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 2nd edition of volume four of the newsletter.

In this edition we highlight some of the details of the comprehensive range of tests we offer. We aim to make the latest tests available and are constantly reviewing our test offering to keep them up to date.

A recent case of feline nasal fungal infection is described in detail by Dr Steve Mills, highlighting how useful special stains can be.

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

IN THIS ISSUE

SNIPPETS

A virtual veterinary conference was undertaken in June. A series of highly informative talks were undertaken by our veterinary team. The sessions were recorded and will be available to registered participants on

https://www.veterinaryeducationtoday.ca/hong-kong

in case you missed them.

Topics covered included:

“Lymphocytosis in dogs and Cats” by Dr Daniela Hernandez Muguiro

“Understanding antimicrobial sensitivity testing” by Dr Vidya Bhardwaj

“Molecular testing in dogs and cats. What has molecular testing shown?” by Dr Fraser Hill

“Common tumours in dogs and cats in Hong Kong” by Dr May Tse.

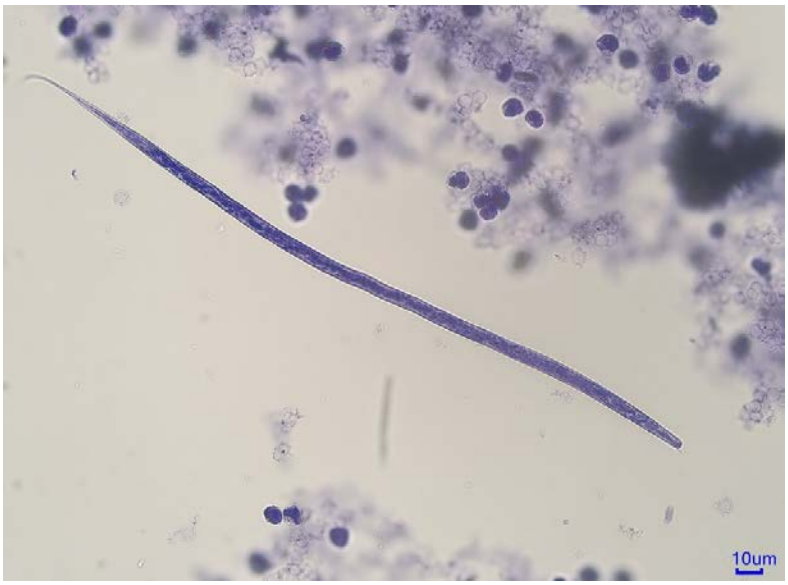
A range of other interesting talks from business management, to legal issues, to wildlife management are also available for review.

TESTING TIPS

Heartworm screening tests

The Knott's test is a useful screen for *Dirofilaria immitis* but should be used as one of a number of tests for diagnosis as infections may consist of non- microfilariae producing males, or immature female yet to produce microfilariae.

The microfilariae of *Dirofilaria immitis* and *D. repens*, and those of *Acanthocheilonema reconditum* should be differentiated. For more accurate evaluation, Heartworm antigen serological testing is recommended.



Knott's test shows a microfilaria of *Dirofilaria immitis* with a tapered head and straight tail (Dog, venous blood 60x).

The recommended testing protocol for evaluation of Dirofilaria status in a dog or cat consists of antigen serology testing and a microfilarial test done at the same time. If both tests are done, the results may be interpreted as described below:

Antigen positive and microfilaria negative

- Single infection with female worm
- Immature adult worms (antigens will not be detected for 5-7 months and 7-8 months post-infection in dogs and cats, respectively)
- Immune-mediated clearance of microfilariae
- Animal on monthly preventatives, or after microfilaricide treatment

Antigen negative and microfilaria positive

- Microfilariae other than *Dirofilaria immitis*
- Heartworm antigen not present, or present in levels too low to detect
- Adult heartworm dead and antigen cleared, but microfilariae still present
- Animal transfused with microfilaremic blood
- Prenatal transfer of microfilariae
- Immune-mediated clearance of antigen-antibody complexes
- Antigen destroyed due to improper storage or treatment of sample
- Microfilarial contamination of lysing solution, dye or filter chamber

These tests results should be correlated with other diagnostic tests, such as thoracic radiographs, electrocardiogram, blood chemistry, and urinalysis.

