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May 1, 2008, AHL User’s Guide and Fee Schedule

A few points of note about the latest edition:
The User’s Guide is posted on both the public side and the clients-only side of the AHL website.
The Fee Schedule is available only on the clients-only side of our website, at http://www.labservices.uoguelph.ca/customer-login/index.cfm. Please see below for client log-in sign-up information.

Equine tests can be found in the Companion/other animal tab.

For clients in specialty practice, we would be happy to provide the Fee Schedule for you sorted by test name, method, etc., within your species of primary interest. Please contact Helen Oliver at (519) 824-4120, ext 54538.

We’ve minimized price increases, but please consult the new Fee Schedule before quoting prices on tests. Many of the sampling / comments (usual days tested) fields have been modified, and many reflect pricing changes. Almost ALL tests are now priced per test (except for Virus isolation on food animals, and Egg inoculation for both avian and mammalian virology).

**Bacteriology setup charge** applies only to: 1) Bacterial culture & susceptibility; companion/other, Food/fiber producing animals, and 2) Bacterial culture; abortion case, and Abortion case with Campylobacter culture.

**Cremation, private, return** has been added at $20.00.

**Histopathology** charges are now inclusive of slide costs.

**IHC panel set up charge** applies to IHC, Bovine abortion panel or IHC, Porcine respiratory panel. Other IHC charges are all per slide.

**Mycoplasma set up charge** - $15.00 applies only to: Mycoplasma culture, food. **AHL**

AHL LIMS & courier update

*Jim Fairles*

We have recently done a small upgrade to our LIMS (Laboratory Information Management System, aka “Sapphire”), so the on-line “look” of the program has changed. This program supports several features:

1. **On-line access to results** – Please contact us for a user name and password. Advantages:
   - Long-term access to results – repository of data.
   - Robust searching capability.
   - Backup for email and fax reports.
   - Ability to view results from any Internet-based computer.
2. **On-line submission of specimens.** You can log-in your case directly, rather than filling out a submission form. Advantages:
   - Case is coded as “In Transit” and we’re alerted.
   - Avoids duplication of paper form - form is printed to send with specimens.
   - Control of syntax, spelling and tests ordered.
   - Case number is known before sending specimens.

**Couriers** – AHL has several options for overnight and same-day couriers – please call for availability.

Purolator Courier – overnight – all Ontario (incoming collect, must order waybills from AHL).

Mississauga - Oakville - Burlington - Hamilton Guelph and area, Kitchener, Waterloo, Cambridge
**Clostridium perfringens**: Avoiding diagnostic confusion  

*Durda Slavic, Josepha DeLay*

Selection and interpretation of diagnostic tests associated with *Clostridium perfringens* can be very challenging, partly because of the number of different tests available. In addition, *C. perfringens* is considered a part of normal gastrointestinal flora in variety of animals and in humans, and it is also a relatively common organism in the environment. Not all *C. perfringens* isolates, however, have equal pathogenic potential. The pathogenic potential of *C. perfringens* is well correlated with the production of different exotoxins. Most practitioners are familiar with the four major exotoxins of *C. perfringens* (Table 1). These exotoxins were used to develop the toxin typing scheme for *C. perfringens* that is still widely used (i.e., types A, B, C, D, E). These exotoxins are also mainly responsible for the many deleterious effects caused by *C. perfringens*, such as tissue necrosis, hemolysis, and local neurologic dysfunction. Recently, 2 additional toxins; beta 2 and enterotoxin, have been shown to contribute significantly to *C. perfringens* disease. Beta 2 toxin is associated with diarrhea in young pigs, whereas enterotoxin is known to cause food-borne diarrhea in humans and enterocolitis mainly in dogs and horses. One should keep in mind that beta 2 toxin and enterotoxin are not used for the toxin typing scheme of *C. perfringens* and as such are not associated with any particular type. For example, the beta 2 toxin gene can be found in type A as well as in type D *C. perfringens*.

