

The Next Generation of Diagnostic Tools – Putting them in practice today

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INTRODUCTION

Advances in technology and the ability to standardize testing methodologies have resulted in cutting edge diagnostic tests becoming commonplace in veterinary practice, particularly over this past decade. Significant strides have been made concerning the diagnosis of infectious diseases and there are even more advanced tests on the horizon. Screening tests for cancer and heart disease have also been developed that help veterinarians diagnose some of these conditions sooner, allowing for treatment to be initiated earlier and often resulting in better long term outcomes for the patient. Many of these tests will be discussed here, including a brief description of how they work, but emphasizing the application of these tests in clinical practice.

No discussion of diagnostic tests is complete without a description of **sensitivity** and **specificity**. These terms are critically important when evaluating the usefulness of a particular test in various situations, so it is equally important that we understand exactly what they mean.

SENSITIVITY: in general terms, when you want to be confident that you have RULED OUT a certain disease or condition, you want a highly *sensitive* test. In other words, you want the test to be really good at finding any evidence of the disease in the patient, so if you get a negative test result, you can be fairly confident that the patient does not have that condition. An easy way to remember what sensitivity means is that sensitivity has an “N” in it, so you want to be able to trust **NEGATIVE** test results; if a test has poor sensitivity and you get a negative test result, do additional tests to get a more definitive diagnosis. (“SnNout”)

e.g.: the low dose dexamethasone suppression test has 97% sensitivity, so if you get a negative test result you can feel pretty confident that you have ruled out Cushing’s disease.

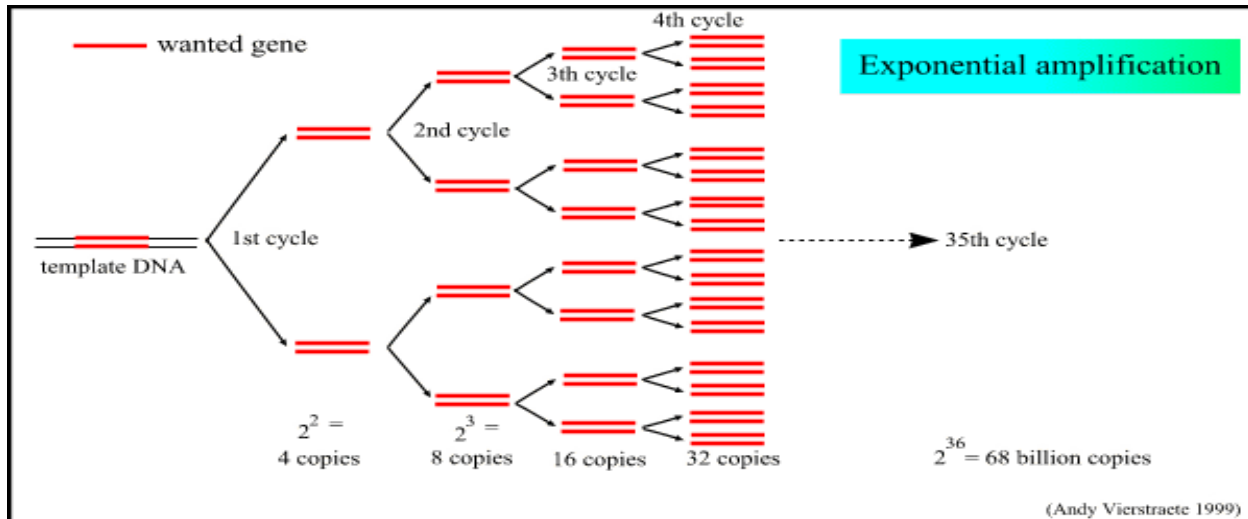
SPECIFICITY: in general terms, when you want to be confident that you have RULED IN a certain disease or condition, you want a highly *specific* test. This means you are minimizing the likelihood of false positive test results, so you would have good confidence that you have diagnosed a particular disease with a positive test result and it is unlikely that an animal would be treated inappropriately in this situation. An easy way to remember what specificity means is that specificity has a “P” in it, so you want to be able to trust a **POSITIVE** test result; if you get a positive test result on a test with poor specificity, either do additional confirmatory tests or proceed very cautiously with treatment. (“SpPin”)

e.g.: the bladder tumour antigen test has a specificity of 41% in animals with urologic disease; many conditions can give a false positive reading (significant glucosuria or proteinuria, pyuria, hematuria), so you should do additional confirmatory tests (abdominal ultrasound and cytology) before definitively diagnosing an animal with transitional cell carcinoma.

