



SEPTEMBER, 2011

## September Focus: In-House vs. Reference Lab Testing, Part II

*We hope you enjoy this issue of ANTECH Insights. Please pass it along!*

In our [July issue](#) of ANTECH Insights, we examined the economic trade-offs between in-house and reference lab testing. This month, we take a look at the other oft-debated side of the same coin: potential accuracy trade-offs between in-house and reference lab testing, examining the following questions:

- How does the overall accuracy and reproducibility of routine in-house lab testing compare to reference lab results?
- If there are significant differences, where are the greatest concerns in terms of medical impact?
- What steps can veterinary hospitals take to improve the accuracy of testing they elect to perform in-house?

As with our previous issue, where we presented study findings that illuminated significant cost differences between reference lab and in-house testing, we hope this issue provides food for thought regarding the best mix of testing modalities within your practice.

### ANTECH study reveals accuracy gaps in common in-house test procedures

In late 2009, ANTECH conducted a study to compare the most common tests performed by veterinary hospital technicians with comparable reference lab testing. The study focused on the following routine procedures:

- Clinical chemistry profiles
- CBC with differential (WBC count)
- Urinalysis with sediment evaluation
- Fecal ova & parasite analysis

**Study design.** Fifteen animal hospitals (2-4 DVM each) participated in the study, which replicated an external quality control/proficiency check. Test samples were sent to each hospital to be run and have results returned for evaluation versus baseline results determined by ANTECH. Ten (10) separate species-specific specimens (canine and feline) per procedure were sent to each hospital (150 total samples per procedure) over a 5-day period, along with six (6) urinalysis specimens per hospital (90 total urine samples). Additional aliquots of the same samples were sent to other ANTECH lab locations with similar handling for comparison to baseline results and to confirm sample stability.

### At a Glance:

[ANTECH study reveals accuracy gaps in common in-house test procedures](#)

Summary of study comparing in-house vs. reference lab testing accuracy

[Antech Data Corner](#)

Feline Abnormal High T4 as Function of Age

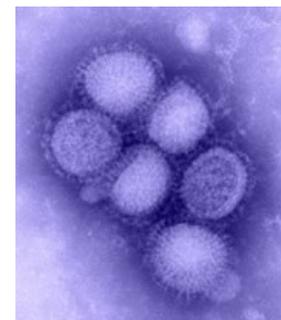
[H1N1 case detected by FastPanel® PCR](#)

Brief case history

### Case History:

**H1N1 Case detected by FastPanel® PCR**

H1N1 influenza (swine flu) has been reported in several companion animal cases from 2009 to 2011. The FastPanel® PCR Canine Respiratory Profile (test code: T995) tests for 11 respiratory pathogens, including three influenza virus strains: H1N1, H5N1, and H3N8 (canine influenza virus). The FastPanel PCR Feline Upper Respiratory Profile also includes the H1N1 influenza virus.



Results were interpreted based on variance from the baseline results, determined by running the samples in duplicate at ANTECH's Irvine lab and again at another ANTECH lab location to confirm they matched the original results (following transport with the same protocol as samples shipped to the 15 participating hospitals). In general, a variance of +/-20% of the baseline value was considered acceptable<sup>1</sup>.

**What were the biggest discrepancies?** In general, the accuracy gaps between the in-house and reference lab procedures were most significant where the reliance on microscopy skills was the greatest. Frequent errors related to the operation and interpretation of hematology instruments were also observed.

**Fecal analysis: over 2/3rds positives missed.** All fecal samples used in the study were positive on original evaluation (ZnSO4 centrifugal flotation). ANTECH re-analyzed the samples on the same day they were delivered to participating hospitals to verify the sample was truly positive for the parasites originally reported and to ensure that transportation issues did not affect the results. ANTECH was blinded to the original results for these samples.