After treatment, *Dirofilaria immitis* microfilariae can persist for up to 6 months and heartworm antigen can persist for up to 4 months, so repeat testing must be done after that time to confirm that treatment has been effective.

If the animal is clinically normal, follow up testing in 6 months with an occult serological test is recommended if the initial testing is inconclusive.

Because of the nature of this disease in cats, veterinarians may want to consider including Heartworm antibody testing for a more accurate assessment of infection status. Since worm maturation increases the chances of a positive antigen test, repeating a test in 1-2 months in suspected cases is often helpful.

Test	Samples required	Turnaround time
Blood examination for microfilararia	Whole blood in EDTA tube	24 hours
Knott's test	Whole blood in EDTA tube	24 hours
D repens and D immitis antigen	Serum (clotted blood) in serum tube	1-3 days

Checking for the BRAF gene in cases of Transitional Cell Carcinoma/Urothelial Carcinoma (TCC/UC)

The CADET® BRAF analysis for canine TCC/UC can be used for the diagnosis, or screening of high-risk breeds, monitoring, and identification of metastasis.

A single mutation in exon 15 of the BRAF gene is present in 85% of canine (TCC/UC). BRAF analysis is based on identification and quantification of wild-type (with no mutation) and mutated BRAF alleles recovered from cells in urine. A comparison between the level of BRAF wild-type and BRAF mutant alleles provides a quantitative measure of cells recovered from the urine sample. These data are used to calculate the detection threshold/technical sensitivity for that specific sample. And, if a BRAF mutation is detected, the relative proportion of mutant alleles (fractional abundance).

Most cells with the BRAF mutation are heterozygous. This means the BRAF mutation is generally detected in only one of the two copies (alleles) of the gene in each cell. Therefore, the fractional abundance of the mutation typically does not exceed 50%. The percentage of cells detected with the mutation is thus up to double the percentage of mutant alleles detected.

There are 2 possible outcomes from BRAF analysis:

- **Detected:** the fractional abundance is provided, which may be used as baseline to monitor changes in urinary BRAF mutation levels during treatment.
- **Undetected:** the detection threshold indicates the sensitivity of the sample assessed and whether the specimen is eligible for analysis with BRAF-PLUS.

The reported biological sensitivity of BRAF to detect canine TCC/UC is 85%. The technical sensitivity (detection threshold) is sample dependent, but is reported to typically be >99.9%. The specificity is currently >99%.

CADET® BRAF-PLUS analysis

This analysis provides further evaluation of those dogs that present with clinical signs consistent with TCC/UC, but for which no BRAF mutation is detected. The same DNA sample used for BRAF is further evaluated with probes to detect other mutations. These mutations are present in 2/3 of dogs with TCC/UC in which the BRAF mutation is not present. After analysis, the data are used to calculate the relative number of copies of each of the target regions and determine if the DNA sample evaluated has signatures consistent with canine TCC/UC.

The BRAF-PLUS analysis increases the combined biological sensitivity to detect a canine TCC/UC, but is less useful as a preclinical screening assay. The reported specificity is >99%.

Sample requirements

The urine sample should be placed in the specialized container (provided by CityU VDL) that contains a small volume of a colourless liquid preservative. This preservative protects the urine specimen during transit to the testing laboratory. The analysis requires **40 mL of free-catch urine.** Each sample container indicates the proper

volume of urine that should be added. It may be difficult to collect this volume of urine, therefore, it is best NOT to try to collect urine directly into the specialized container. It is best to use a household container (e.g., glass jar or plastic cup for males, and a flat rimmed container such as cookie sheet, for females) to collect the free-catch urine sample. Make sure the container is cleaned and fully-dried before using. Then, the collected urine can be transferred into the specialized container with the preservative. This should be done **within 15 minutes** of sample collection. Additional collection tips are given with each specialized collection container.

If you want to proceed with testing, please contact CityU VDL and ask for this test (BRAF mutation in urine), so we can we can send out a container with the next courier run to your clinic.

