Diagnotic Tests — User Error in Individual Bird Medicine
"A Bird in the Hand is Worth Two in the Sink"

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How to perform a "Sink Test"*

Step 1 *Collect a blood sample*
Step 2 *Look at it carefully (preferably under a good light)*
Step 3 *Pour the blood down the sink*
Step 4 *Write report with the diagnosis "no significant abnormalities found"*

** PLEASE NOTE: This test was developed in Australia and has no clinical or research value whatsoever.*

Introduction

Client expectations have increased. Many clients want a report to confirm the diagnosis given by the clinical veterinarian. If cost to the client is not a problem then blanket testing can, in most cases, aid diagnosis and cover the veterinarian from litigation. There have been many discussions on which test and in which order these tests will give the best results. The integral idiosyncrasies of each type of test have also been widely discussed. However there is very little practical information on intrinsic test problems due to veterinary and laboratory error on sample utilisation and interpretation. When results are printed, owners treats them as complete, foolproof facts. It is therefore important to recognise our own practical interpretation errors.

There are many refereed journal articles and proceeding papers on the problems with certain tests. There are many on the use of a good history and physical examination. The other area is to make sure that these tests, once chosen and the results are revealed, can be utilised in the best possible manner. However most interpretation of tests, aside from what is in the textbooks, is open to variations. It is fine to quote a price for a test and run it. But one problem is that once you have the results, what errors could have crept into the test? Another problem is that due to a failure of the test to run correctly, you have no written results to give the owner.

Failing to proceed and understand the test can turn it into a "SINK TEST".

The following is a list of practical problems with some tests in use and interpretation.

This is not a list of a test's relative sensitivity and specificity nor is it a complete list but rather some common practical concerns our clinic has experienced.

Chlamydia in the live bird — the relatively new tests in Australia

(a) **Immunocomb test**

- Immunocomb is IgG – yet only IgM for up to first 1-2 weeks.
  So may not be useful if want to perform a new bird check if from pet shop as it may still be negative for IgG.
- Control can be quite a light grey. Most results may also have a light grey positive spot. These light results are hard to interpret.
- Test has one pair of forceps for all tests in the kit thereby risking contamination.
- Not individual tests kits — have all tests on same kit holder.
  Problem of placing the test in the wrong sink hole.
- Have to be heated to 25°C but hard to verify.
  We place it on top of Clearview incubator to heat it up.
- Does not answer the owners' common zoonotic question of "Is the bird shedding?"
- How long can blood be stored after collection?
  Calls to Labdiagnostics and Biogal: unsure and suggest a "few days".
- Always more difficult at house calls to take blood then a swab.
- Ab test may need rising titre so low results may be positive but hard to explain to owner who lives far away and may have to come back.

(b) PCR — see Section 2

(c) Clearview (not a new test)

- Many commonly perceived faults. Cheap, simple but not reliable.
- Does give an idea of whether a bird is passing chlamydia.
- Needs gram stain as well and preferably only conjunctiva and choanal swab rather than faecal.

3. PBFD

(a) Ag Ab needs growing feather for antigen.
Need to recognise that some carriers may be negative.

(b) PCR — see Section 2.

4. PCR — see Section 2

5. Gram Stains

- Poor for megabacteria
- Fungi often missed
- Does not show mycobacteria
- Stains hard to replicate and thickness is personal
- Culture and Sensitivity — in Australia commonly have varying results to the gram stain
- Unless video microscope used, clients often have no concept of the test as there is no written result.

6. Culture and Sensitivity

- Labs are very good at reporting many results as streptoccocus.
- Small amounts lost on preservative medium in transport medium. (Try restaining the same sample using the culture medium swab as results often differ.)
- Overseas recommend cloacal swabs yet seems to result in no bacteria.
- In-house cultures hard to interpret and do not usually have the technical ability to utilise inhibition and promotion media products to allow certain bacteria to grow.
- Anaerobic cultures —
- can be of questionable value in Australia;
- not many veterinary clinics have the transport mediums; and
- labs often poor correlation with clinical expectation
- Labs non-specific concerning microbes being cultured as there is no commercial exotic animal laboratory in Australia.

7. Biochemistry

Underlying problem of small quantity of blood does not often allow repeat test if there is an error

In-house

- **Reflotron**
  - Reliability?? — possibly harder quality control
  - Good as with lack of dilution
  - No protein
  - No bile acids — often cannot assure owner there is no liver problem. Especially fatty liver.
  - Often no blood left to do bile acids at laboratory.

- **Idexx** a problem in-house to dilute.
  - Dilution in-house how many people using micropippette tubes.
  - Lipaemic samples can lead to sample rejection. Quite common in birds on fatty diets.
  - Amylase rejected in lipaemic samples and often seems to be lipaemic and high amylase.
  - No bile acids — often cannot assure owner there is no liver problem. Especially fatty liver. Often no blood left to do bile acids at laboratory.

Individual Tests

- **Dilution** in general pushes to limits of the reagents. Some tests are known to be affected at some dilutions but many still unknown.
  - Uric acid suspect on dilution
  - Calcium and protein definitely affected by dilution

- **Protein** tests very poor in-house using refractometer.

- **Amylase** very dependent on the lab fudge factor eg At IDEXX/VPS the fudge factor is 2.5x (IDEXX/VPS figures come out considerably higher than Idexx and Reflotron).

