Dog's urine, an indication of health and illness

Introduction

The kidneys receive about of quarter of the blood flow and are very sophisticated filters where unwanted breakdown products such as urea and drugs found in the body are excreted. The kidneys also assist in controlling the water balance in the body. Thus the urine can be used to test for some diseases, like diabetes mellitus where there is an increase in sugar in the urine, it can also be used to test for some drugs like most antibiotics that are excreted by the kidneys. Some chemicals filtered through the kidneys will result in crystal formation. The urine is also an indicator of kidney and urine bladder health. Can the kidneys concentrate the urine? Is there blood or kidney and white blood cells in the urine? Is there infection of the kidneys or bladder? Are there bacteria or fungi in the urine? If there are can they be grown and tested for susceptibility to commonly used antibiotics.

Objective

Analyse urine collected from sick dogs to determine their health status.

Materials and Methods

To do this, you will analyse the urine using the following tests:

1. Observation (volume, colour, smell, cloudiness)
2. Culture a urine sample and perform a bacterial count, identification and antibiotic susceptibility tests (see description in a separate section)
3. Examine the urine using a "multistick"
4. Sediment the urine by centrifugation
5. Specific gravity of the urine supernatant.
6. Urine sediment examination to look for cells, crystals and micro-organisms under the microscope
7. Testing the urine supernatant for the presence of antibiotics

Use disposable gloves, provided for all the work you will be doing. Also remember for your own safety that you must follow all the rules of the laboratory.

DAY 1

Specimen

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

1. Observation

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

a. Note down the method of urine collection.
b. Note and record the volume of the urine sample. A 3mL sample volume is ideal. Interpretation of the microscopic examination is based on a standard starting volume of 3mL.
c. Note the color and turbidity (clarity) of the urine sample.

2. Culture a urine sample

a. Collect 10uL of the urine using a sterile plastic disposable loop (blue one) and streak onto a split agar plate. One side has blood agar and the other side MacConkey agar. You will be shown how. Take the opportunity to describe what you do in your laboratory book.
b. Incubate overnight the urine on the agar in a 5% CO_2 enriched in air incubator set to 37°C. You will examines these agar plates the following day.

3. Multistick examination of the urine

Drip a Multistick strip into the well mixed urine sample. Replace the lid on the test strip immediately. Read the results 60 seconds after dipping or by according to product instruction by comparing the colors to the test pad colors on the vial. Record the results. **Do not report the results of the leukocyte, nitrite, or urobilinogen test pads as they don't work for dogs.**

3. Sediment the urine by centrifugation

Place the urine sample using a plastic Pasteur pipette in a centrifuge tube and centrifuge the urine sample for 5 minutes at 1500rpm.

If the protein value from Multistick strip is considered to be significantly increased then further quantification of urine protein may include one or more of the following tests:

i. Determination of urine protein concentration using an automated chemistry analyzer.
ii. Determination of urine protein to creatinine ratio

4. Determine the specific gravity (USG) on the supernatant.

a. Decant the supernatant quickly into a clean test tube (eg. Quickly turn the tube upside down over the clean tube; don't worry the sediment will remain in the upturned tube**).**
b. Wipe the glass area (specimen area) under the plastic flap with 70% alcohol.
c. Place a drop of urine supernatant on the glass area and cover with the plastic cover, taking care not to create air bubbles.
d. Looking through the eye-piece observe and record the specific gravity. This is the area on the scale where the light and dark zones meet.

5. Urine sediment examination

Do the microscopic evaluation of the urine sediment (**Note: lower the condenser or reduce the diaphragm aperture of the microscope to increase visibility of the sediment constituents.**)

Note that you will be given a brief introduction to the use of the microscope before you will be allowed to use it.

a. Use 10ul urine sediment for unstained and 10ul urine sediment + 10ul Sedistain for stained preparations.
b. Scan on low (10x) or high dry (40x) power for crystals, bacteria, debris and fat. Quantify on high power as **scant** (5%); **mild** (5-25%), **moderate** (25-75%) or **abundant** (>75%).

Note: percentages are based on approximate area of the 40x field occupied by these components.

c. Scan on low (10x) power and numerically quantify casts.
d. Scan 15-20fields on high (40x) power and average the counts for cellular elements,- report as a numerical range up to 100/HPF. If >100/HPF report such as or, if excessive, as "field obscured by RBCs, WBCs".
e. Report sperm only as present (do not quantify)
f. Use 100x to specify bacterial morphology (rods, cocci, chains) or aid in identification of anything obscure at lower magnification.

DAY 2

Urine culture

1. Semi-quantify the bacterial colonies on the blood

One colony usually originates from one living bacterium as bacteria divide asexually by binary fission.

1+ Growing on primary inoculum

2+ Also growing on first streak lines

3+ Also growing on 2nd streak lines

4+ Growing on all streak lines

2. Primary identification tests (You will be provided with a laboratory manual that will describe the detailed methods of each test)

a. Describe the bacterial colony on blood agar: colour, size, haemolysis
b. Describe the bacterial colony on MacConkey agar: colour and presence
c. Gram'stain of the bacterium and look at the stain using 100x oil objective. Record your results
d. Preliminary identification tests which include the catalase, oxidase, spot indole and motility tests. Record all your results

e. Complete identification using commercial tests – these will be incubated overnight at 37°C.

3. Antimicrobial susceptibility tests (The methods will be provided in the Laboratory manual)

You will be testing your test bacteria for susceptibility to a number of commonly used antibiotics used to treat urinary tract infections using 2 tests:

a. Disk-diffusion test. For this test you will also be setting up an antibiotic disc control using known bacteria that have a known response to each of the antibiotics you will be testing. This is to ensure that the antibiotics in the disks are still active.
b. Minimum inhibitory concentration (MIC) test

These will be incubated overnight at 37°C.

DAY 3

1. Reading of urine culture tests

Additional reagents will be added to the commercial tests and the results recorded

Final identification of the bacterial will be carried out, either using provided identification tables (In the Laboratory Manual) or in an on-line data-base.

Record the bacterial Genus and Species identified

2. Reading of antimicrobial susceptibility tests

a. The zone of inhibition of growth (ZOI) diameters for both the test bacterium and the bacterium control are read and recorded on a provided sheet in millimetres.
b. This provided sheet will also provide you with the interpretative values for each antibiotic. Note that the quality control bacterial ZOI should fall within a given range to PASS. If they are outside the range, they will FAIL and it means that we cannot trust the results that that antibiotic gives. The test bacteria ZOI will record as SUSCEPTIBLE (S); INTERMEDIATE (I) OR RESISTANT (R).
c. The highest dilution of antibiotic that gives no visible growth of bacteria will be considered as the MIC.
d. The recording sheet will also provide you with interpretative MIC values.
e. Compare the results of both tests. Note that you will not be able to do this for all the antibiotics.

3. Detection of antibiotics in urine

If there is time, the urine sample will also be tested for the presence of antibiotics. This will be done by adding 100 uL of urine to 900uL of brain heart infusion broth with 1% glucose and phenol red indicator that contains 1x 10^5 colony-forming units of *Geobacillus stereathermophilus* and incubating it in a heating block set to 80°C for 10 minutes and then lowered to 60°C for 3 hours.

Note that you will have to do this test first thing in the morning so that you can read it after tea.

4. Data Evaluation

Use the rest of the time to sort out your data into tables in a way that you can compare your different results. You will need to get that ready for a presentation the next Day. Please make sure that you take lots of pictures as you can use them in your presentation.