Out of the 123 positive samples for which ANTECH received results, only 28% (35 samples) were correctly classified. The table below summarizes the results:

Organism	Number identified by in-house labs	Number known positives	% Correct
Giardia	0	64	0%
Coccidia	13	30	43%
Roundworm	19	21	90%
Hookworm	2	5	40%
Taenia	0	2	(not meaningful)
Whipworm	1	1	(not meaningful)
<b>TOTAL</b>	<b>35</b>	<b>123</b>	<b>28%</b>

<sup>1</sup>When a sample is split, acceptable variance between the two specimens is 10% or less, according to general laboratory standards; if more than two split samples are evaluated, there should not be more than 20% variance between the high and low end values.

## Case History: (continued)

The following case history was recently shared with us by Brian Thompson, DVM, Hobart Animal Clinic.

**Patient:** Molly, 3 month old female Labrador Retriever, Hobart, Indiana

**Initial exam:** "Molly was boarding at the time. She had a pirulent nasal discharge; not much of a cough. We ordered the FastPanel profile and a culture of the discharge and started Molly on antibiotics (Clavamox)."

**FastPanel results:** "The sample was positive for H1N1, Bordetella bronchi-septica, and canine para-influenza. We believe the CPiV positive may be due to recent vaccination."

**Case outcome:** "Molly was better within a couple of days after starting antibiotics. Within a week (after returning home), she was much better, with symptoms almost completely resolved."



*NOTE: The shedding period of influenza viruses is short. Samples should be submitted within 7 days of the onset of clinical signs for accurate detection by PCR.*

[Click here](#) to review the Veterinarian FAQ originally published by the AVMA in 2009, with a 2011 update.

**CBC results: significant WBC count and HCT errors.**

Approximately 20% of the 150 in-house CBC results revealed errors significant enough to lead to inappropriate clinical decisions. The variances were largely attributed to:

<p><b>1. Frequent, marked disparities in WBC counts</b></p>	<p>==&gt; Some pets with normal counts were reported to be neutropenic                  ==&gt; Some pets with inflammatory leukograms were missed</p>
<p><b>2. HCT results significantly below baseline</b></p>	<p>==&gt; Resulted in erroneous assessments of anemia in some patients                  ==&gt; Some errors appeared to be caused by obvious obstructions within the counting chamber that would typically be caught as an error, but many were more subtle errors that often go unchecked</p>
<p><b>3. Unheeded machine error flags</b></p>	<p>==&gt; ANTECH noted error flags on several results indicating some form of machine error (possible aspiration error, fibrin clot, etc.); these results were not re-run by participating hospital technicians to check or verify the values</p>
<p><b>4. Incomplete mixing of specimens</b></p>	<p>==&gt; Some clinics indicated they did not employ CBC (lavender top) tube rockers to ensure complete mixing of the specimens</p>

**Urinalysis results: false positive bilirubin and protein measurements common.**

While the variances between in-house urinalysis and reference lab results were smaller than the CBC results, the following errors were most common:

<p><b>Urine chemical analysis:</b></p>	
<p><b>False positive bilirubin results</b></p>	<p>==&gt; Many errors were attributed to manual interpretation of color changes</p>
<p><b>False positive protein measurements</b></p>	<p>==&gt; Attributed to known limit of urine strip proteins (reference labs perform SSA test for confirmation)</p>
<p><b>Urine microscopy:</b></p>	
<p><b>Bacterial cocci false positives</b></p>	<p>==&gt; <b>25%</b> of samples with negative sediments were reported by in-house labs to have bacterial cocci</p>
<p><b>Inconsistent crystal identification</b></p>	<p>==&gt; Struvite crystals identified as Oxalate in 10 of 90 specimens</p>

**In-house chemistry: closest correlation with reference lab results.** The 150 in-house chemistry profile results generally showed good correlation for most analytes, with a few notable exceptions:

<b>Total bilirubin values</b>	==> 20% of the chemistry profiles performed at the hospitals reported errors in bilirubin values significant enough to cause false positive or false negative interpretations.
<b>Enzyme values</b>	<p>==&gt; Enzyme results were highly variable with limited linearity which can cause underreporting of end point or no value;</p> <p>==&gt; High enzyme values gave no result on one in-house machine (dilutions were not routinely performed to give a value).</p>
<b>Amylase results</b>	==> In-house amylase values commonly varied from baseline by more than 25%, which could lead to false positive results in normal patients and false negatives in patients with disease.

