香港城市大學
City University of Hong Kong

VETERINARY DIAGNOSTIC LABORATORY

CITYU VETERINARY DIAGNOSTIC LABORATORY

MESSAGE FROM THE DIRECTOR

Welcome to the latest issue of the newsletter.

In this newsletter we highlight more new technology and tests available at CityU VDL in Hong Kong including identification of fungi by molecular methods, ALP cytology staining for osteosarcoma, PBFD diagnosis in a bird, and new antibiotic panels for urine testing. We also profile Dr Andrew Ferguson, our newest pathologist.

- Dr Fraser Hill, Anatomic Pathologist, Director of CityU VDL

IN THIS ISSUE

What's new at CityU VDL

NEW TEST OPTIONS

ALP test for osteosarcoma

Diagnosis of osteosarcoma by ALP staining of cytology slides is now an option at CityU VDL. Dr Daniela Hernandez Muguiro and the clinical pathology team have worked up the application of this test in Hong Kong. If you suspect an osteosarcoma in an animal, collect multiple samples for cytology and indicate your suspicions on the submission form. Some of the slides can then be retained unstained, for possible ALP later staining. If the cytology is suggestive of osteosarcoma and sufficient and suitable cellular material is present on the slides this test could be undertaken. Standard immunostaining pricing applies (contact the laboratory for the latest pricing).

Due to the complexity of preparing reagents and running this test the turn around time is up to seven days.

Fungal Identification by PCR and sequencing

Identification of fungi by morphological methods or culture is difficult and prolonged. At CityU VDL the molecular team has developed a method using a pan fungal PCR combined with sequencing to rapidly identify fungi in suitable samples. If fungal infection of tissue is prolific, fresh tissue or wax shavings from formalin fixed parafin embedded (FFPE) tissue can be used to extract fungal DNA. This DNA can then be sequenced and matched with the on-line data base of fungal organisms to identify various fungi.In samples where the fungal infectious load is low, this type of testing will be unsuccessful so it is best used after fungi have been visualised on histopathology.Another option is to increased the fungal concentration by culture and then attempt identification on the cultured material.

The turnaround time for this test is up to 14 working days.Contact the laboratory for the latest pricing.

