more different types of colonies seen, this is likely a contaminated sample.

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## Knowledge check



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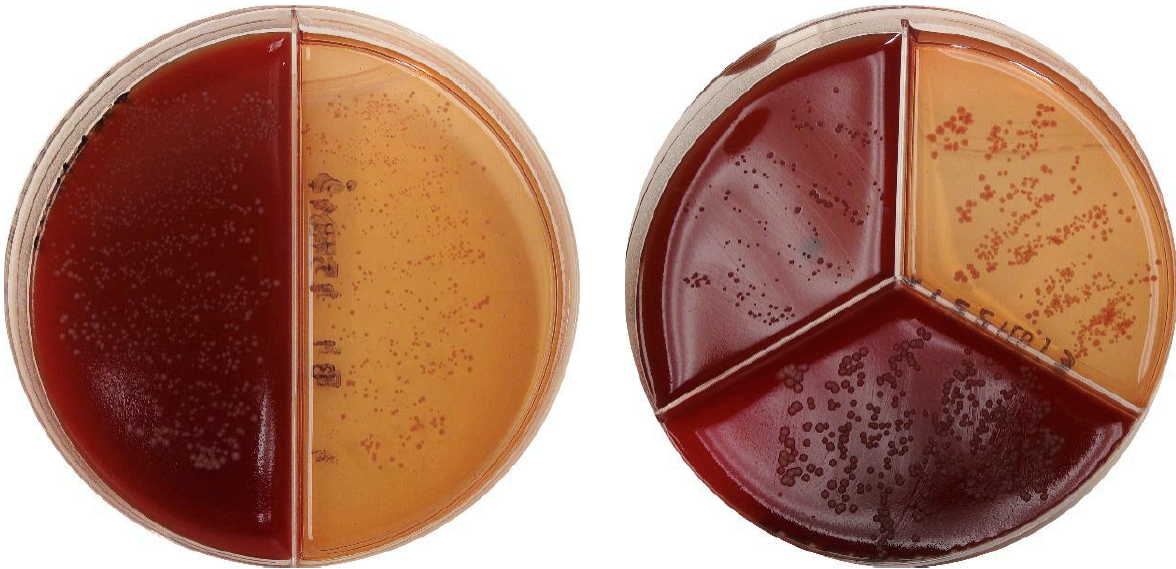
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## Step 2: Identification of pathogens

What if there is growth on all three plates with a single type of colony?

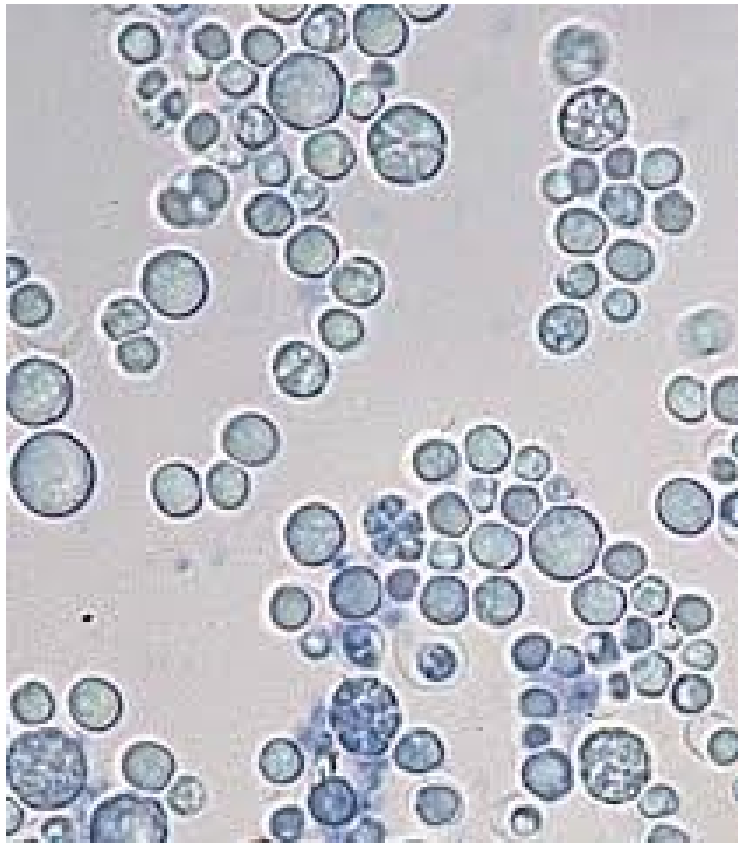
### Prototheca

If growth on **all sections** look very similar and appear as small, dry whitish colonies we suspect *Prototheca*. *Prototheca* is an alga that is considered a contagious pathogen and will grow on many different types of media. *Prototheca* has devastating health implications for the cow so for this reason, we need to do additional tests to positively identify this pathogen.



### Prototheca

*Prototheca* has a very distinct microscopic morphology, thus if suspected we lift a few colonies from the plate and prepare a wet preparation. At 40x objective, these algae are round (about the same size as a WBC) with a Mercedes Benz or floral appearance to them



Prototheca wet mount with several of the “floral” morphology



Prototheca wet mount with several of the Mercedes-Benz morphology

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## Knowledge check



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## What if we only find single colonies on one or more plates?

The first step is using our plates to attempt identification of the pathogen using our differentiating media.

## Hemolysis patterns on the blood agar plate

Different mastitis pathogens have different hemolysis pattern of the blood agar plates<sup>1</sup>. The three types of hemolytic activity include:

### Alpha ( $\alpha$ ):

Partial lysis of RBCs (cell membrane remains) resulting in a **green** or **brown** discoloration around colony from the conversion of hemoglobin to methemoglobin

### Beta ( $\beta$ ):

Lysis and complete digestion of RBCs surrounding the colony leaving a colorless area (I.E. *Staphylococcus aureus*)

### Gamma ( $\gamma$ ):

AKA non-hemolytic is the term referred to as the lack of hemolytic activity.

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1. Focus plates are a type of blood agar plate.



Blood Agar Plate (BAP) with alpha, beta, and gamma hemolysis

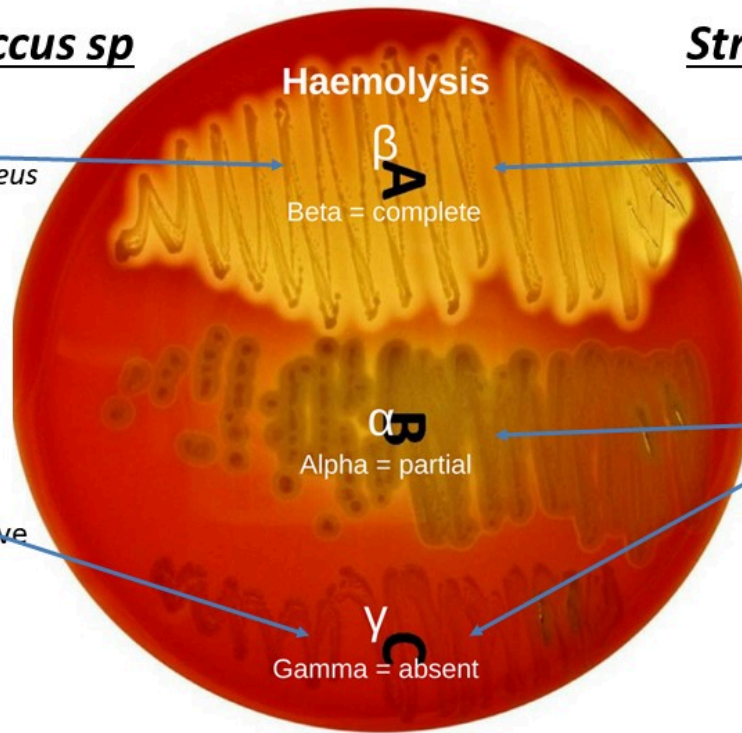
In the urine culture laboratory, we evaluated the different hemolysis patterns seen on our blood agar plates. Below are examples of the common hemolysis patterns that we observe with *Staphylococcus* versus *Streptococcus* spp. Other organisms can cause hemolysis (this is why we do additional biochemical tests).