Tests for Infectious Diseases

PCR

- Stands for “polymerase chain reaction”
- An enzyme (called DNA *polymerase*) is used in a series of *chain reactions* to copy a specific portion of DNA unique to the organism that you are looking for
- Allows amplification of a tiny amount of DNA to an amount that can be detected and analyzed
- Highly sensitive and specific, but *a negative test does not definitively rule out that disease*
- Real-time PCR is better than traditional PCR—know what your lab is using!



- Tips for submission:
 - When submitting swabs, always use plastic-stemmed swabs (e.g. from Culturette tubes)
 - Submit all swabs dry, in a sterile container
 - Keep all samples (swabs, feces, secretions, biopsies) chilled while in transit to the lab

PCR tests for specific infectious diseases: when to use them and what to submit

- **Leptospirosis:**
 - An important differential diagnosis with acute renal, liver or even pulmonary disease
 - Collect samples **PRIOR** to antibiotic treatment (in some cases, as little as one antibiotic administration may be enough to give a negative test result)
 - Whenever possible, send in both urine **AND** whole blood to be tested (a significant number of cases tested at the IDEXX PCR lab were positive on only one of the two sample types; rarely were both urine *and* blood positive)
- **Canine Distemper**
 - Previous vaccination will interfere with serologic tests, so PCR is a great test to perform
 - A variety of sample types can be tested (conjunctival or deep pharyngeal swabs, whole blood, feces, CSF)

- The lab can *quantitate* a positive result so if the number of viral particles in the sample far exceeds the typical “background number” characteristically associated with a recent vaccination, you can assume the dog has a real infection
- **Feline Hemotropic Mycoplasma** (formerly *Hemobartonella*)
 - Review of a blood smear is NOT sufficient to rule out this disease! (In an internal IDEXX study from 303 anemic cats, only 10 samples were identified with FHM by trained technicians compared to 88 samples identified by PCR analysis)
 - Three types of FHM are screened for with this test (*Mycoplasma haemofelis* (large form), *Candidatus Mycoplasma haemominutum* (small form), *Candidatus Mycoplasma turicensis* (small form))
 - FHM is usually associated with regenerative anemias but is frequently a contributing factor in sick cats with non-regenerative anemias
 - All potential blood donors (clinic cats??) and FeLV/FIV positive cats with anemia should be screened with this test
- **FIV**
 - A great test to do as a follow-up to a positive screening test (e.g. in-house FeLV/FIV SNAP[®] test) in a cat with known vaccination history (so the positive screening test could just be antibodies to the vaccine) or when the vaccination history is unknown
- **FIP**
 - Please keep in mind that there is really no good ante-mortem test for FIP (i.e. you can't really trust a positive test result and you can't really trust a negative test result); the only definitive test for FIP is immunohistochemical staining on a biopsy sample
 - IDEXX can perform FIP PCR on body cavity effusions, whole blood, feces and CSF but a positive test may only raise your index of suspicion for the disease *in a cat with clinical signs characteristic of FIP*; PCR does not discriminate between true FIP cases and enteric feline coronavirus infection
 - A PCR test performed at the Auburn University College of Veterinary Medicine Diagnostic Lab focuses on detection of mRNA; the theory behind this test is that true FIP virus infects monocytes and macrophages and replicates in these cells; however, an independent study (Can-Sahna et al, [J Feline Med Surg](#). 2007 Oct;9(5):369-72) found this mRNA PCR test to have poor specificity (too many false positive results in healthy cats)
- **Other PCR panels**
 - Various PCR panels are offered for both dogs and cats to test for multiple infectious causes of diarrhea, respiratory diseases, fungal diseases and neurologic disease

Lyme Quant C6 test

- This test looks for antibodies to a particular protein found in the organism that causes Lyme disease (*Borrelia burgdorferi*); a dog will only test positive if it has been previously infected
- Previous vaccination does NOT interfere with this test (unlike IFA, ELISA, and Western blot)
- With ease of travel of companion animals and migration of birds that may carry tick vectors of this organism, Lyme disease should be a differential diagnosis in cases of arthritis and renal disease with protein loss, even in areas where the disease is not prevalent
- SNAP 4Dx or 3DX tests have a Lyme spot that looks for the same protein as the Quant C6 test but only gives a positive or negative result; it is recommended to follow-up a positive Lyme SNAP test with a Quant C6 test to determine if treatment is necessary and to have a baseline value to compare to in the future
- There is no specific test to determine if a dog has protective vaccination titers against Lyme, however Western blot analysis could be used to evaluate for vaccine-related antibodies