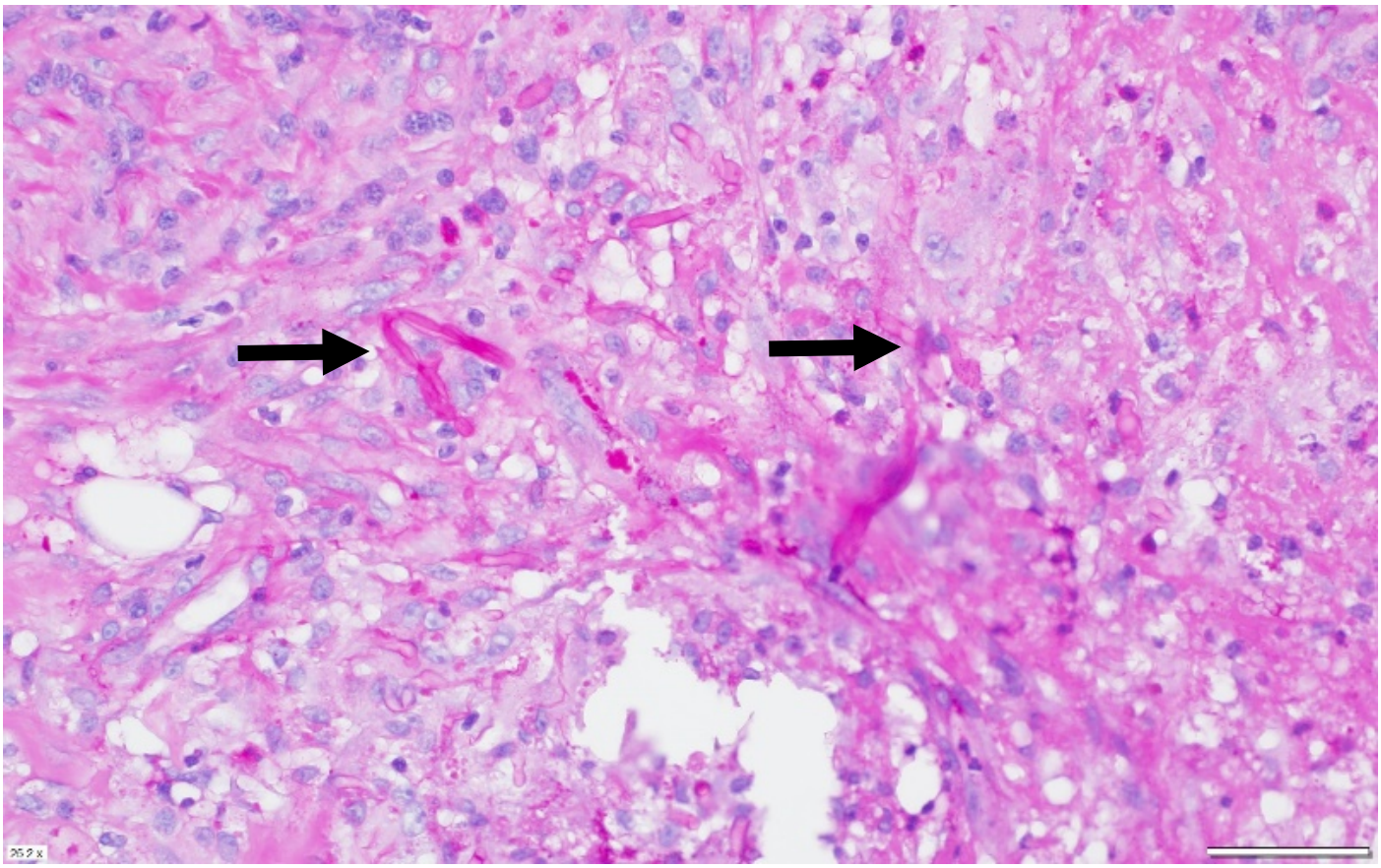


Figure 1: Fungal organisms stain bright pink with PAS stains (arrows) in tissues from a dog identied as *Pythium insidiosum* after sequencing

Psittacine Beak and Feather Disease (PBFD)

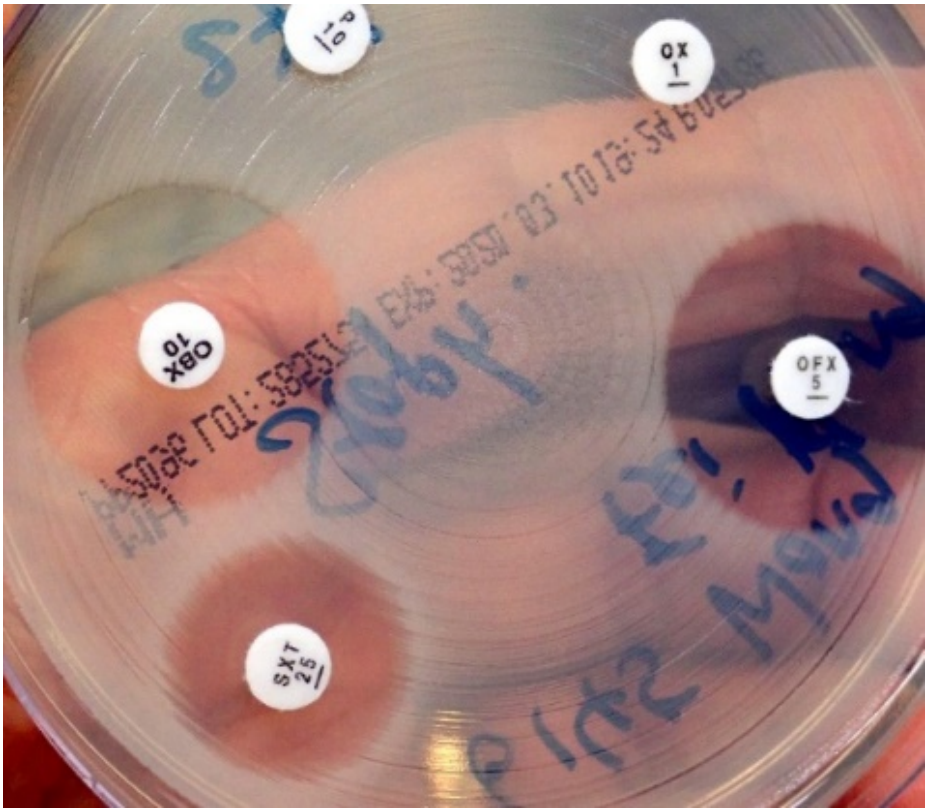
Diagnosis of Psittacine Beak and Feather Disease is possible at CityU VDL. Testing is best undertaken on whole blood collected into EDTA (but feather samples can also be tested) and has a turnaround time of 3 days. Disease is caused by a circovirus affecting actively growing beak and feather cells of birds including Psittaciformes, Columbiformes, Passeriformes and Anseriformes. Early detection of disease and hygeine are imporant in control of infection.