# Hemolysis on blood agar

## *Staphylococcus sp*

**Beta hemolysis**  
Suspect *Staph aureus*  
-> follow up with  
coagulase test

**No hemolysis  
(gamma)**  
"Coagulase negative  
Staphylococcus"



## *Streptococcus sp*

**Beta hemolysis**  
Suspect *Strep. agalactiae*  
-> send to lab for  
confirmation

**Alpha or gamma  
hemolysis**  
"Environmental  
Streptococci"  
-> follow up with  
esculin test

Common hemolysis patterns that we observe with *Staphylococcus* versus *Streptococcus* spp.

## Minnesota Easy<sup>®</sup> Culture Media

The Factor<sup>®</sup> media (type of Blood Agar Plate) is enriched, differential media (gram-positive only) used to differentiate fastidious organisms and detect hemolytic activity. Factor<sup>®</sup> media is different from Blood agar in that it selectively enhances Beta hemolysis of *Staphylococcus aureus*, a significant mastitis pathogen. So colonies with pronounced Beta hemolysis on Factor<sup>®</sup> would be suspect *Staph aureus*, whereas on blood agar they could be *Staph aureus* or *Strep agalactiae* (or something else).

Focus<sup>®</sup> media is also an enriched, differential media, but selects for Streptococci and Strep-like organisms (*Enterococcus*, *Lactococcus*, *Aerococcus*) and enhances Beta hemolysis of *Streptococcus agalactiae*.

## Hemolysis on Minnesota Easy® Tri-plate



Growth on Factor® =  
Gram-positive (Staph or Strep)  
Hemolysis on Factor® =  
Staph aureus suspect

Growth on Focus® + Factor® =  
Strep or Strep-like organisms  
Hemolysis on Focus® =  
Strep ag suspect



(Note faint hemolysis on Factor® compared to  
enhanced hemolysis on Focus®)

Hemolysis patterns on Minnesota Easy® Tri-Plate

## Knowledge check

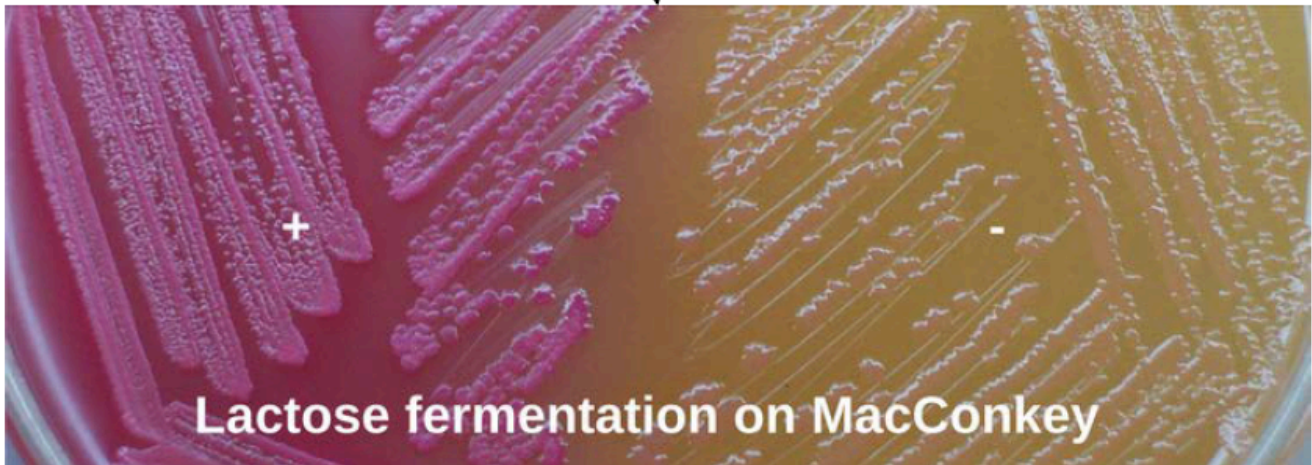


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## Lactose Fermenters

As we discovered in the urine culture laboratories, the MacConkey media in addition to selecting Gram-negative bacteria it also indicator media as the lactose fermenting bacteria will form pink colonies.



Lactose fermentation on MacConkey Plate

- If there are **pink** colonies, then these colonies are **coliforms**. The most common type of coliform is *E. coli*, but we would need to do additional testing to confirm that as other coliforms appear similar on MAC.
- If the colonies are white, tan, or grey, these are non-coliform Gram-negative bacteria

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## Knowledge check



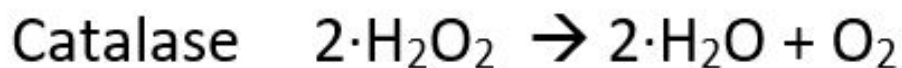
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## Catalase testing

The catalase test is useful in the presumptive identification and differentiation of many bacteria, most notably *Streptococcus spp.* (catalase-negative) and *Staphylococcus spp.* (catalase-positive). Catalase is an enzyme that breaks down hydrogen peroxide into water and oxygen.



When catalase is present in the bacterium, when H<sub>2</sub>O<sub>2</sub> is applied the colony will bubble. Bacteria that do not have catalase will not bubble. Here is a short video demonstrating this reaction.



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While we are using this test to primarily differentiate between *Staphylococcus* and *Streptococcus spp.*, other bacteria and yeast are also catalase positive. Using physical colony characteristics and differential plates is helpful to distinguish these other catalase-positive organisms.

**Table 12.2: Catalase Reaction Results for Various Organisms**

Organism	Catalase Reaction
Bacillus	+
Yeast	+
<i>Prototheca</i>	+
<i>Trueperella pyogenes</i>	–
<i>Staph. sp.</i>	+
<i>Strep. sp.</i>	–

\*Note that the Minnesota Easy® Tri-plate differentiates Staphs and Streps for you.

## Knowledge check

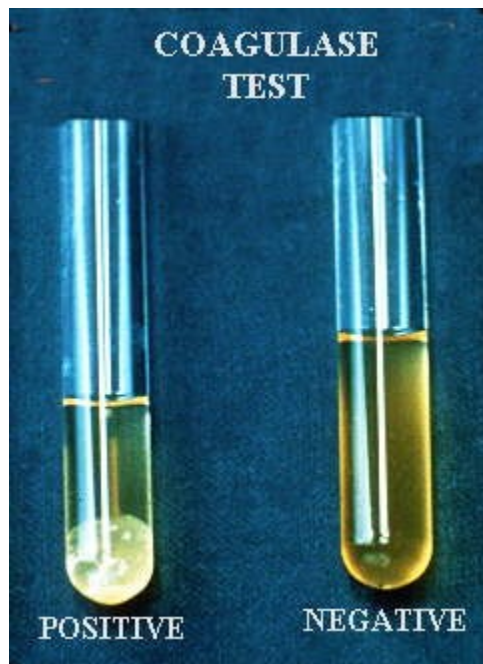


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## Coagulase test

The coagulase tube test aids differentiation of *S. aureus* from other *Staphylococcus* spp. on the basis of their coagulase production. The test detects the presence of “free” coagulase, an extracellular protein enzyme that causes the formation of a clot when inoculated with plasma. ***Staphylococcus aureus* is coagulase-positive.** If a coagulase test yields a negative result, the Staph is placed into an umbrella grouping called “Coagulase Negative Staphylococcus” (CNS). Remember *S. aureus* is a contagious pathogen and CNS are environmental pathogens.



