Pitfalls in interpretation of clinical laboratory data: some tips on how to avoid them

Richard A. Squires BVSc (Hons), PhD, DVR, DipACVIM, DipECVIM-CA,
School of Veterinary and Biomedical Sciences,
James Cook University, Townsville, Queensland, Australia.

Background

Diagnostic reasoning is intricate and error-prone. Success hinges upon the collection and correct interpretation of accurate clinical information. To make a diagnosis, the clinician should first collect a good history and carry out a thorough physical examination. At this stage the patient's problems are identified and appropriate differential diagnoses are listed. If necessary, further diagnostic tests (such as diagnostic imaging, blood tests and urine analysis) can be done to help rule in or rule out differential diagnoses. The clinician then uses inferential reasoning to refine her or his understanding of the patient's problems. More specific diagnostic tests may be necessary at this stage to approach a diagnosis. A 'definitive' diagnosis can be deduced once the patient's problems are understood with a degree of precision sufficient to allow accurate prognostication and formulation of an optimal therapeutic plan (see the flow chart below).

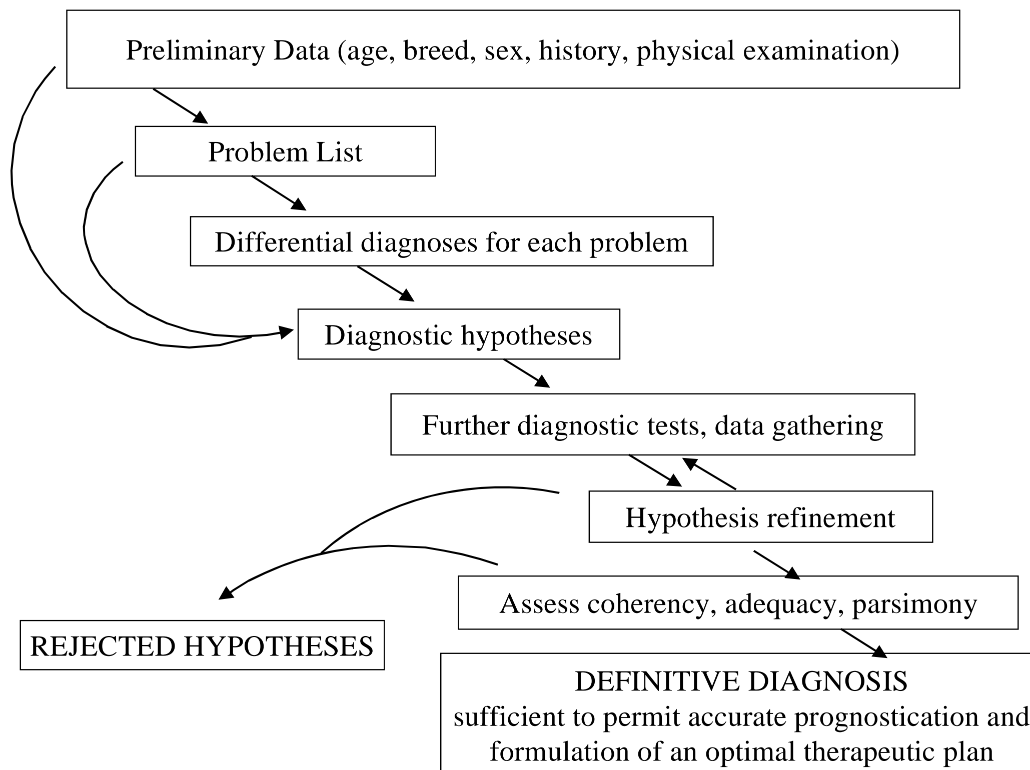

Figure 1 Process of diagnostic reasoning

Clinical laboratory data can help the clinician to reach a definitive diagnosis, by augmenting the historical and physical examination findings. The central role of the history and physical examination cannot be over-emphasised. Laboratory and other specialised diagnostic tests should never be used to 'fish blindly' for diagnoses. Ideally, laboratory tests should be done to confirm or deny differential diagnoses derived from the history and physical examination rather than to generate differential diagnoses *de novo*. This will help to reduce the likelihood of diagnostic errors in several ways:

- Only the most relevant laboratory tests are likely to be selected in the first place;

- over-reliance on laboratory test results will be minimised;
- uncritical acceptance of spurious or artifactual test results is less likely to occur; and
- confusion arising from misinterpretation of irrelevant tests (which should not have been done in the first place) will be minimised.