Continued on p 11

<table>
<thead>
<tr>
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<th>beta</th>
<th>epsilon</th>
<th>iota</th>
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<td>-</td>
<td>-</td>
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<td>+</td>
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Table 1. *Clostridium perfringens* toxin types, associated diseases, and species affected.
**Clostridium perfringens:** (continued from p. 10)

In order to properly diagnose *C. perfringens* disease, request anaerobic bacterial culture or specify on the submission form that *C. perfringens* is suspected. The growth of *C. perfringens* on fecal culture, however, is not enough to confirm its significance. It is the toxin type that determines the significance of *C. perfringens*. For example, in a clinical situation with sudden unexpected death in lambs, genotyping of *C. perfringens* isolated from intestine is necessary to differentiate between type A (potentially normal flora) and type D (responsible for ‘pulpy kidney disease’ or ‘enterotoxemia’, and not a normal contributor to enteric flora). Genotyping of *C. perfringens* isolated in large numbers from the intestine of a foal with diarrhea will allow differentiation between *C. perfringens* type A and the less common type C. Genotyping will also identify beta 2 toxin gene presence in *C. perfringens* type A isolates, which is associated with (but not definitively causal of) neonatal diarrhea in pigs.

The enterotoxin gene (*cpe*) can also be detected by genotyping. The presence of enterotoxin, however, can also be detected by ELISA in feces and intestinal content. The main difference between ELISA and genotyping detection of enterotoxin is that genotyping will detect the presence of gene (*cpe*) but it won’t confirm if the enterotoxin (an actual protein) is made, whereas ELISA will confirm if enterotoxin is being made and is present in the diagnostic material. A positive ELISA result will leave no doubt of the presence of enterotoxin and is a significant finding, whereas the presence of the gene (*cpe*) alone can be of questionable significance because genes are not always expressed and proteins are not always made. For diagnostic purposes, it is also important to differentiate between enterotoxin and enterotoxemia of sheep. Despite the similarity in name, enterotoxin is NOT involved in enterotoxemia of sheep and goats due to *C. perfringens* type D (‘pulpy kidney disease’), and *C. perfringens* enterotoxin ELISA is NOT a useful test for the diagnosis of ‘pulpy kidney disease’. For suspected cases of ‘pulpy kidney disease’, please request bacterial culture with genotyping of *C. perfringens*, if isolated.

Genotyping is also recommended if commercially available or autogenous vaccination is considered. The main components of these vaccines are the various exotoxins. It is important to buy vaccine that will contain most and/or all of the exotoxins detected in the clinical isolates. Otherwise, the vaccine will not confer full protection against the targeted organism.

At present, the most common toxin types detected by our laboratory are *C. perfringens* type A, type A with beta 2 toxin, and type D in caprine and ovine species. *AHL*

**References**
Songer JG, Post KW. Anaerobic gram-positive rods and cocci, the genus *Clostridium*. In: *Veterinary Microbiology, Bacterial and fungal agents of animal disease*. Elsevier Saunders: St. Louis, MO. 2005:261-282.

## Minimum sample volumes for biochemistry testing

*Kristiina Ruotsalo*

‘Is there enough sample?’ This is a common client enquiry fielded by the technologists in our clinical pathology laboratory. The answer of course depends upon what range of biochemistry testing is required.

In general, 1 mL of serum is sufficient for full canine, feline, equine, and bovine biochemistry panels. For pocket pets (ferrets, guinea pigs, rabbits, hamsters) a minimum of 0.5 mL of serum or 0.5 mL of heparinized plasma is required for a full biochemistry profile.

For birds and reptiles, 1.5 mL of heparin blood or 0.5 mL of heparinized plasma is sufficient for full biochemistry profiles.

For extremely small avian and reptilian patients and samples, a Vetscan biochemistry profile can be performed on 0.2 mL of heparin blood or heparinized plasma.

If certain biochemical tests are of greater clinical importance, please prioritize them on the submission form. *AHL*
AHL Lab Reports

RUMINANTS

Caprine arthritis encephalitis virus (CAEV) antibody detection ELISA is now available for goats at the AHL

Susy Carman, Jim Fairles

The AHL now offers the IDEXX ELISA for the detection of antibody to CAEV in goat sera. The test will be reported as Value %, and classified as negative (Value % < 30%), suspect (Value % > 30% to < 40%), or positive (Value % > 40%). Send 1 mL of serum. The test is normally run on Tuesdays. The cost is $7.50 per test.