Coagulase Test

**Bringing it together clinically:** If you have determined that you have *Staphylococcus aureus* from your plates the next step is a catalase test. If your **catalase** test is positive, then the next step you need to perform on the colony is a coagulase test to determine if the pathogen is contagious (*S. aureus*) or environmental (CNS) as this will guide your treatment and control recommendations for the cow.

Here is a video showing the “clotting” that occurs in the test.



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## Knowledge check



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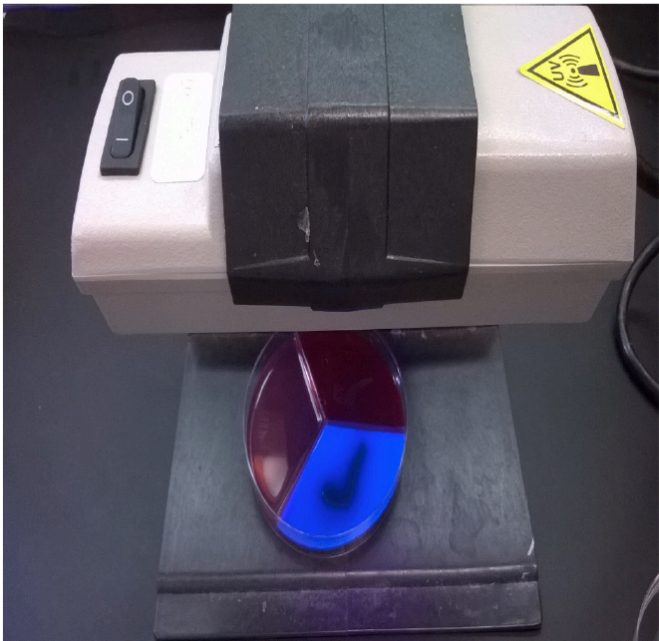
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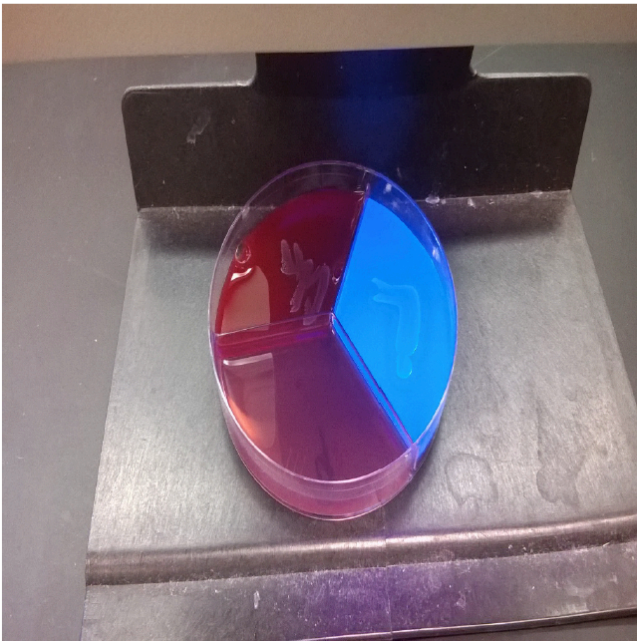
## Esculin reaction

Culture media that contains esculin the (Focus®/MTKT portion of the Tri-plate in our lab) is used to differentiate Enterococci and group D Streptococci based on their ability to hydrolyze esculin. When an organism hydrolyzes the glycoside esculin to form esculetin and dextrose, the esculetin reacts with the ferric citrate in the media to produce a **dark brown** or **black phenolic iron** complex. A **UV light** may be used to more clearly visualize the reaction, although it can be seen without the UV light.

Positive Esculin Reaction



Negative Esculin Reaction



Positive and negative esculin reaction results

Table 12.3: Esculin Reaction Results for Various Organisms

Esculin +	Esculin –	Esculin Variable
Enterococcus sp.	Strep agalactiae	Aerococcus sp.
Strep. uberis	Strep dysgalactiae	
Lactococcus sp.		

This is a useful test because the majority of esculin-positive Strep and Strep-like organisms (not Aerococcus) are typically more pathogenic than the non-ag/non-hemolytic esculin-negative *Streptococcus dysgalactiae*. *Streptococcus uberis*, *Enterococcus* and *Lactococcus* are more difficult to cure and more likely to cause chronic mastitis.

## Knowledge check



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### Key Takeaways

- Most coliforms are lactose fermentors and detected on the MacKonkey agar
- Staphylococcus and Streptococcus can both have complete hemolysis (beta) on the BAP
- If you determine that the colonies are Staphylococcus or Streptococcus, the next step is a catalase test to differentiate
- If you determine Staphylococcus, the next step is a coagulase test to determine if it is an environmental or contagious Staphylococcus
- If you determine Streptococcus, the next step is an esculin test and may aid in prognosis
- Prototheca grows on all types of differentiating and selective media. We use wet mounts for identification

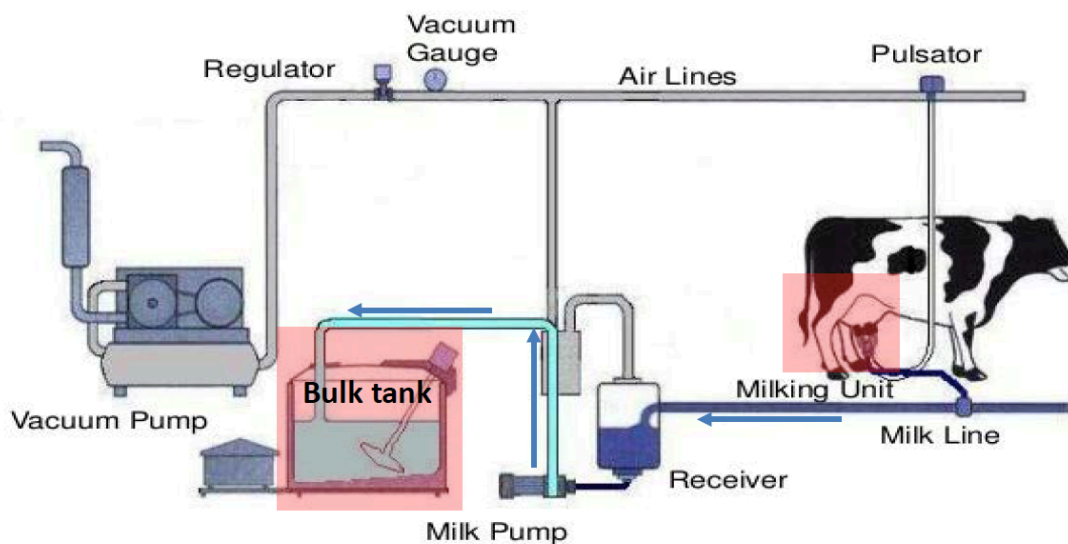
# MODULE 12.5: EVALUATING MASTITIS AT THE HERD LEVEL

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## Bulk Tank Culture

Bulk tank culture is an important part of the mastitis control program to determine if there are any **contagious pathogens** at the **herd** level.