**Discussion of results with David Lewis, BVSc, PhD, DACVIM, Director of Consultation Services, ANTECH Diagnostics**

**Q: Why were the results so disparate in some tests?**

**A:** I would attribute it to a combination of equipment limitations and the demanding skill sets required for some of these procedures, particularly those involving microscopy. Regarding equipment, we know hematology instruments are more volatile and prone to technician errors in sampling – it can take months of specific training for technicians to gain proficiency at understanding and correcting for the known limitations of their equipment. Regarding microscopy skills, both veterinary schools and veterinary technician programs have limited time to dedicate to training and teaching this very exacting skill.

**Q: What should practitioners be most concerned about in light of these results?**

**A:** Out of all the common errors that emerged from the study, I would be most concerned about the missed positive fecals – the results revealed too many missed opportunities to diagnose, treat, educate and follow-up on a great number of parasitized patients. My next two greatest concerns would be the false positive bacteria identified in many urinalysis results – leading to unnecessary treatments for UTI — and the missed hematology findings.

Regarding the hematology instruments, I would ask all my techs:

1. Are they running Quality Control samples each day to verify the machine is working correctly?
2. Do they know what to do with instrument flags?
3. Do they automatically question and re-check abnormal results?
4. How do they prep samples for aspiration? Are samples always well-mixed? Is specimen identification maintained throughout the process?
5. Are they reviewing blood smears to verify abnormal results; and are they adept at identify platelet clumping, and common morphological abnormalities (for example—blast cells, bands, toxic change, mast cells, nucleated red cells).

I would make sure the hospitals policies and protocols are very clear, and that you check in regularly to make sure they are followed consistently.

**Q: Do you think the in-house fecal testing results are representative of the average hospital's capabilities?**

**A:** I know that some hospitals do a very good job of ova & parasite identification, particularly practices in which there is a veterinarian or technician with a particular interest in & aptitude for Parasitology. Parasitology quality is more likely compromised if this is not the case. Often, technicians do not have the experience or ongoing training that is needed to do an excellent job recognizing the different types of ova, and because of the need to multi-task, often do not have the time that is really necessary to devote to reading fecal samples.

In this study, the 15 participating hospitals were aware that their ova & parasite identification skills were being evaluated, i.e., this wasn't a blind study for the hospitals. Most of the samples were 3+ (10 to 50 eggs or cysts under the entire cover-slip) or 4+ positive (>50 eggs or cysts). That made the multiple missed parasites even more surprising and eye-opening.

**Q: What are some simple steps hospitals can take to improve their parasitology accuracy?**

**A:** Some quick check-list items I would recommend:

- Do the techs have an SOP to follow?
- How carefully are they supervised to ensure they are setting up and reading samples correctly?
- If they are setting up multiple fecals simultaneously, are they carefully maintaining specimen identification throughout each step in the process – the fecalizer or centrifuge tube, the slide that is viewed, and where they record their result?

In general, and of course I'm biased, I recommend that hospitals send out their routine fecal testing, because ova and parasite identification IS an exacting skill, and a reference lab like ANTECH (because of our scale) is able to dedicate highly-trained technicians to the task, monitor their performance in a very robust fashion, and perform quality assurance processes that just aren't realistic in a typical hospital setting.