This test is not suitable for use with sheep sera for detection of antibody to Visna/maedi virus (VISNA - MVV). Unique antigenic strains of MVV are present in Canada, and antibody to these unique strains is not identified by either IDEXX or VMRD CAEV/MVV ELISA kits. Sheep sera should be forwarded to the CFIA for testing in ELISA using MVV strains specific to Canada.

For more information contact Dr. Susy Carman, 519-824-4120 ext 54551, scarman@lsd.uoguelph.ca.

Sudden unexpected death in cattle

Jan Shapiro, Beverly McEwen, Brian Binnington

Between January 1, 2001 and December 31, 2007, 51 beef cattle and 44 dairy cattle submitted to the Animal Health Laboratory in Guelph and Kemptville for necropsy had a history of sudden death. In this article, “sudden death” is defined as being unexpected, the animal being found dead with no recent clinical signs having been observed.

The gastrointestinal system was the most common body system involved in sudden death, involving 27% of beef cattle and 20% of dairy cattle cases, with all age groups affected. The most common diagnoses were primary rumen bloat, perforated abomasal ulcer, acute mesenteric torsion, traumatic reticuloperitonitis, torsion of the abomasum, and rupture of a liver abscess into the caudal vena cava with embolic spread to the lungs. There were single cases of impaction of the rumen and emaciation, necrotizing E. coli cholecystitis and cholelithiasis, with rupture of the gall bladder and septic peritonitis, and peritonitis due to puncture of the duodenum by a piece of wire.

Severe pneumonia was diagnosed as the cause of sudden death in 21% of beef cattle and 14% of dairy cattle cases. Most cases of respiratory disease had an infectious cause, with Mannheimia haemolytica and Pasteurella multocida the most frequently isolated bacterial pathogens, and BRSV the most common viral pathogen detected. Two dairy cattle that died within minutes to 6 h after vaccination had lung lesions consistent with an anaphylactic or anaphylactoid reaction. Two other cases had acute severe idiopathic pulmonary edema.

Cardiovascular system lesions were interpreted as the cause of sudden death in 8% of beef cattle and 14% of dairy cattle cases. Congenital heart defects were diagnosed in Holstein cattle aged 3 h, 1 mo and 2 yr respectively. Acute arterial rupture (uterine artery, splenic artery), idiopathic multifocal myocardial necrosis and fibrosis, and idiopathic myocardial or multisystemic arteritis were also diagnosed.

Emaciation due to inadequate nutrition was diagnosed in 10% of beef cattle cases, and no dairy cattle cases. Four % of beef cattle and 4% of dairy cattle dying suddenly had soft tissue and boney lesions of severe trauma.

Gross necropsy and histology results supported the history of death associated with being cast, entrapped or strangled by a neck chain or stanchion in 4% of beef cattle and 9% of dairy cattle. These cases were submitted to support an insurance claim for mortality. However, in a few bovine sudden death cases that were submitted for insurance, significant pre-existing or underlying disease, specifically acute pneumonia, acute mastitis and lymphosarcoma, was found.

For 10% of beef cattle and 20% of dairy cattle, there was no cause of death established, i.e., there were no gross or histological lesions, and no evidence of sudden death due to toxic or physical insults.

One dairy herd submitted 2 lactating cows from a group of 23, 16 of which died suddenly due to electrocution while tied to a head-rail in a new barn. A 20-day-old veal calf was diagnosed with severe acute myocardial degeneration and mineralization attributed to doxycycline toxicity.

While it is tempting for owners not to investigate sporadic cases of sudden death in cattle, it is important to note that 25% of cases submitted in the 7-yr period reported here died from unexpected infectious disease, most with implications for herd health or management. Also, in over 75% of cases, histologic examination and/or other laboratory testing was considered necessary to make or confirm a diagnosis and etiology, or to rule out the presence of disease not detected by gross examination.
AVIAN/FUR/EXOTIC SPECIES

Fatal infection with the giant liver fluke *Fascioloides magna* in a small group of fallow deer

Brian Binnington, Andrew Peregrine, Mary Lake, Douglas Campbell

In February 2008, a dead 3-year-old female and in March a 3-year-old male fallow deer were submitted to the Animal Health Laboratory-Kemptville for necropsy. A 2-year-old female fallow deer had died earlier, but was not examined. Four fallow deer were housed in a 1 acre enclosure with a covered shelter and trees but no standing water sources; deer were either found dead or had experienced a brief period of staggering gait, recumbency and death.