Testing for Ionised Calcium

Testing for ionised calcium is now undertaken on serum samples (rather than heparin plasma) and this is the preferred sample type due to the possible binding of calcium to heparin and decreasing the ionized calcium if not in the proper ratio to blood.

Use these steps for sample collection and preparation:

1. Collect blood into red top/plain tubes (preferred to lithium heparin)
2. Allow samples to clot, then spin down.
3. Maintaining anaerobic conditions, collect the serum (can uncap tube briefly to remove serum or using a syringe and needle, withdraw serum through the cap, making sure all air is expelled from the syringe and needle). Do not mix serum with air.
4. Place serum in another plain tube, maintaining anaerobic conditions as much as possible (go through the cap with needle and syringe, if using).
5. Keep cool and submit to laboratory ASAP.

The turnaround time is: 24 hours

NB: Only use plain tubes and not gel separator tubes, as the gel can falsely increase ionised Calcium.

Case report: Feline fungal rhinosinusitis

Dr Steve Mills

A 12-year-old, male, castrated British Shorthair cat presented to a primary care clinic in Hong Kong for chronic left-sided nasal discharge, obstruction, and enlargement of the left submandibular lymph node. CT imaging revealed multiple irregular to polypoid sinonasal structures. Biopsies of the lesion were performed and submitted to CityU VDL for histologic evaluation. The formalin-fixed specimen underwent standard processing. Paraffin infiltrated sections were stained with Hematoxylin-Eosin and examined microscopically (figure 1).

The sinonasal mucosae was expanded by marked oedema and densely cellular, nodular to coalescing inflammatory infiltrates, predominated by eosinophils (figure 2). Macrophages were also abundant intermingling, with fewer neutrophils. Loose lymphocytes and plasma cell aggregates were sometimes seen cuffing the margins. A histologic diagnosis of sinonasal polypoid eosinophilic granuloma was made and two additional stained sections were prepared (Gomori's methenamine silver, (figure 3) Periodic acid-Schiff (figure 4)) to investigate for a fungal etiology. Special stains revealed intra-lesional fungal organisms, characterized by loose aggregates or tangles of irregular linear and branching hyphal structures. The histologic diagnosis was revised to fungal rhinosinusitis (FRS), presumptive *Aspergillus* spp. infection.

Acute or chronic sinonasal disease in cats is regularly encountered in a primary practice setting. Common differential diagnoses include nasopharyngeal polyp, foreign body, infectious rhinitis/sinusitis, tooth root abscess/fistula, or neoplasia. Patients may present with a history of nasal discharge, excessive sneezing, stertor, facial swelling, epistaxis, and/or regional lymphadenopathy. The clinical workup is variable and depends on multiple factors, but imaging modalities (radiograph, CT, rhinoscopy/nasopharyngoscopy) and histopathology can be high-yield diagnostic tests.

Aspergillus spp. comprise a large genus of saprophytic fungal organisms found pervasively in the natural environment. A subset of this group are opportunistic pathogens that cause disease in humans and companion animals primarily through inhalation of aerosolized spores. Aspergillosis is uncommon to rare in dogs and cats, but upper respiratory infection is the most frequently encountered form of disease and may be invasive or non-invasive (Greene, 2012).

Aspergillosis in cats is caused by species from two subgenera, *Fumigati* and *Circumdati* that cause a spectrum of disease ranging from non-invasive sinonasal Aspergillosis (SNA) to invasive sino-orbital Aspergillosis (SOA) (Barrs, 2020). Immunosuppression, such as occurs secondary to Diabetes Mellitus, steroid administration, or systemic viral infection increases the risk of infection, but immunocompetent patients are also vulnerable. Persian cats appear predisposed to SNA or SOA, perhaps attributable to a heritable defect of innate immune function (Barrs, 2020, Whitney and Barrs, 2019). Definitive diagnosis of Aspergillus spp. infection typically requires adjunctive testing in addition to microscopic visualization, such as culture, serology, or molecular techniques and these are all available at CityU VDL.