An analogy concerning Joel Chandler Harris's Tar-Baby (1879; part of his Uncle Remus tales) may be helpful. The more Brer Rabbit struck out at the Tar-baby (for not answering his questions) the more he became stuck fast. Similarly, the more ill-considered diagnostic tests that a clinician requests, the more likely he or she is to pay heed to spurious or artifactual results, strike out and request further tests and get stuck fast to one or more diagnostic misconceptions.

These notes will provide information intended to help clinicians avoid possible pitfalls in the interpretation of clinical laboratory data. Most of the information presented here will concern interpretation of routine haematological parameters, serum biochemical profiles and urine analyses. A few comments will be made concerning more specialised tests. It will be assumed that a careful history has been taken, a physical examination has been carried out, differential diagnoses have been considered, and specific clinical laboratory tests have been deemed necessary to help pursue a diagnosis.

What is normal? What is abnormal?

An 'abnormal' laboratory test result is generally defined as one that lies outside of the reference range. The reference range is determined by analysis of random samples from a representative population of normal animals. 'Representative' is the key word here: it is very important that the population used to determine the reference range is truly representative of the patient under study. Differences in species, breed, age and sex must be taken into account. For example, it is entirely inappropriate to evaluate feline haematological and serum biochemical values using canine or human reference values. Similarly, laboratory values for healthy young puppies differ from those of healthy adult dogs. Ideally, each clinical laboratory would establish its own reference range for veterinary patients of varying species, age, breed and sex and would provide the most applicable reference range when reporting laboratory results for a particular patient. Unfortunately most laboratories have established reference ranges only for adult dogs and cats of mixed breeds and sexes. It is the responsibility of the clinician to find out whether the provided reference range is truly representative of the patient under study. If it is not, the clinician must try to persuade the laboratory to develop a more suitable reference range, or may have to rely upon published reference ranges provided by other laboratories. Sometimes, when dealing with a confusing case, conscientious clinicians decide to submit several "control" samples from animals of similar breed and age to their patient. While this does not substitute for development of proper reference range, it is sometimes helpful.

Once samples from a suitable representative population of normal animals have been analysed, statistical methods are used to determine a reference range that usually includes 95% of normal animals. This means that for a particular analyte, about 2.5% of normal, healthy animals will have a value below the lower limit of the reference range and about 2.5% of normal, healthy animals will have a value above the upper limit of the reference range. Furthermore, consider a typical 15-test serum biochemical profile done on a normal, healthy animal. The probability that ***all 15 results*** will be in the normal range is $(0.95)^{15}$, or 0.46. Therefore, more often than not, a healthy animal will have at least one spurious abnormal value on a 15-test biochemical profile. This simple calculation highlights once again the danger of uncritical acceptance of abnormal laboratory test results, and demonstrates the need for interpretation of laboratory data in light of other, relevant clinical information.

A laboratory test result slightly above or below the limits of the reference range should be evaluated particularly critically: the patient may be an outlier in perfect health, or may be showing subtle evidence of mild or early disease. To help discriminate, the test result should be interpreted in light of the history and physical examination. If appropriate, the test can be repeated, or alternative tests that address the same diagnostic issue can be done.

Initial approach to clinical laboratory data

Whenever an abnormal laboratory test result is obtained, the clinician should consider several questions to diminish the likelihood of misinterpretation:

- Does this result confirm diagnostic hypotheses generated from the history and physical examination, or is it quite unexpected (and therefore worthy of particularly sceptical scrutiny)?
- Is the magnitude of deviation from normal small, medium, or large?
- Although it is 'flagged' as abnormal, could the result be normal *for this particular patient*, given the species, breed, age and sex?
- Could the abnormal result be a consequence of sub-optimal specimen collection or handling?
- Could the abnormal result be a consequence of artefact, or interfering substances in the specimen? (*e.g.,* is there a comment on the report stating that haemolysis, lipaemia or hyperbilirubinaemia was present in a blood specimen?)
- What further steps (if any) are necessary to corroborate the abnormal finding and approach a definitive diagnosis?