Necropsy findings for the 2 deer were similar. The female had sanguineous fluid in the thoracic cavity. Linear slightly raised red to black streaks were present over the pleural surfaces of the lungs. The male had sanguineous fluid in the abdominal cavity and extensive hemorrhage in the omentum and mesentery. Livers were moderately enlarged, mottled with red to black areas and a soft friable texture. Black tortuous linear streaks were present on the capsular surface (Fig. 1). On cut surfaces, there were tortuous black tracts, soft black circumscribed areas and cavities up to 3 cm diameter that contained dark red to black fluid or clotted blood. The hepatic lymph nodes were enlarged and black. Located within the sanguineous peritoneal fluid of the male were 27 trematodes (flukes) that were red-brown, elongated oval, dorsoventrally flattened and from 2-5 cm long and 1-2 cm wide (Fig. 2). Flukes were not identified in the fresh liver of the female but emerging flukes were found in formalin-fixed sections of liver.

Histopathology of the liver demonstrated extensive areas of hepatocyte coagulation necrosis with hemorrhage. Macrophages containing dark-brown to black, granular, birefringent pigments (pigmented macrophages) were present in areas of necrosis. Fibrosis and accumulations of lymphocytes, plasma cells and pigmented macrophages were present in portal regions. Localized pools of blood surrounded by fibrovascular tissues containing lymphocytes, plasma cells and numerous pigmented macrophages were present in the acinar parenchyma. One of these blood pools contained a fluke. Several flukes were forwarded to OVC Pathobiology and AHL-Guelph, and were identified as *Fascioloides magna* that appeared to be immature flukes.

The natural host for *Fascioloides magna* is the deer. In North America, the common definitive and reservoir hosts are wapiti (elk), white-tailed deer and caribou. In the definitive host, e.g., fallow deer in Europe, the liver infection is usually subclinical and the mature flukes are encapsulated in a fibrous capsule that communicates with the biliary system through which the eggs pass and are excreted in the feces.

The life cycle of *Fascioloides magna* in the definitive host is complex. Encapsulated adult flukes in the liver excrete undeveloped eggs via the biliary system and the feces. Eggs embryonate and miracidium larvae develop for 27-44 days until release from the egg. The ciliated miracidium penetrates a freshwater snail and develops through multiple stages. After 40-69 days, motile cercariae emerge from the snail and encyst on vegetation. Definitive hosts such as deer ingest the encysted metacercariae on vegetation and metacercariae emerge from the cyst in the gastrointestinal tract. Immature flukes penetrate the intestinal wall and wander in the abdomen to the liver and sometimes other organs. The time from infection with metacercariae to egg-producing adult flukes is 3-7 mo.

The fatal infection in these fallow deer is unusual, and the source of infection in these deer was not determined with certainty. The presence of infected snails and grasses on the small lot is possible, however, for the male that arrived in November 2007 and died in March 2008, this is an unlikely source of infection during the particularly cold snowy winter conditions during this period. The deer may have acquired the infection earlier on the source farm that had numerous deer, a large pond, and had previously housed elk from western Canada that are often infected with liver flukes. The female deer, however, had been resident since Jan 2007; therefore it is unlikely that immature flukes would have remained dormant until Feb 2008. The diet consisted of hay harvested from an arid pasture and cob corn, which are unlikely sources of encysted metacercariae.
Real-time PCR for the identification and differentiation of Porcine circovirus 2 strains 2A and 2B at the AHL Susy Carman

The AHL offers a quantitative real time PCR for the identification of PCV-2. For living animals, send serum or semen. Quantification (copies/mL) will be reported for serum. Send lung and tonsil for pigs presented for post mortem. Tissues from aborted fetuses can also be tested, including thymus, lung, liver, spleen, and lymph node. Tissues from the same animal will be pooled. The charge is $27 per test.

A second real time typing PCR is now available to differentiate 2A (RFLP 422) and 2B (RFLP 321) strains of PCV-2. PCR typing is available on request for PCR positive samples for an additional charge of $27 per test.

For more information contact Dr. Susy Carman, 519-824-4120 ext 54551, scarman@lsd.uoguelph.ca.