The patient in this case had invasive FRS (presumptive SOA) based on histologic identification of fungal hyphae within the sinonasal submucosae. This is the most common form of Aspergillosis in cats (Tamborini, 2016). Clinical signs that may be associated with SOA such as exophthalmos, third eyelid prolapse, keratitis, or conjunctival hyperemia were absent, but submandibular lymph node enlargement is common. The lesion itself was primarily eosinophilic and macrophagic. Well-developed granulomas, reactive fibrous encapsulation, and fungal invasion of adjacent tissue and paranasal structures such as cartilage or bone were lacking in the submitted biopsy specimens. However, the lesion was consistent with reported histomorphology associated with SOA in cats, which can vary widely (Barrs, 2020). Histological assessment was also limited by the incomplete (incisional) nature of the biopsy.

In summary, Aspergillosis is an important differential diagnosis in feline patients presenting with sinonasal or peri-orbital disease. Although it can be considered uncommon to rare, the majority of fungal rhinosinusitis cases in cats are invasive. A firm diagnosis, appropriate characterization (ie. SNA versus SOA), and prognosis often depends on a combination of histopathology, imaging modalities, and adjunctive testing.

1. Dey MJ and VRD Barrs, Feline Sinonasal and Sinoorbital Aspergillosis Fumigatus Complex and Penicillium Infections (pp. 659-666) IN Greene (Ed.). 2012. Infectious Diseases of the Dog and Cat, 4th ed, Elsevier.
2. Barrs VR and JJ Talbot. 2020. Fungal Rhinosinusitis and Disseminated Invasive Aspergillosis in Cats. Vet Clin Small Anim 50, 331-357.
3. Whitney J, Haase B, Beatty J and VR Barrs. 2019. Genetic polymorphisms in toll-like receptors 1, 2, and 4 in feline upper respiratory tract aspergillosis. Vet Immuno and Immunopath 217, 109921.
4. Tamborini A, Robertson E, Talbot JJ, and VR Barrs. 2016. J Fel Med and Surg Open Reports 1-6.

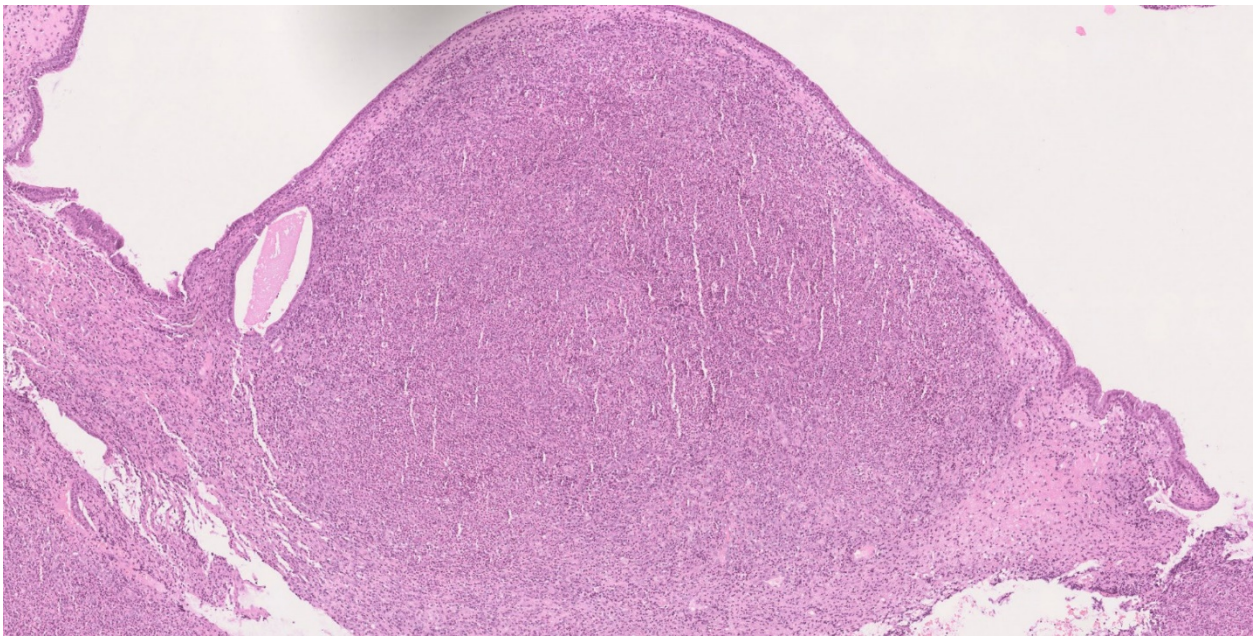


Figure 1 – low power view of the lesion showing the tissue expanded by inflammatory cells. HE stain 20 x