- **Glucose** dependent on time to laboratory and stress. Do urine glucose as well. Never call a bird diabetic on one sample without full profile and haematology for underlying inflammation.

- **Bile acids** use a lot of blood so may not leave enough blood for full biochemistry.

- **Injections affects blood sampling.** S/C fluids and I/M injections (especially Psittavet) can affect CK and AST. AST and CK can go up easily in handling.

- **Cholesterol** up when fasting according to Sue Jaesch (or is it?)

- **Cholesterol** up in breeding birds.

- **GLDH** still on laboratory profiles but needs more investigation

8. Haematology

- Every user different result.
- Large lymphocyte compared to monocytes.
- Lab different result and have trouble with smudge cells.
- Most labs in Australia do so few that there is no technical expertise.
- Coverslip to slide method is best — easier for laboratory technicians and in-house staining, storage and reading.
- Coverslips from many companies in Australia are very greasy and will smudge the cells. Need to wipe clean slide prior to use.

9. Urinalysis

- Well recognised problems with usefulness of urine sticks.
- Microscopic analysis — all results need to be interpreted in the light of cloacal contamination.

10. Cytology

- See 8. Haematology. Though more room for error in interpretation.

11. PCR

- **What are the problems with PCR testing at present?**

i. Poor perceptions of quality control in Australian labs.
ii. Over-diagnosis of disease using one positive result.
iii. Poor understanding of the disease epidemiology by the commercial labs as no veterinarians are involved. This lack of veterinary input can lead to the extraction of incorrect tissues and misdiagnosis of disease on poor results.
iv. Poor understanding of PCR technology by veterinarians leading to poor sample collection and incorrect disease treatments and prognosis on the results of a PCR test.

- **What can PCR testing do for us?**
  - Highly sensitive to nucleic acid
  - Highly specific to the selected nucleic acid
  - Highly accurate for determining if an infectious agent is present

- **Chlamydophila tests**

  - Highly specific and useful.
  - Compares to the gold standard of culture yet needs only very small amount of DNA to be positive in a PCR compared to culture. Can be used to see if an animal is shedding even minute amounts of a pathogen.
  - Faecal samples can have DNAses (enzymes-like material that destroy DNA) to chlamydophilosis. This can make a test negative. Choanal and conjunctival samples are best. If faecal samples are taken, these may need to be extracted and amplified separately to choanal and conjunctival samples.
  - Blood samples are at present poor samples for chlamydophilosis testing, as there are poor levels of bacteraemia with latent infections. This may be due to the particular life cycle of chlamydophilosis. There is still some concern that a test for chlamydophilosis using the correct samples may still not be 100% accurate due to the many strains of chlamydophilosis.
  - *The test is useful if the bird:*
    - is about to be stressed eg transport
    - is going into zoonotic exposure area
    - is being placed with other birds in closed environment situation

If there is a negative result on sick bird is unlikely to be chlamydophilosis ie **few false positive in sick birds**.

  - *The test is not useful as it:*

    - does not distinguish negatives to test
    - does not distinguish between dead and live DNA

- will not give a level of exposure
- suggests exposure and possible shedding but may be false positive when concerns disease clinical signs
- relies on time factor for results. Often need results a soon as possible.

- **PBFD Tests**
  - PCR testing for Psittacine Beak and Feather Disease (PBFD) is highly sensitive and useful.
  - There are some veterinarians who claim PBFD is a diagnosis made on clinical symptoms, however a disease profile is a must for legal reasons and for a true diagnosis. A negative PCR can usually rule out PBFD from feather picking or other underlying diseases. There are also diseases associated with PBFD eg megabacteria.
  - A PCR test for PBFD is usually performed on blood and feathers with the feathers crushed into the blood. If possible, ask your lab to run the blood and feathers separately to decide if the patient has viraemia. If negative on blood it may be environmental contamination on the feathers.

  - *This test is useful as it:*
    - is very sensitive so should pick up a positive exposure in a young bird
    - can pick up if bird has been in the presence of PBFD and so warn owner to watch for disease
    - is most useful in birds not showing any signs of PBFD
    - often at retest worth doing HA HI or doing HA HI on other birds in contact to see if have any resistance
    - can be used on birds with no blood feathers

  - *This test is not useful as it:*
    - often uses feathers and blood which can lead to false positives as feathers may have contamination of viral particles (from bird shop etc)
    - does not give level of exposure
    - does not give indication of ability to survive or become carrier
    - is possible DNA is not conserved between species leading to possible poor amplification (eg lorikeets)

- **Polyoma Tests**
  - Specific and useful as equal to any gold standard for Polyoma.
  - Because of the course of the shedding of the virus temporally PCR may be able to determine where in the course of the disease the bird is (ie are viral particles in feathers blood or faeces). Especially in Polyoma disease positive does not always equal disease. Disease is in most species self-limiting and age specific so don't use for any sick bird. For most species there is no point testing after 16-20 weeks of age.

  - *The test is useful as it is:*
    - good for buying new young eclectus or asiatic
    - useful in cockatiels that are young and have multiple illness

- good for aviaries to decide on whether to stop breeding till disease has run its course
- useful in budgies at varying ages
- useful to distinguish PBFD from Polyoma in young cockatoo spp

- *The test is not useful as it:*
  - does not distinguish viral particles simply passing through in the faeces in a healthy immune bird
  - does not distinguish if cause of clinical signs
  - as can have polyoma with no clinical illness and may just be present in an ill bird with no effects

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