Infection is reported world wide and virus was detected in EDTA blood from a bird in Hong Kong recently.

News from the Microbiology Department

Dr Vidya Bhardwaj

Urinary Antibiotic Sensitivity Panel Update



As part of a regular review process, CityU VDL microbiology has been evaluating antibiotic sensitivities in the common urinary pathogens isolated from dogs and cats in Hong Kong over the past 12 months. CityU VDL takes great efforts to ensure accurate and reliable testing, and makes every effort to ensure results can be interpreted readily and used to guide clinical decision making.

Based on the patterns seen, a new urinary antibiotic sensitivity panel has been designed and will be implemented in the next few weeks. These changes are in-line with the guidelines prescribed by the Clinical and Laboratory Standards Institute (CLSI).

Figure 2: Antibiotic sensitivity testing is undertaken by placing antibiotic infused discs on a bacteria covered agar plate and checking for clear zones where the bacteria are inhibited

Cephalosporins and beta-lactam combination agents are commonly used in the treatment of urinary tract infections. These antibiotics are commonly found in higher concentrations in urine and hence, the criteria used to define resistance is different for urine compared to serum. Assessing antibiotic susceptibility using MIC is a more accurate and reliable method compared to disc diffusion. If an organism tests as “sensitive” in disc diffusion tests, these results can be considered reliable. However, if an organism demonstrates multi drug resistance in disc diffusion testing, it is better to confirm this using MIC testing.

Figure 3: MIC plates are supplied with titrated concentrations of antibiotics included in a series of wells. Known concentrations of bacteria are placed in each well, the plate incubated and the minimum concentration of antibiotic inhibiting the bacteria measured by optical density.

The standard Urinary Panel performed by antibiotic sensitivity testing (AST) includes:

Amikacin	Doxycycline
Ampicillin	Enrofloxacin
Amoxicillin-clavulanate	Gentamicin
Azithromycin	Imipenem
Cefazolin	Marbofloxacin
Cefovecin	Nitrofurantoin
Ceftazidime	Orbifloxacin
Ceftriaxone	Piperacillin-tazobactam
Chloramphenicol	Pradofloxacin
Clindamycin	Trimethoprim sulfamethoxazole

The key changes in the new urinary panel tested AST are set out here. The antibiotics listed below will no longer be tested on the standard urinary AST panel. Options for MIC testing or extrapolation from other information are possible alternatives and are explained in detail below:

Cephalexin: Urinary breakpoints for cephalexin have only been developed for MIC testing and ideally the MIC method should be used to assess cephalexin sensitivity. Failing that, it is better to extrapolate cefazolin results (which are accurate for urinary pathogens). Performing disc diffusion tests for cephalexin on urinary pathogens leads to a potentially inaccurate result.

Cefpodoxime: Results for disc diffusion tests on urinary pathogens are not as accurate as MIC testing.

Cefuroxime: Data (Page 6) shows limited advantage in using cefuroxime compared to cefazolin.

Ciprofloxacin: Ciprofloxacin is a precious antibiotic in human therapeutics and its use in animals is discouraged. Its pharmacokinetic distribution does not support its use as a systemic antimicrobial agent in dogs.

Ofloxacin: Interpretation criteria for ofloxacin are based on studies in humans and have not been updated for veterinary use. CityU VDL data suggests that laboratory testing of veterinary isolates with ofloxacin does not accurately reflect the clinical picture, can be misleading and could result in case mis-management so is no longer available.

Ticarcillin-clavulanate: Testing for piperacillin-tazobactam can be performed accurately and reliably using the disc diffusion method and results for piperacillin-tazobactam can be readily extrapolated to ticarcillin-clavulanate. Ticarcillin-clavulanate should only be used in veterinary medicine, when all other therapeutic options are exhausted.