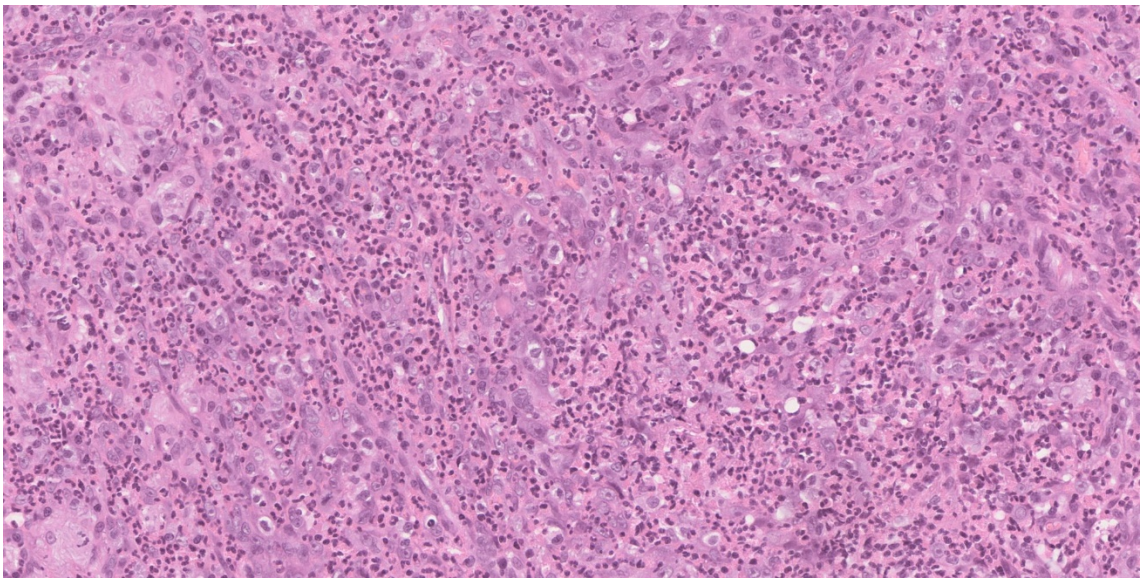


Figure 2 – Eosinophils predominate in the inflammatory infiltrate mixed with macrophages HE stain 100x