On the horizon: FISH

- Stands for “fluorescence *in situ* hybridization”
- Uses fluorescent probes of DNA (or RNA) that bind to complimentary portions of DNA (or RNA); the probe then gets incorporated into the chromosome and the fluorescence can be visualized using special microscopes
- Has been used in the research setting to detect certain types of bacteria that are embedded in tissue (e.g. “Boxer colitis” was found to be due to *E. coli* bacteria deeply invading the colon)
- This test is not yet commercially available

Tests for Neoplastic Diseases

Immunophenotyping for lymphocytosis (canine/feline) and canine lymphoma

- This test uses flow cytometry to detect certain cell markers that can determine which types of cells make up the population of cells being evaluated
- What is flow cytometry? In simple terms, cells “flow” one-by-one past a sensor; the sensor can determine what the cell is based on size (e.g. CBC analysis, differentiating RBCs, WBCs and platelets) or based on markers (like fluorescence) that can be attached to the cells
- Useful in cases with very elevated lymphocyte counts; this will help determine if there is one main type of lymphocyte making up the population (neoplasia) or many different types of lymphocytes (seen in reactive lymphocytosis, where the immune system is “reacting” to something)
- Can be helpful prognostically in cases of lymphoma to determine if it is T-cell or B-cell in origin (“**T** is **T**errible, **B** is **B**etter”)
- Flow cytometry has also become valuable in diagnosing mediastinal masses; these are most commonly due to either thymoma or lymphoma and the cells from these tumors (thymocytes and lymphocytes, respectively) have characteristic markers
- As more cell markers become validated in animal species, this test will become more widely used

Proliferation Profiling for Canine Mast Cell Tumours

- Mast cell tumours (MCT) can be relatively benign or highly aggressive, resulting in systemic disease
- Michigan State University has developed a “MCT Prognostic Panel” which evaluates three markers to assess risk for systemic disease:
 - *Immunohistochemistry for Ki67* – determines the number of cells in the sample currently proliferating
 - *Determination of the number of AgNORs* in neoplastic mast cells – this estimates the speed of cell proliferation
 - *Immunohistochemistry for PCNA* – determines the cell-cycle phase of proliferating cells
- A PCR test is also performed to assess for mutations in the c-kit gene; cancers caused by c-kit mutations tend to be highly aggressive but respond well to tyrosine kinase inhibiting therapies (e.g. Palladia)

Bladder Tumour Analyte Test

- Used as a screening test for transitional cell carcinoma (TCC)
- Specificity is poor (41% in animals with urologic disease) but sensitivity is high (90%)
- Never use this test to diagnose TCC; use abdominal ultrasound combined with traumatic catheterization to get cytology samples as a confirmatory test for this neoplasia
- A useful application might be to screen geriatric HEALTHY breeds of dogs predisposed to TCC (ScottiEs, WestiEs, sheltiEs, beagles, wire-haired fox terriers) during their annual wellness exams

Tests for Cardiac Disease

Cardiopet® pro-BNP

- This is a cardiac biomarker that is increased in the blood of dogs and cats with heart disease
- Becomes elevated in response to increases in intracardiac hydrostatic pressure, increased cardiac wall stress, angiotensin II, myocardial hypoxia, and increased sympathetic tone
- Can act as a useful screening test for heart disease in geriatric animals (e.g. 6% of cats with heart disease do NOT have a heart murmur...)
- Also used to help distinguish heart disease from respiratory disease (e.g. the coughing patient...)
- An echocardiogram is strongly recommended with any elevated Cardiopet pro-BNP result

Cardiac Troponin I (cTnI)

- Another cardiac biomarker that gives an indication of myocardial damage
- A commercially available immunoassay for dogs, cats and horses is available
- May be useful in evaluating at-risk dogs for heart disease (e.g. Doberman pinschers, dogs with hemangiosarcoma (to identify cardiac involvement), animals with pericardial effusions)
- Some studies have shown too much overlap in the cTnI value between healthy and sick animals to be diagnostically useful in some disease conditions
- In some conditions, this test may be more useful prognostically