Avian Disease Testing: What's New and What's Accurate

Teresa L. Lightfoot DVM, DABVP-Avian

There are many laboratory tests available for evaluation of specific psittacine diseases. These tests can be an important tool in aiding in avian diagnosis when used and interpreted appropriately.

1. ***Chlamydophila spp*- Include *C. psittici*, *C. felis*, and *C. pneumonia*, formerly *Chlamydia psittici*.**
   National Association of State Public Health Veterinarians (NASPHV) defines confirmed case as :
   - Isolation of organism from clinical specimen
   - Identification of Chlamydial antigen by immunofluorescence of the bird's tissue
   - ≥ 4-fold change in serologic titer in 2 specimens from the bird obtained at least 2 weeks apart and assayed simultaneously at the same lab
   - Identification of the organism within macrophages in smears of the tissue stained with Gimenez or Macchiavello stain.

   A. Antibody tests: Indicate exposure to the organism with host immune response
      1. IFA (Immunofluorescent antibody Testing)
         Samples required-Serum
         Technique-Fluorescent-labled anti-chlamyophila antibodies bind to host antibody
         Notes-Polyclonal antibodies used, in some species titers may be falsely low
      2. CF (Compliment fixation)
         Samples required-Serum
         Technique-Compliment binds to ABY-AG complexes in positive samples
         Notes-May be more sensitive than IFA
      3. EBA (Elementary body agglutination)
         Samples required-Serum
         Technique-Detects anti elementary body IgM, elementary bodies are infectious form
         Notes-May be useful in detection of early infection

   B. Antigen tests: Indicates presence of chlamydial antigen in host
      1. IFA (Immunofluorsecent antibocy test)
         Samples required-Serum, tissue impression smears
         Technique-Similar to antibody test
         Notes-Useful in combination with other tests, false negatives and positives possible
      2. ELISA (Enzyme-linked immunosorbent assay)
         Sample required-Serum
         Technique-Enzyme-activated color change caused by the presence of AG-ABY complexes
         Notes-Developed for human testing of C. trachomatis, false positives& negatives occur

   C. DNA tests
      1. PCR (Polymerase chain reaction)
         Sample required-Whole blood, choanal, cloacal, or fecal swabs
         Technique-Amplification of target DNA to produce detectable levels
         Notes-Sensitive and specific, method not standardized, does not indicate live organism

   D. Isolation of organism
      1. Culture
         Sample required-Infected tissue(liver) or exudates(choanal swab)/excrement (feces)
         Technique-Organism grown in tissue culture or chicken embryo
         Notes-Sample must contain organism, difficult to culture, special requirements for shipping and handling
      2. Hisopathology
         Sample required-Infected tissue (biopsy or necropsy samples)

Technique-Micrscopic identification in tissues using special stains
Notes-Best in post mortem diagnosis, samples must contain organism

2. **Proventricular Dilitation Disease (PDD)**: Recent research has demonstrated compelling evidence of avian bornavirus (ABV) etiology
    A. Histopathology-Characteristic lesions suggestive of disease
        1. Biopsy
        Sample required- Full thickness crop or proventricular sample containing vessel and associated nerve ganglia
        Technique-Microscopic demonstration of lymphoplasmacytic ganglioneuritis
        Notes-Proventricular biopsy may be contraindicated due to poor healing, negative results do not rule out disease
        2. Post mortem biopsy
        Samples required-Proventriculus, crop, brain, possibly adrenal glands, heart, spine, and mesenteric plexus
        Notes-Most useful in multi-bird households or aviaries
    B. DNA Probe-Identifies ABV DNA
        1. PCR (Polymerase chain reaction)
        Samples required-Whole blood, verify with lab before sending
        Technique-Amplification of bornavirus-specific DNA sequence to produce detectable levels
        Notes-Still under development, best used in conjunction with clinical and radiographic signs, as well as crop biopsy for ante-mortem diagnosis
3. **Polyoma Virus**: Clinical disease occurs in young psittacines
    A. Serology-Useful in documenting exposure, does not indicate viral shedding
        1. Antibody titers
        Samples required-serum
        Technique-Virus neutralizing (VN) or ELISA, contact lab for specific method
        Notes-Vaccination will not interfere with test in neonates (no production of neutralizing antibody); adults do, however titer usually lower than those after infection
    B. DNA Probe-Identifies Polyoma virus DNA
        1. PCR (Polymerase chain reaction)
        Samples required-Whole blood, cloacal and choanal swabs
        Technique-Amplification of avian polyoma DNA sequence to produce detectable levels
        Notes-Swab PCR may be more useful in detecting shedding, both PCR and serology together of most diagnostic value
4. **Psittacine Beak and Feather Disease (PBFD)**: Avian circovirus, two variants (PsCV1, PsCV2) vary in pathogenicity
    A. DNA Probe-Identifies avain circovirus DNA
        1. PCR (Polymerase chain reaction)
        Samples Required-Whole blood, environmental swab
        Technique-Amplification of PsCV DNA
        Notes-Some labs use sequence common to both, others specific for each variant, birds may still shed virus after PCR negative until next molt, positive PCR in clinically normal birds should be retested in 3 months
    B. Histopathology
        1. Feather biopsy
        Samples required-Blood feather with surrounding skin
        Technique-Microscopic demonstration of viral inclusion bodies
        Notes-Positive birds remain infectious until affected feathers have molted
5. **Aspergillosis**: *A. fumigatus* and other species ubiquitous in the environment can cause infection

- Multiple tests exist and definitive diagnosis may be difficult
- Tests run as a panel increase likelihood of diagnosis in infected patients

A. Antibody titers
   1. ELISA (Enzyme-linked immunosorbant assay)
      Sample required-Serum
      Technique-Enzyme-activated color change caused by the presence of AG-ABY complexes
      Notes-Many infected birds have negative or weak positive titers

B. Antigen titers
   1. ELISA (Enzyme-linked immunosorbant assay), Antigen capture assays
      Sample required-Serum
      Technique-Similar to antibody technique
      Notes-Infected birds may have negative or weak positive titers, strong positive antigen titers often associated with negative antibody titers
   2. Galactomannan-Dominant *Aspergillus* antigen
      Sample required-Serum
      Technique-Commercial assay, reported as an index
      Notes-Positive results depend on course of and location of infection, repeated testing may be necessary

C. Culture, cytology or biopsy may also yield diagnosis, but may be difficult to obtain

6. **Mycobacterium**: Several species capable infection, including *M. avium* and subspecies, *M. intracellulare*, *M. genevense*, difficult to diagnose

A. Cytology
   1. Acid-fast staining
      Sample required-Feces
      Technique-Acid-fast stains used to identify organisms
      Notes-Must be distinguished from non-pathogenic saphrophytes, may not be detected in fecal samples

B. Histology
   1. Biopsy or post-mortem diagnosis
      Samples required-Infected tissue (often GIT)
      Technique-Demonstration of acid-fast organisms in paraffin-embedded tissues
      Notes-Samples may be difficult to obtain ante-mortem

C. Organism isolation
   1. Culture
      Samples required-Infected tissue or fecal sample
      Technique-Mycobacterial or other special media used to grow organism
      Notes-Takes several months to grow, many labs do not offer this test

D. DNA probe
   1. PCR (Polymerase chain reaction)
      Samples required-feces, fresh tissue, paraffin-embedded tissue
      Technique- Mycobacterium DNA sequence to produce detectable levels
      Notes-Can differentiate between species/subspecies and much quicker than other tests, false negative if organism not present in sample