Porcine circovirus 2 antibody detection ELISA is now available at the AHL Susy Carman

The AHL now offers a competitive ELISA for detection of antibodies to Porcine circovirus 2. The results of this test will be reported as sample/negative (S/N) ratio, and each classified as positive (S/N ratio <0.15), doubtful (0.15 < S/N ratio <0.20) or negative (S/N ratio >0.20) for the presence of antibodies in sera. Titers can be determined using 4-fold dilutions of sera, with each dilution tested separately. Send 1 mL of serum. The test is normally run on Tuesdays. The cost is $7.50 per test.

For more information contact Dr. Susy Carman, 519-824-4120 ext 54551, scarman@lsd.uoguelph.ca.

Porcine circovirus 2 cluster identification 2004 – April 2008 Beverly McEwen, Josepha DeLay, Susy Carman, Murray Hazlett, Margaret Stalker, Jim Fairles

Porcine circovirus 2 (PCV-2) infection identified at the AHL by PCR and IHC has significantly decreased since 2005 (Figure 1). Temporal cluster analysis using time-space scanning software (1) revealed a significant high rate of positive cases in 2005 for both IHC and PCR (dotted ovals, Figure 1). Significant low rates of PCV-2 were identified by PCR in 2006 from September to December and by IHC from January to April 2008. Only the primary clusters are included in this article, however, there is overlap of secondary and tertiary, low and high rate temporal clusters for IHC and PCR (data not shown). From these data, it cannot be confirmed if this decrease was related to vaccination of swine for PCV-2 instituted in 2006. Trends in PCV-2 infection will be updated in future Newsletters.


Figure 1. Identification of Porcine circovirus 2 (PCV-2) of infection by PCR and IHC. Low rates of infection are designated by circles with a solid line and high rates by a hatched line; shading within the ovals surrounds the clusters identified by IHC.
Acute myelitis due to rabies in a vaccinated horse
*Murray Hazlett, Ruth Barbour, Alexander Wandeler*

A 15-month-old female thoroughbred horse was presented clinically as a tie-up 1 h after appearing normal and being brought into the barn. Caudal muscles were hard, and the horse became increasingly agitated. The use of detomidine (Dormosedan; Pfizer Canada, London, ON; 7 mg IV) to allow internal and external examination of the pelvis and internal organs, caused an exaggerated lack of coordination, but had no impact on this horse’s attitude, and it remained extremely agitated despite sedative doses. Over the next 42 h this progressed to extreme weakness, hyperesthesia of the hindquarters and tail area, hypermetria and a proprioceptive deficit that was severe in hindlimbs but milder in front. The horse was constantly kicking out with hind legs, and was unable to empty her bladder. After the first day, she exhibited extreme pain over her back, especially from sacrum region to the tailhead. By 24 h, the horse was unable to rise, and prior to euthanasia at 40 h she was unable to stand, despite desperate attempts to rise. The filly continued to kick violently in lateral recumbency during manipulation of the tail, bladder catheterization and rectal examination. She was sent to the AHL for a necropsy the day of euthanasia.

Significant findings at necropsy were confined to the spinal cord, where there was irregular dark discoloration with some hemorrhage in gray matter starting in the region of the first thoracic vertebra and extending caudally the remaining length of the cord. (Fig 1). Rule-outs included *Equid herpesvirus 1* (neurotropic), *Rabies virus, Sarcocystis neurona*, and (less likely due to the time of year) *West Nile virus* and *Eastern equine encephalitis virus*. Significant microscopic lesions were confined to this same region, and were characteristically severe nonsuppurative myelitis, most severely involving gray matter where there was also frequently hemorrhage, but also with some white matter involvement, especially adjacent to grey matter (Fig 2).

Frozen brain and spinal cord submitted to ADRI Nepean for rabies fluorescent antibody testing showed no staining in brain sections submitted, but strong staining in the submitted section of thoracic cord. Subsequent IHC staining of medulla oblongata sections showed a few infected neurons, with a few questionably affected neurons at the level of the vertebra C3. Cord sections at the level of T1 and L1 were both heavily infected.

Spinal cord smears were examined with indirect immunofluorescence using a panel of 16 different monoclonal antibodies. The selected panel provides distinctive reaction profiles for most of the recognized indigenous *Rabies virus* variants. The observed Mab results were consistent with the profile established for infections with the Arctic *Rabies virus* variant. Arctic *Rabies virus* infections were very widespread in foxes in the second half of the 20th century, but are now seen mostly in skunks in restricted parts of Ontario.