Responsible use of Cephalosporins

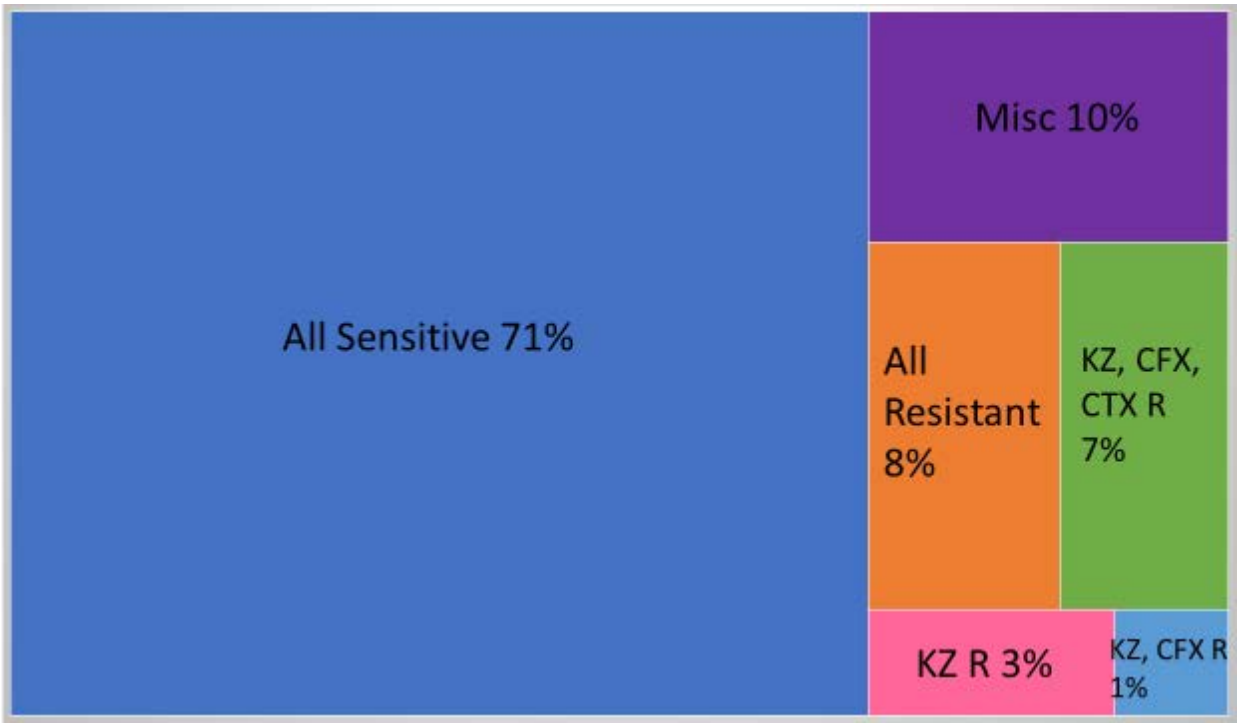
Gram negative enteric bacteria (*E. coli, Proteus mirabilis, Klebsiella spp.*, etc.) are the most common causes of urinary tract infections in dogs and cats. This group of bacteria commonly develops resistance to antimicrobial agents and thus sensitivity testing is warranted.

Cephalosporins are widely used in veterinary and human medicine against most bacteria as they have a broad spectrum of activity. Resistance to these antimicrobial agents is prevalent worldwide and is driven by their use and abuse.

CityU VDL has examined the sensitivity patterns of the common urinary pathogens to four cephalosporins (cefazolin, cefuroxime, ceftriaxone and ceftazidime) over the last 12 months (Chart 1).

1. Cefazolin is a first-generation cephalosporin that has good activity against gram positive bacteria as well as gram negative enteric bacteria. It is commonly used for surgical antibiotic prophylaxis.
2. Cefuroxime is a second-generation cephalosporin that is less effective against gram positive bacteria but has increased activity against gram negative bacteria.
3. Ceftriaxone and Ceftazidime are third-generation cephalosporins that have extended gram-negative bacteria coverage. These antimicrobial agents are commonly used to treat those infections that are resistant to first- and second-generation cephalosporins. These, especially ceftazidime, are very precious as they are considered last line drugs for infections such as meningitis in humans.

Chart 1: Schematic representation of sensitivity patterns within gram-negative enteric bacteria isolated from urine samples in the microbiology laboratory at CityU VDL



KZ- Cefazolin, CFX- Cefuroxime, CTX- Ceftriaxone

What are the implications of this data (summarised in Table 1)?