In the milking parlor, the milk from all cows is commingled into a single tank (bulk tank). When screening for contagious pathogens, milk is collected from the bulk tank for 3 to 5 consecutive days, and submitted for culture.



<https://image.slidesharecdn.com/ee29f7bb-f578-4854-b53e-27e0b0dd9ea7-161025094828/95/how-we-milk-our-cows-20-638.jpg?cb=1477388967>

Bulk Tank Culture

**What are the contagious pathogens we are trying to detect?**

- Staph aureus
- Strep ag
- Mycoplasma
- Prototheca

If these contagious pathogens are present in bulk milk, it means there are likely cows infected with those pathogens in the herd.

The bulk tank culture is also used to monitor milking time hygiene by tracking the level of environmental bacteria (Environmental Streps, Coliforms) in the milk. The presence of those organisms in bulk milk is primarily due to contamination during milking, NOT from cows infected with those organisms.

---

**Key Takeaways**

- Mastitis is a common problem in dairy herds worldwide and can lead to tremendous economic losses
- The California mastitis test (CMT) is a screening tool to identify subclinical mastitis, specifically elevated somatic cell counts
- Bulk tank cultures screen for environmental and contagious pathogens at the herd level
- Individual milk cultures are used on an individual cow with clinical mastitis to characterize the specific environmental or contagious pathogen(s) that are causing disease

You have now reached the end of Module 12. If you are enrolled in CVM 6925, please go to the Canvas page and take the quiz: "Module 12: Mastitis quiz." There is an assignment that accompanies the in-person laboratory for this module.

## MODULE XIII

# MODULE 13: INTRO TO DERMATOPHYTES

### Module Objectives

1. Memorize the 3 most common dermatophytes in small animals
2. Memorize which species glow under a Wood's lamp
3. Describe which biological form is seen on cytology versus DTM culture
4. Compare and contrast colony features of dermatophytes versus contaminants
5. Compare and contrast the microscopic differences between macro and microconidia for *M. canis*, *M. gypseum*, and *Trichophyton*
6. Correctly identify the 3 macroconidia from dermatophytes listed above when applied to a clinical scenario



# MODULE 13.1: INTRODUCTION TO DERMATOPHYTES

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## Intro to Dermatophytes

Dermatophytes are a group of cutaneous fungi transmitted to animals by direct contact with **arthrospores** usually through a superficial wound from contact with another animal (zoophilic), in the environment (geophilic), and those adapted from a human host (anthropophilic).

These infections are found worldwide and more commonly observed in younger or immunocompromised animals. On the animal, dermatophytes infect **keratinized structures** (stratum corneum, hair, and nails) resulting in most commonly in hair loss, plaque lesions, and hyperkeratosis (thickening of the skin). In people, these lesions result in a red rash forming a “ring” thus the common name for this disease is “ringworm”.



Moriello, K.A. Using a Wood's Lamp. NAVC clinician's brief. 2009.

Dermatophyte Infection

***In veterinary medicine, there are 3 genera of dermatophytes of medical importance including:***

- *Microsporum spp.*
- *Trichophyton sp.*
- *Epidermophyton sp.*

From those 3 genera, there are three species of veterinary significance in small animals and large animals.

**Table 13.1: Dermatophytes of Veterinary Significance**

Organism	Classification	Major source	Common hosts	Fun facts
<i>Microsporum gypseum</i>	Geophilic	Saprophytic in the soil of warm and humid climates	Dogs and cats	Challenging to control because it is ubiquitous in the environment
<i>Microsporum canis</i>	Zoophilic	Cats	Dogs and cats	The most common cause of dermatophytosis in small animals. Can be found on cats without clinical disease
<i>Trichophyton mentagrophytes</i>	Zoophilic	Rodents	All species	Rodent control is an important aspect of prevention and control

## Knowledge check



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# MODULE 13.2: COMMON TESTS USED TO IDENTIFY DERMATOPHYTOSIS

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## Common tests used to identify Dermatophytes

In this section, we will discuss the common patient-side testing done to diagnose dermatophytosis. These tests are Wood's lamp, trichograms, and fungal culture.

### Wood's lamp

This test is a **screening** test that uses a black light to identify *Microsporum canis*. *M. canis* produces a metabolite that coats the hair shaft and glows a bright green apple florescence. Other dermatophytes do not produce this metabolite and do not fluoresce under the Wood's lamp. This test is quick and easy but only can identify approximately 50% of *M. canis* infections, as not all infection produces enough metabolite to glow. This means a negative result does not rule out the presence of *M. canis* (the test has a low sensitivity). Additionally, many other substances including fibers, detergents, urine, and semen will fluoresce under a woods lamp. To increase specificity, broken hair from the affected part of the lesion should be plucked and evaluated for fluorescence and a trichogram concurrently performed. Fungal culture should be used for a definitive diagnosis.