It is interesting that this horse had been vaccinated with a killed 3-year vaccine product at 4 and 5 months of age. Suspected failure of rabies vaccine in horses has been documented previously in a study of 21 rabid horses, where histories indicated that 5 had been vaccinated between 4 to 24 months prior to disease development. Newer third-generation DNA vaccines that are now used in horses for West Nile disease, and have been studied for use in equine rabies, may hold promise for better protection in the near future.

Efforts by the OMNR have greatly reduced rabies in Ontario to the point where in 2007 there were only 106 confirmed cases of rabies in Ontario (no horses). Six cases of skunk rabies (arctic fox strain) were diagnosed in the last quarter of 2007 in Wellington County. Although some coyotes had been seen in the area of the farm in this case, foxes or skunks were not. This case illustrates the need to consider rabies as a rule-out in any horse showing neurologic signs, regardless of vaccination status, and raises the question of whether spinal cord testing for rabies should be routinely considered in horses showing acute spinal cord disease, especially when humane considerations require euthanasia relatively early in the disease course. *AHL.*

References

Figure 1. Thoracic cord, level of T1, showing discoloration due to necrosis and hemorrhage (arrows).

Figure 2. Histology of figure 1, showing large mononuclear cell cuffs around blood vessels (arrows) with hemorrhage.
Canine leptospirosis update
Beverly McEwen, Davor Ojkic, John Prescott

In 2007, the proportion of *Leptospira* spp. seropositive dogs was the lowest in the past 9 years (Table 1), although from Jan-Apr 2008, the rate had increased to 17%. The marked reduction in submissions may reflect increasing confidence by veterinarians in their ability to diagnose infection, the effect of increasingly widespread vaccination against serovars *grippotyphosa* and *pomona* in reducing canine infection, weather conditions in the fall of 2007, or other factors.

Leptospirosis usually occurs in late summer and fall, however, seropositive dogs were identified during the entire year and were from all regions in Ontario, including the greater Toronto area. The infection is transmitted to dogs in rural, suburban and urban locales by reservoir species including raccoons, skunks, other dogs, mice or rats, with raccoons thought to be the main reservoir of infection for dogs in Ontario. A number of anecdotal and one published report have identified a few cases of human infection acquired from dogs in Ontario or Québec in the last 2-3 years.

Table 1. Percent canine serological diagnoses, AHL, by year, for all *Leptospira* serovars

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<th>Suspicious n (%)</th>
<th>Negative n (%)</th>
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<td>1266</td>
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<td>2007</td>
<td>699</td>
<td>81 (12)</td>
<td>172 (25)</td>
<td>446 (64)</td>
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*Data from 2005-2007 includes all canine serological testing for *Leptospira* sp. submitted to AHL including Ontario and other provinces.

*L. grippotyphosa* has recently replaced *L. autumnalis* as the most frequent serovar reacting in the microscopic agglutination test (MAT), and the frequency of seropositivity to *L. bratislava* has dramatically decreased (Table 2). However, deducing the infecting serovar from MAT titers for acutely infected dogs is highly problematic, because of extensive cross-reactivity of sera with different serovars. The range of serovars is used because it can sometimes help in interpreting seroreactivity, most notably in distinguishing immunization from infection.

Table 2. Percent seropositivity of canine sera to various *Leptospira* spp. serovars, 1998 – 2007

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<td><em>L. canicola</em></td>
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<td><em>L. hardjo</em></td>
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** - serology not done

Since May 1, 2008 we have modified our *Leptospira* MAT procedure to harmonize it with WHO recommendations. Furthermore, the same panel of seven reference antigens (*autumnalis, bratislava, canicola, grippotyphosa, hardjo, icterohaemorrhagiae, pomona*) is now used for all samples regardless of the sample species. Titer is the reciprocal of the highest dilution of the sample that reacted with a reference antigen. A positive MAT titer (100 or greater for serum samples) indicates the presence of antibodies against leptospires. Clinical significance of positive MAT titers must be interpreted in light of clinical presentation, vaccination history, supplementary laboratory results and other relevant information. **AHL**

References