**Q: How does ANTECH evaluate the performance of its network of labs?**

**A:** It's a 3-part process:

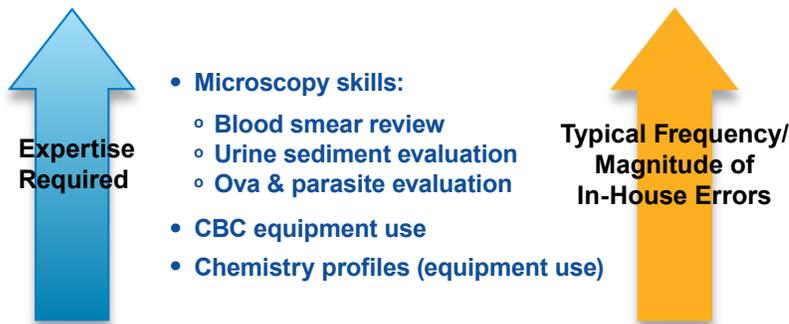
- Daily internal multi-level quality control values are directly reported to online monitoring programs and reviewed by lab supervisors and our QA department. Any variances beyond acceptable values of control materials are noted by technicians, then corrective actions are taken and documented.
- Additionally, all re-checks of lab results requested by clients or the QA department are evaluated for significant variances (errors) and discussed weekly with our lab supervisors, managers and QA department.
- Finally, we also engage in external QC processes monitored by independent agencies. All external QC studies are evaluated and discussed internally, and any issues that surface are quickly addressed.

If you have questions about ANTECH's QA/QC process, feel free to email us your question at [newsletter@antechmail.com](mailto:newsletter@antechmail.com).

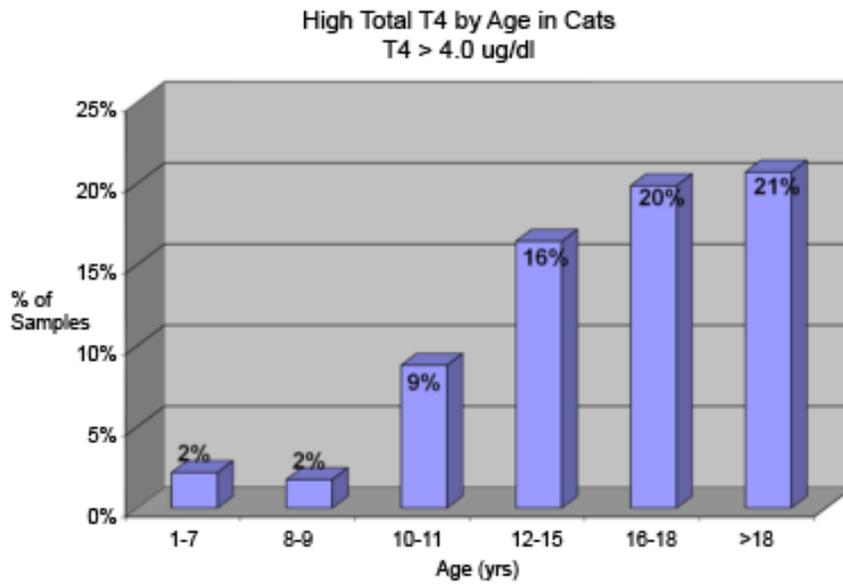
## ANTECH Take-Aways:

- ~ Outsourcing ova & parasite testing to a qualified reference lab will result in significantly higher accuracy for most hospitals
  - o Avoid missed parasites (false negatives)
  - o Avoid mis-identified parasites (false positives)
- ~ Unnecessary UTI treatment is a common byproduct of in-house urinalysis errors, with bacterial Coccidia often incorrectly identified
- ~ In-house hematology accuracy is most often compromised by incorrect sample handling and erroneous evaluation of results

**Summary.** The frequency and significance of laboratory errors for in-house testing is directly correlated to the level of training and expertise of the technicians performing the tasks, as illustrated below. Based on the current study in this newsletter and previous studies in human and veterinary literature, veterinary hospitals that rely on in-house technicians for routine microscopy should both monitor their ongoing proficiency (with split samples to the reference lab) and recognize that with severely ill patients, samples will tend to have increasingly uncommon findings – and a greater associated risk of erroneous testing.



## ANTECH Data Corner: Feline Abnormal High T4 as Function of Age



In a sample of over 250,000 T4s submitted as part of total body function tests (CBC, chemistry screen, T4), more than 50% of cats over the age of 12 had T4 values above 4.0 ug/dl. T4 values in this range support a diagnosis of hyperthyroidism. These findings are consistent with what has been previously described in the literature. Hyperthyroidism is a common endocrine disease in geriatric cats and this recent data shows that the age distribution does not appear to have shifted.