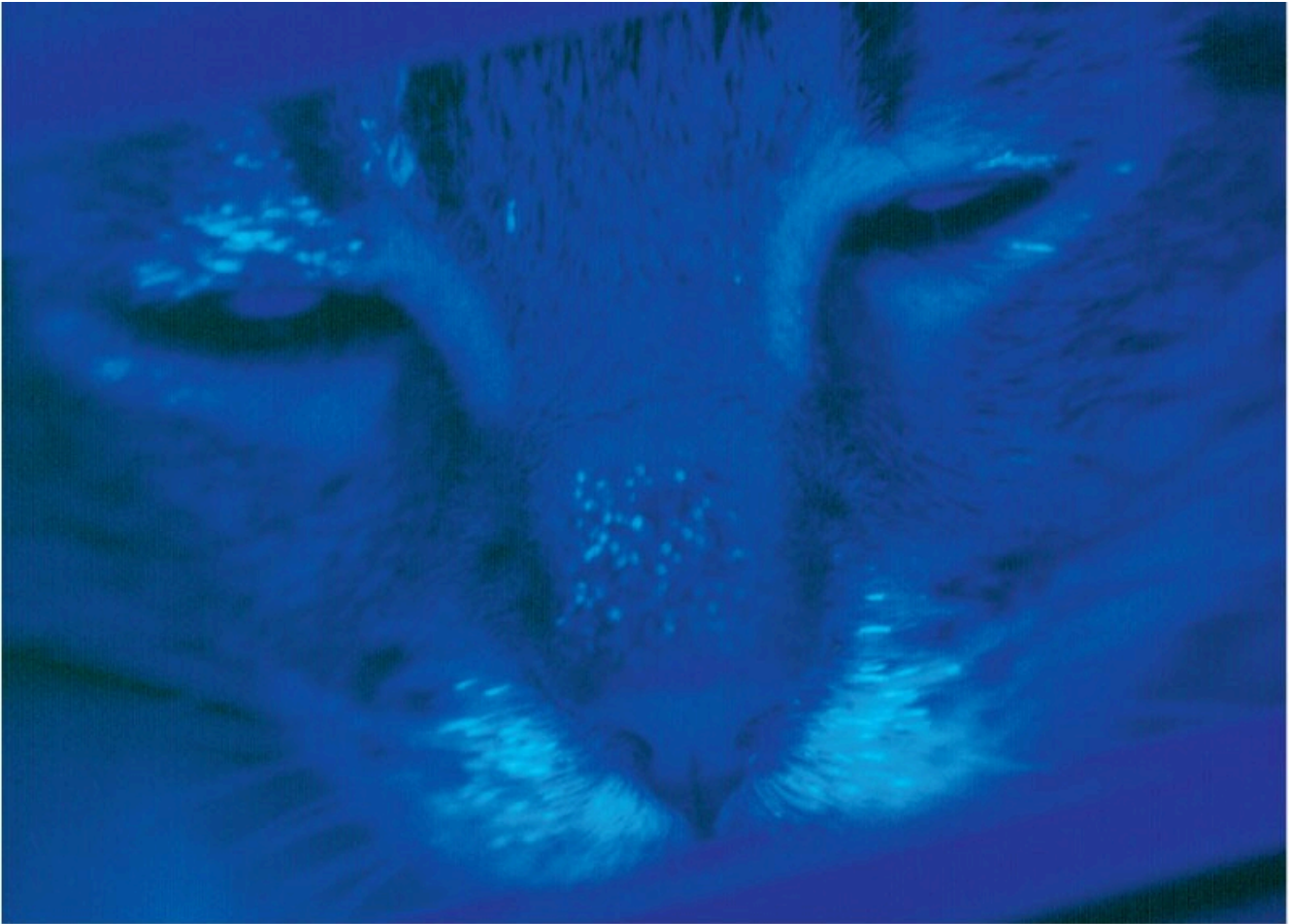


Fig. 56-5. Wood's light examination of a cat with dermatophytosis showing positive fluorescence results.

Photograph by Craig Greene © 2004 University of Georgia Research Foundation Inc.  
Greene: Infectious Diseases of the Dog and Cat, 4th Edition  
Copyright © 2012, 2006, 1998, 1990 by Saunders, an imprint of Elsevier Inc.

Wood's light examination of a cat with dermatophytosis showing positive fluorescence results

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## Knowledge check



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## Direct Microscopic Hair Exam or Trichogram

This test involves hair being plucked from the patient and mounted on a slide with mineral oil or potassium hydroxide incubated overnight for identification of arthrospores (Image A and B). You can also use the Wood's lamp on the hair that was plucked from the patient to help determine if the arthrospore identified is possibly *M. canis*.

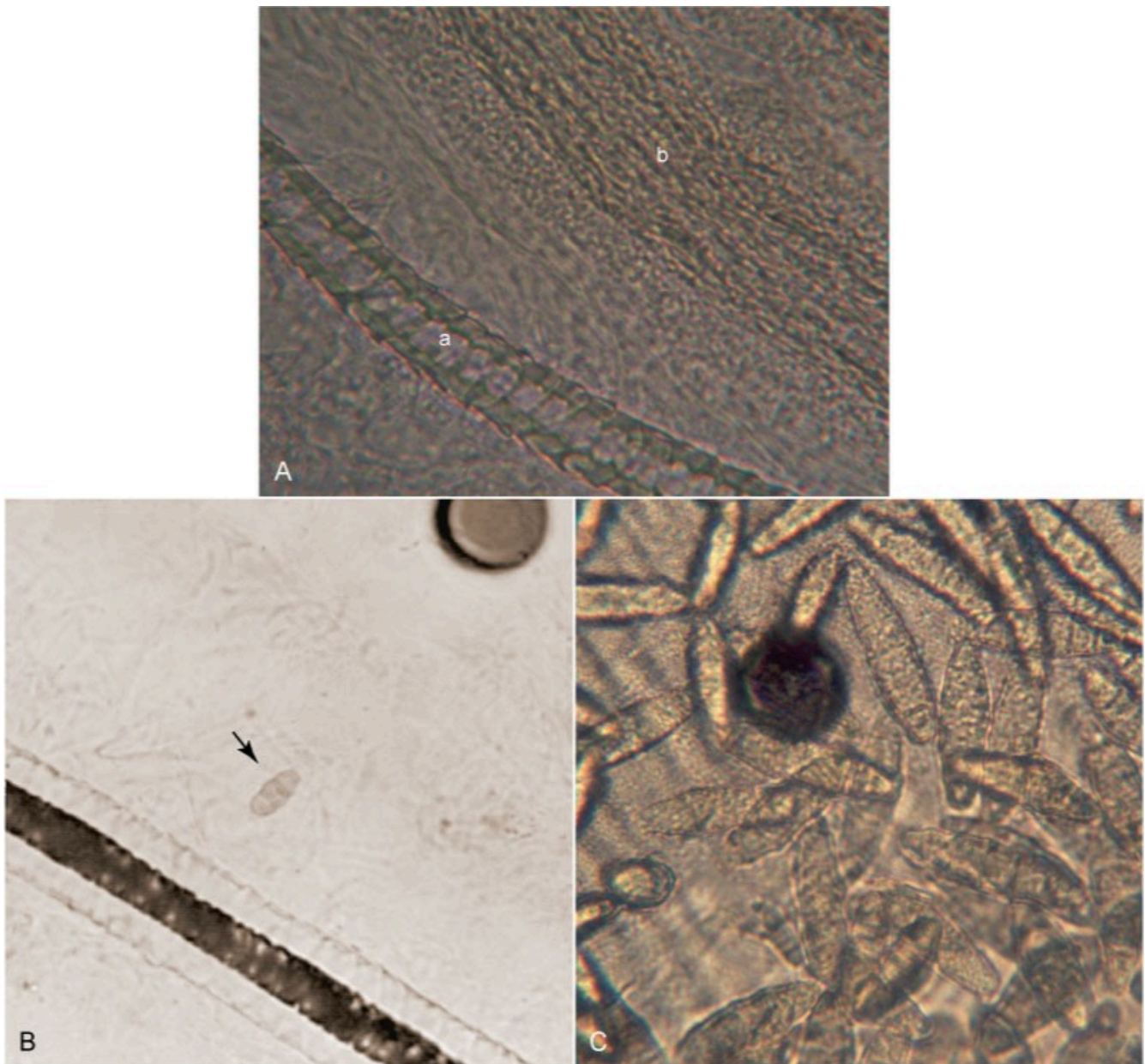


Fig. 56-6. **A**, Microscopic appearance of infected hairs cleared with KOH. The lower hair (*a*) is normal; note that the upper hair (*b*) is swollen, misshapen, and has tiny spherical ectothrix spores on the surface (unstained,  $\times 100$ ). **B**, This KOH-digested microscopic preparation of hair and scale from a dog contains a multiloculated fungal spore that may be mistaken for a dermatophyte macroconidium. It is an *Alternaria* spore (arrow). Dermatophytes never produce macroconidia in tissue. **C**, Microscopic appearance of macroconidia from a positive dermatophyte test medium culture, displaying typical morphology for *Microsporum* spp. (unstained,  $\times 100$ ).

Courtesy University of Wisconsin Teaching Materials, University of Wisconsin, Madison, WI.  
 Greene: Infectious Diseases of the Dog and Cat, 4th Edition  
 Copyright © 2012, 2006, 1998, 1990 by Saunders, an imprint of Elsevier Inc.

Above is an image that identifies what a normal hair (A) and an infected hair (B) with dermatophyte arthrospores looks like. Image (C) is an unstained macroconidium taken from culture (DTM). This form would not be found on hair, only on culture (DTM).

## Knowledge check



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## Fungal culture

Culturing dermatophytes requires specific dermatophyte test media (DTM) to promote the growth and proliferation of macroconidia from the arthrospore. DTM is one of the most common in-clinic laboratory tests used to definitively diagnose dermatophytosis.