Table 1: Sensitivity and resistance percentage values of gram negative bacteria isolated from urine samples in the microbiology laboratory at CityU VDL

Antimicrobial agent sensitivity	Percentage of isolates
All four antimicrobials SENSITIVE	71%
All four antimicrobials RESISTANT	8%
Cefazolin RESISTANT	3%
Cefazolin and Cefuroxime RESISTANT	1%
Cefazolin, Cefuroxime, Ceftriaxone RESISTANT	7%
Miscellaneous results*	10%

** Includes incomplete data sets, inaccurate results and all results that do not fit into the above patterns*

Can Cefazolin continue to be used for surgical antibiotic prophylaxis or as an effective antimicrobial agent in Hong Kong?

- Ideally antimicrobial sensitivity testing should be performed in every situation. But, failing that, the results show that cefazolin was effective in at least 70% of tested isolates.
- Therefore, cefazolin should continue to be used for routine surgical prophylaxis and in situations where sensitivity testing is not performed.

Should we use a third-generation cephalosporin instead?

- Using ceftriaxone over cefazolin only has a benefit in 4% of the tested isolates. Using ceftazidime instead of cefazolin has a benefit in only 11% of the tested isolates.
- There is absolutely no justification for using these precious antimicrobial agents without performing sensitivity testing, preferably MIC testing.

Does cefuroxime have a place in veterinary medicine in Hong Kong?

- Based on these results it appears that cefuroxime has an advantage over cefazolin in only about 3% of the isolates.

Antimicrobial resistance is a serious global issue and is driven by use/abuse of antimicrobial agents. It is our responsibility to use these drugs responsibly, making choices driven by science.

Reference: International Society for Companion Animal Infectious Diseases (ISCAID) guidelines for the diagnosis and management of bacterial urinary tract infections in dogs and cats. Weese JS, Blondeau J, Boothe D, Guardabassi LG, Gumley N, Papich M, Jessen LR, Lappin M, Rankin S, Westropp JL and Sykes J (2019). The Veterinary Journal 247, 8-25.

TESTING TIPS

Sample submission- previous case numbers

The specimen accessioning team make every effort to match up previous cases and information so the pathologists can follow a cases progress. To assist the team, if you are working with an on-going case please put any previous CityU VDL case numbers in the history. If there are additional laboratory findings from other laboratories, include these as well.
If the case is a referral to your practice and previous laboratory diagnostics have been undertaken at the referring clinic, please include those case numbers too.
Thanks for your assistance with this as it will help our pathologists understand the case and provide the best opportunity to make a diagnosis.

Diagnosis of Dermatophytosis in Dogs and Cats

Utilising a number of tests is recommended by the World Association for Veterinary Dermatology (Vet Dermatol, Moriello et al, 2017, 28, 266-e68) for the diagnosis of dermatophytosis in dogs and cats.

The recommended approach includes; using a Wood's lamp, direct examination, dermatophyte culture (using a tooth brush technique), response to treatment and biopsy of nodular or unusual infections. PCR detection of dermatophyte DNA can be helpful in confirming infection or the absence of infection in successfully treated cats but results need to be considered in the clinical context. Dermatophyte DNA will also be detected from non-infected fomite carriers and dead fungi on successfully treated animals (false positive).

CityU VDL is able to offer dermatophyte culture, PCR testing and biopsy for case investigations.

STAFF PROFILE

Dr. Andrew Ferguson- BVSc, GCVSt (Diag Path), MANZCVSc (Pathobiology) - Anatomic Pathologist

Dr Andrew Davis Ferguson is a member of the Australian and New Zealand College of Veterinary Scientists in Pathobiology and has experience working in veterinary diagnostic laboratories within government, research and commercial settings across several countries. Over the last ten years Andrew has worked for various offices of WHO and OIE, and has also consulted for FAO including in both technical and management roles, promoting collaborative approaches in the fields of laboratory strengthening, diagnostic testing, and zoonotic disease. Most recently he has been working with the AFCD Tai Lung Veterinary Laboratory providing pathology support to their team but has now moved to CityU VDL to take up the position of Senior Veterinarian (Diagnostic Services) and coordinating the specimen accessions team. He enjoys interacting across fields and looks forward to working with clinicians and veterinary practices to ensure they receive the best value out of our testing services.

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