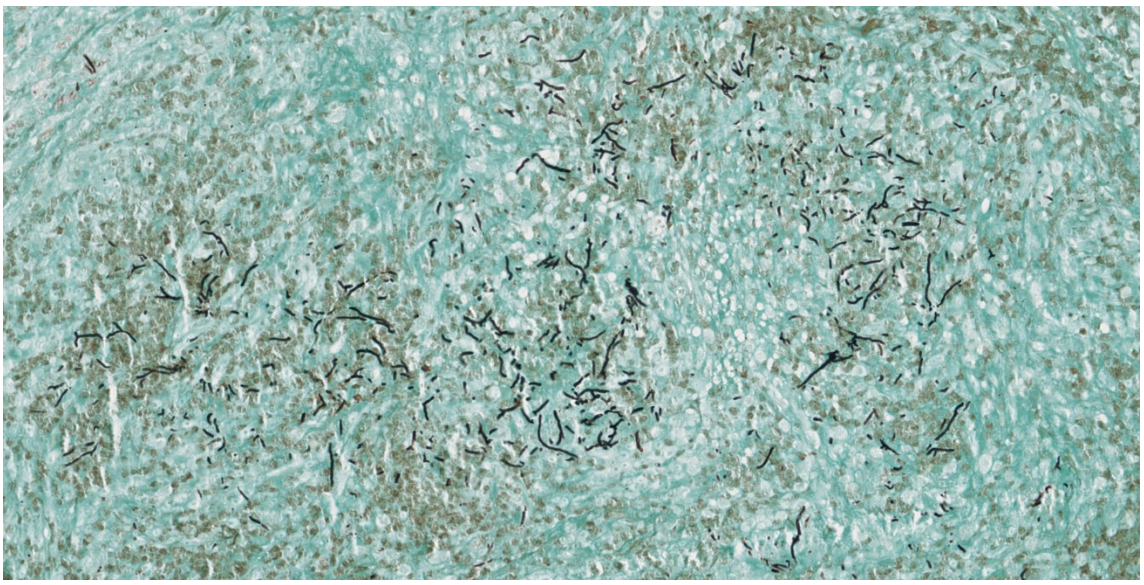


Figure 3 fungal hyphae stain black within the lesion Gomori's methenamine silver 100x

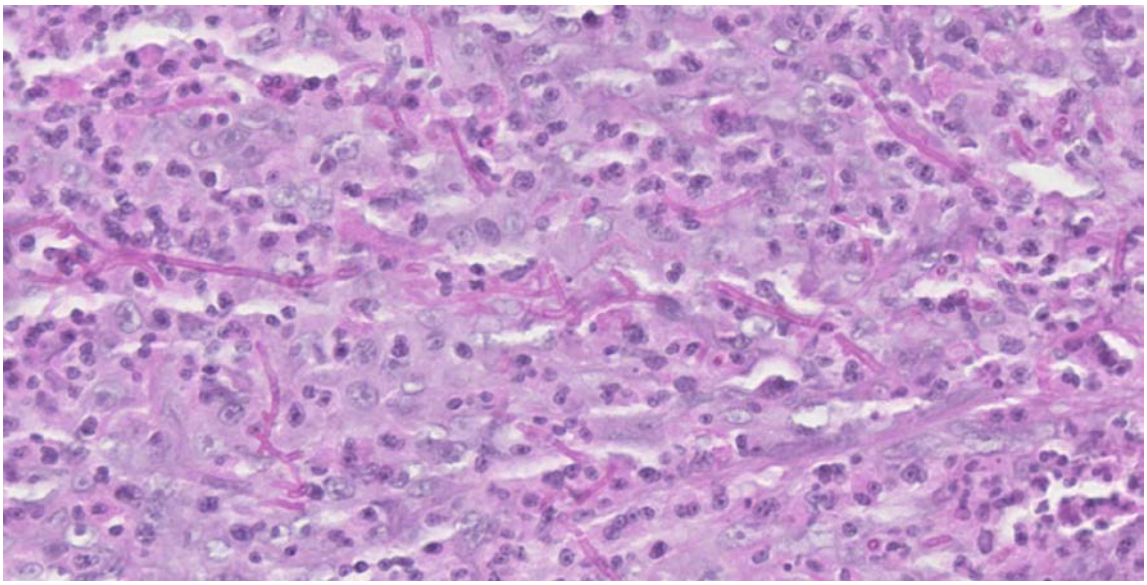


Figure 4 Elongated and branching fungal hyphae in the lesion Periodic acid-Schiff 400x

STAFF PROFILE

Accessioning section:

Dr Andrew Ferguson (BVSc, MANZCVSc, Team leader and Pathologist)

Miss Angela Lai (BSc, Senior Laboratory Assistant)

Miss Melody Yip (BSc, MLT II, Technologist)

Miss Cherry Leung (Laboratory Assistant)

Miss Christie Lai (BSc, Laboratory Assistant)

Mr Steven Flores (BS, Quality Assurance Manager)

Led by Dr Andrew Ferguson and supervised by Miss Angela Lai, the accessioning team performs a key role in the laboratory of safely and accurately checking the samples submitted, accurately entering the data into the laboratory information management system, distributing the samples to the sections and returning the results to clients, all the while answering the telephone and dealing with client inquiries.