## Collection technique

The most important step for this test is collecting enough **arthrospores** on the patient to result in a positive culture. A common technique for sample collection is using a sterile toothbrush that is brushed over the entire animal. In cats, the regions of most importance to brush over are ears and near eyes. Alternatively, plucking individual hairs from the leading edge of the lesion that appears broken. Increased success has been demonstrated when a wood's lamp is used to help identify glowing hairs for sampling. For more info on the collection technique: <http://veterinarymedicine.dvm360.com/how-perform-and-interpret-dermatophyte-cultures> (Links to an external site.).

## Culturing

The collected hairs are gently embedded into the testing medium. (image below). The DMT plates should be allowed to warm to room temperature before inoculating with sample



Embedding collected hairs into the testing medium

Dermatophytes culture best in a dark room that is incubated between 75-86 degrees F (24-30 degrees C). DTM cultured at room temperature is more likely to result in false positives with environmental contaminants. These plates should be monitored daily for growth and color change. Growth can take up to 21 days depending on the dermatophyte genus. Because of the time, it takes to produce a positive result, you will not be performing a DTM in the laboratory. You will be given samples to evaluate from previously cultured and positive DTM cultures to diagnose.

## Interpretation

A positive culture is identified by the **simultaneous** formation of **buff-colored** fungal colonies and color change of the media from **yellow** to **red** as the fungus utilizes proteins in the media (see images below). The positive culture must be confirmed with the microscopic evaluation of **macroconidia** to rule out environmental contamination. Environmental contaminants and non-specific fungi that normally colonize animal skin will result in a positive color change of the test medium, but the colonies grown are generally not buff in color.



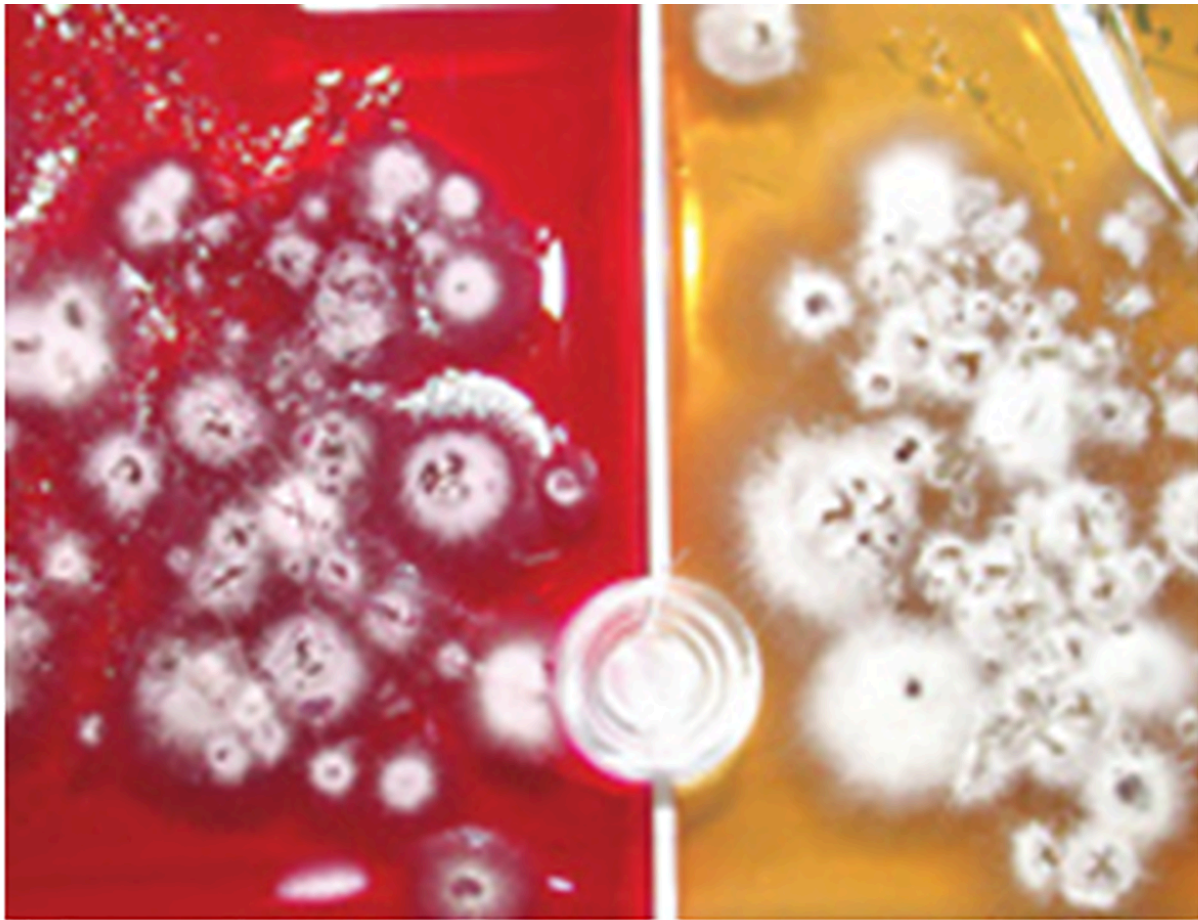
Interpreting a dermatophyte culture



Interpreting a dermatophyte culture



Interpreting a dermatophyte culture

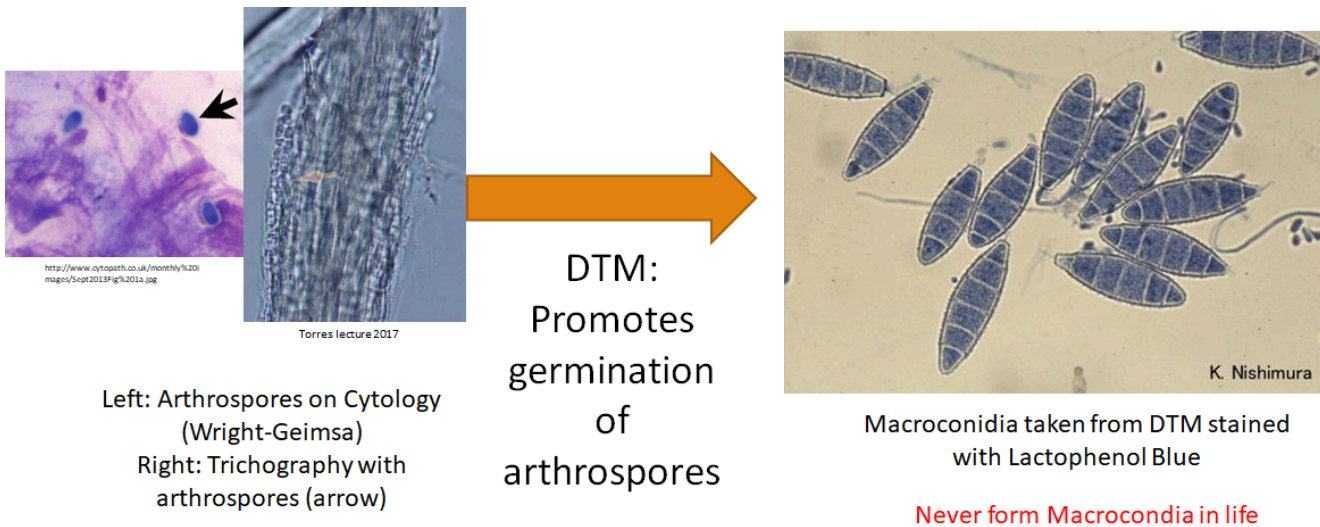


<http://veterinarymedicine.dvm360.com/how-perform-and-interpret-dermatophyte-cultures?id=&k=&date=&%0A%09%09%09&pageID=2>

### Interpreting a dermatophyte culture

Once a color change is detected, colonies are sampled for microscopic identification to determine the dermatophyte genus and to confirm that the patient is infected with dermatophytes. Acetate tape is used to collect colonies and **lactophenol blue stain** is used to identify the macroconidia.

**We cannot definitively determine the genus or species of dermatophyte based on the cytologic characteristic of the arthrospore, that is why we use DTM to germinate the arthrospores to the macroconidia stage.**



Using DTM to germinate arthrospores to the macroconidia stage

### Key Takeaways

- The Wood's lamp is a screening test for *M. canis*. Other dermatophytes will not fluoresce
- Trichograms evaluate the hairs for arthrospores.
- Dermatophytes from buff colonies on DTM, other color colonies are contaminants.
- We use the morphology of the macroconidia to determine the specific genus or species of dermatophytes.
- Differentiating different dermatophytes are important for counseling of control measures.

## Knowledge check





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<https://pressbooks.umn.edu/cvdl/?p=463#h5p-101>



*An interactive H5P element has been excluded from this version of the text. You can view it online here:*

<https://pressbooks.umn.edu/cvdl/?p=463#h5p-102>

# MODULE 13.3: THREE MOST COMMON VETERINARY DERMATOPHYTES OBSERVED ON DTM

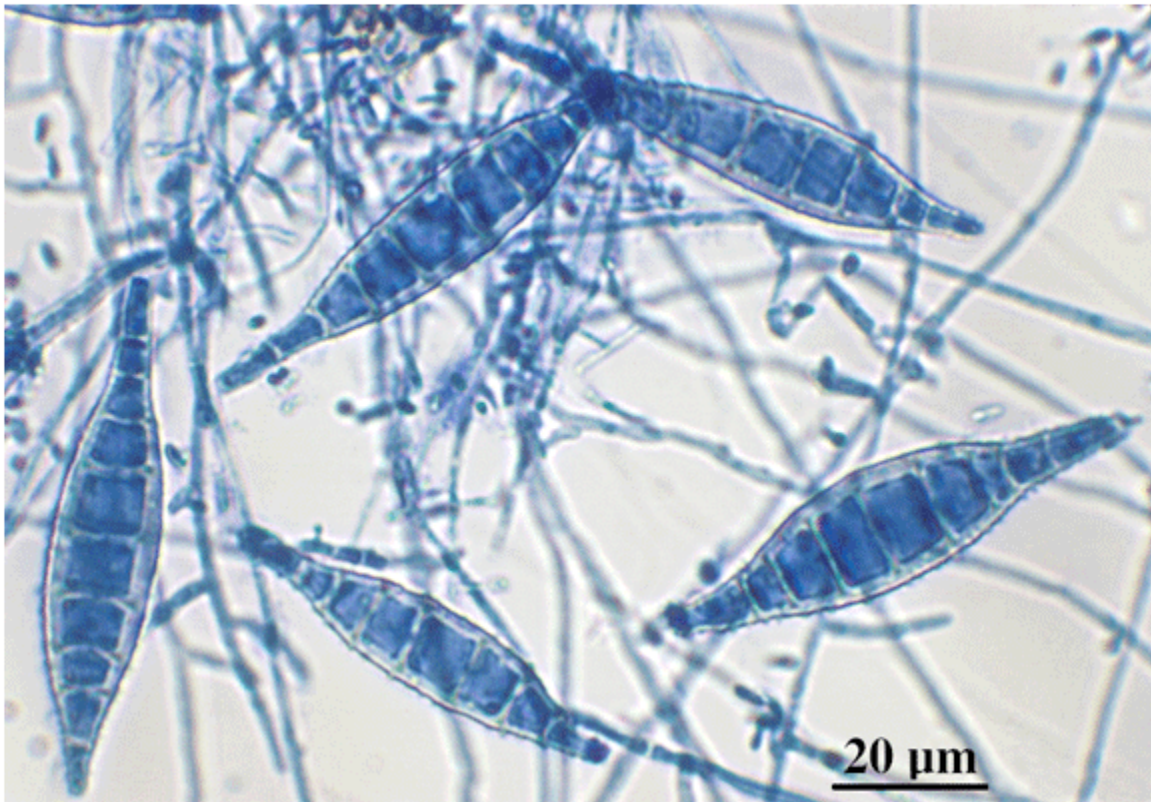
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## The three most common veterinary dermatophytes observed on DTM

In the laboratory you will be asked to identify the 3 most common dermatophytes commonly cultured on DTM; *Microsporum canis*, *Microsporum gypseum*, and a *Trichophyton sp.* Below are descriptions and images of those fungi.

### *Microsporum canis*

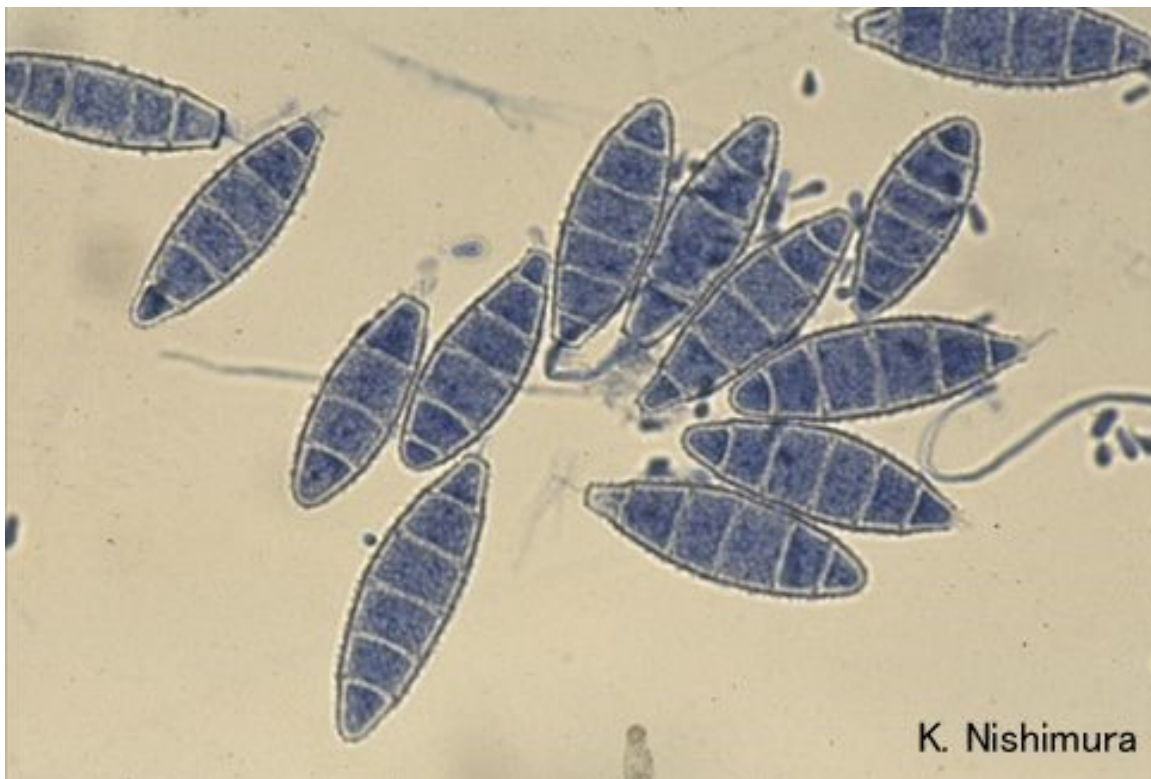
The macroconidia are multicellular (5-15 cells), spindle-shaped with rough, thick-walled. The macroconidia have a terminal knob. They produce fewer macroconidia than *M. gypseum*.