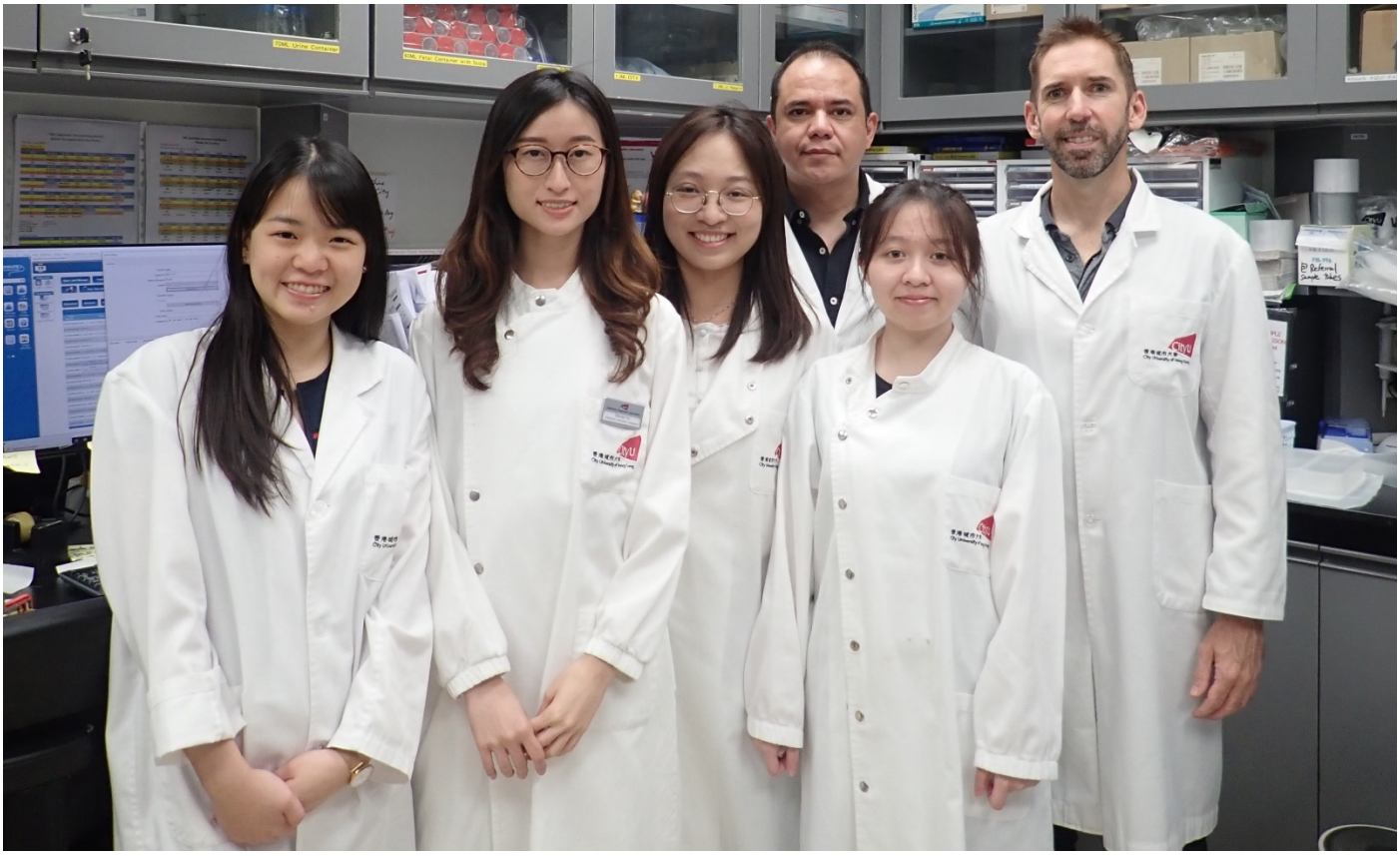


Figure 4: The CityU VDL accessioning team includes from left to right: Miss Christie Lai, Miss Melody Yip, Miss Angela Lai, Mr Steve Flores, Miss Cherry Leung and Dr Andrew Ferguson.

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