*Microsporum canis*

## *Microsporum gypseum*

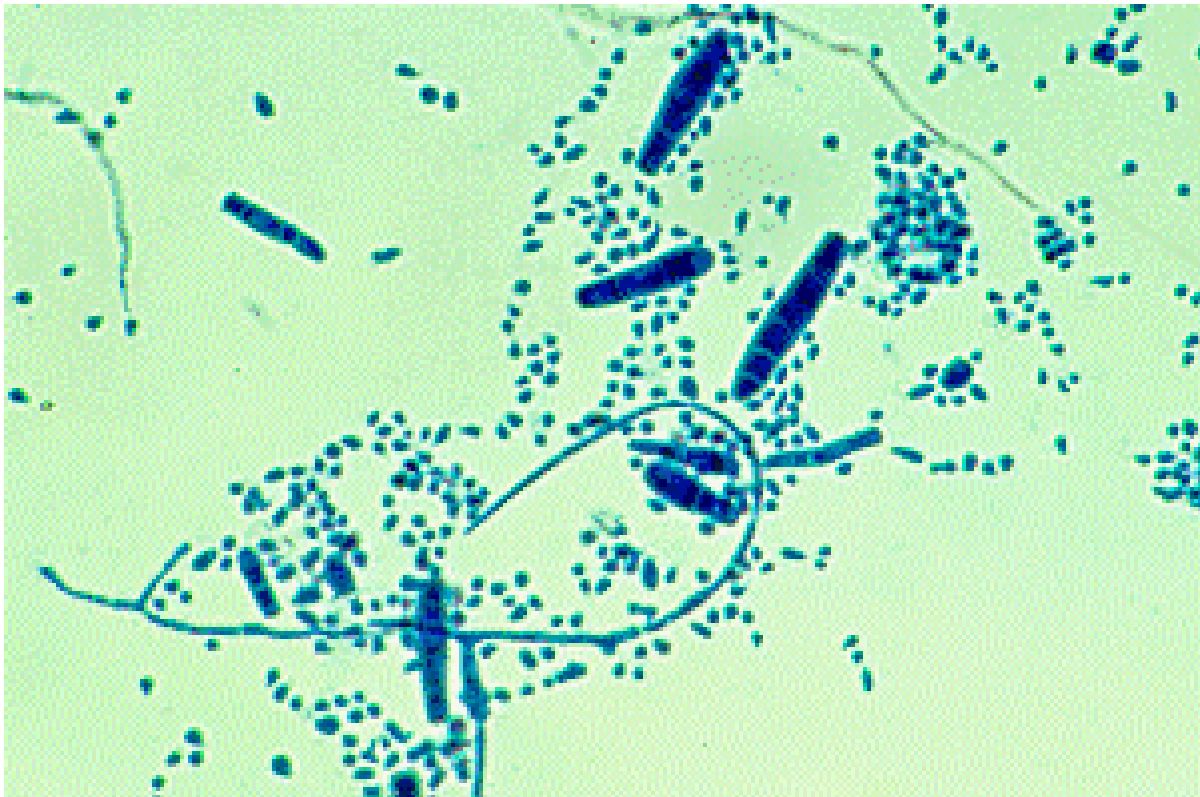
The macroconidia are numerous, multicellular (4-6 cells), symmetrical, ellipsoidal, with thin walls. The ends of the macroconidia differ in shape. The distal end is rounded where the proximal end can be truncated from where it attached to hyphae.



*Microsporum gypseum*

### *Trichophyton sp.*

The microconidia are single-celled, spherical in shape, and numerous. The macroconidia are cigar-shaped, multicellular (2-5 cells), thin-walled, and are rarely seen. These can be differentiated from *M. canis* as they have 6 compartments formed by the septae.



*Trichophyton sp.*

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## Knowledge check



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<https://pressbooks.umn.edu/cvdl/?p=468#h5p-103>

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## Key Takeaways

- Dermatophytes are common cutaneous fungal pathogens in all species
- Microsporum and Trichophyton are the 2 most common genera in veterinary medicine
- On DTM, the dermatophyte fungal colonies are buff in color and accompany a color change to red on the agar
- Saprophytic or contaminant fungi will grow on DTM, but do not routinely cause a color change and the colonies are not buff
- Microscopic evaluation of the colonies is an important step in the diagnosis and may influence the control steps based on the species of dermatophyte observed (i.e. geophilic vs. zoophilic)

You have now reached the end of Module 13. If you are enrolled in CVM 6925, please go to the Canvas page and take the quiz: "Module 13: Dermatophyte quiz." There is an assignment that accompanies the in-person laboratory for this module.



# GLOSSARY

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## arthrospores

very primitive spore type, formed by the breaking up or disarticulation of fungal mycelia

## bacteremia

is the presence of bacteria in the bloodstream.

## Biohazardous waste

also called infectious waste or biomedical waste, is any waste containing infectious materials or potentially infectious substances such as blood.

## biosafety

the application of safety precautions that reduce a laboratorian's risk of exposure to a potentially infectious microbe and limit contamination of the work environment and, ultimately, the community.

## coliforms

Umbrella term for bacteria that has originated from the GI tract. This is most commonly E. coli.

## colony-forming unit (CFU)

a bacterial cell or cluster of cells that give rise to a colony on a plate.

## contagious mastitis

Contagious mastitis is the “cow-associated” form where infected cows are the reservoirs for bacteria and spread occurs cow to cow at milking time

## cystitis

inflammation of the urinary bladder.

## Enterobacteriaceae

large family of Gram-negative bacteria that includes *E. coli*.

## environmental mastitis

Mastitis pathogens that are found outside the milking parlour. Environmental organisms are found in bedding, soil, walkways, on pasture or any surface with which the cow or her manure comes in contact.

## gradual metamorphosis

This is a type of development in which the immature stage (nymph) is a smaller version of the adult.

## Heinz body

an erythrocyte inclusion from denatured hemoglobin

## hematuria

the presence of blood in urine.

## host-specific

capable of living solely on or in one species of host.

## metarubricyte

An immature erythrocyte just prior to development to a polychromatophil. This stage still has a nucleus. Also known as a nucleated red blood cell.

## new methylene blue stain

is a supravital stain that is used in veterinary medicine to stain residual ribosomal RNA (reticulin) or Heinz bodies

## pollakiuria

means frequent, abnormal urination during the day.

## pseudoparasite

an object or organism that resembles or is mistaken for a parasite.

## Pyelonephritis

Upper urinary tract infection that often involves the kidneys.

## pyuria

an abnormal increase in WBCs in the urine.

## questers

or "quest", is the act of a tick seeking out a blood meal.

## spurious

an incidental finding. An example of a spurious parasite is finding rabbit coccidia in a dog fecal exam.

## stranguria

characterized by painful, frequent urination of small volumes that are expelled slowly only by straining and despite a severe sense of urgency, usually with the residual feeling of incomplete emptying.

## Tamm Horsfall Mucoprotein

Tamm-Horsfall protein (THP) is exclusively produced by renal tubular cells of the distal loop of Henle and is the most abundant urinary protein in mammals.

## turbidity

(of a liquid) cloudy, opaque, or thick with suspended matter.

## Urine Specific Gravity

laboratory test that shows the concentration of all chemical particles in the urine.

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## Module 8

[Cornell University College of Veterinary Medicine eClinPath](#)

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## Module 11

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