The information provided in the remainder of these notes will help the clinician to answer these questions in future. The rest of the notes are divided into sections dealing with patient factors, specimen factors and human factors that can contribute to misinterpretation of clinical laboratory data.

Patient factors that can cause pitfalls

Species

Reference ranges for a multitude of clinical laboratory parameters have been established for dogs and cats. It is clear that there are important differences between the species. Some veterinary clinical laboratories used to report feline test results alongside canine reference ranges. Fortunately, this practice is now rare. However, 'human' hospital clinical laboratories may report canine and feline patient results alongside human reference ranges. If in doubt, contact your clinical pathology laboratory to ascertain that the correct reference range is being provided.

Important differences between canine and feline clinical laboratory parameters are provided in Table 1. Compared with cats, dogs have a higher blood haemoglobin concentration, a higher haematocrit and larger red blood cells. The upper limit of the normal feline lymphocyte count is higher than that of the dog.

A noteworthy difference between dogs and cats concerns the serum activity of alkaline phosphatase (ALP). ALP is a membrane-associated enzyme that is released into plasma from a variety of tissues. The two most important tissues, from a diagnostic point of view, are bone and hepatobiliary tissue. Canine hepatobiliary tissue produces copious amounts of ALP in response to cholestasis. Substantial amounts are also released into plasma from growing or diseased bones. Increased serum alkaline phosphatase (ALP) activity is a sensitive indicator of cholestatic disease in skeletally-mature dogs, but is rather non-specific. This is mainly because dogs produce a glucocorticoid-induced isoenzyme of ALP in response to a diverse variety of illnesses. The glucocorticoid-induced isoenzyme is produced by the canine liver, but is distinct from the hepatic isoenzyme produced in response to cholestasis. Both isoenzymes contribute to the total ALP measured in canine serum. To complicate matters further, the cholestasis-associated isoenzyme of ALP can also be induced by medications, including anticonvulsant drugs and corticosteroids. Thus drug therapy, cholestasis, or a variety of other illnesses associated with increased circulating endogenous glucocorticoids can all lead to a prompt, dramatic increase in canine serum ALP activity.

In contrast, cats do not produce a glucocorticoid-induced isoenzyme of ALP, and feline ALP production does not increase dramatically in response to cholestasis or drugs. The plasma half-life of the major isoenzyme is short in cats (six hours) compared to dogs (about 70 hours). Consequently, a modest elevation in serum ALP activity is much more significant in a cat than in a dog, and is a more specific indicator of hepatobiliary disease.

Another difference between dogs and cats of direct relevance here concerns urinary excretion of bilirubin. Normal cats do not excrete dipstick-detectable bilirubin in their urine, but dogs of both sexes may do so. In dogs, hyperbilirubinuria precedes hyperbilirubinaemia and clinical jaundice as cholestasis progresses. In cats with only modest elevation of serum ALP, the presence of hyperbilirubinuria may portend cholestatic disease. Other causes, such as haemolysis must, of course, be ruled out. Conversely, bilirubin is frequently detected in the urine of normal dogs. Absence of bilirubin from the urine of a dog with

substantially elevated serum ALP activity should prompt the clinician to consider other causes for the enzyme elevation.

Table 1 Differences between canine and feline clinical laboratory values

Laboratory Value	SI Units	Feline	Canine	Species differences
Routine haematology *				
Haematocrit	L/L	0.24-0.45	0.37-0.55	Cats normally have a lower haematocrit and haemoglobin concentration than dogs
Haemoglobin	gm/L	80-140	120-180	
Mean corpuscular volume	fl	40-55	66-77	Feline red blood cells are much smaller than those of most dogs
RBC morphology				Heinz bodies may be present on a proportion of the RBCs in normal feline blood. Heinz bodies are not typically seen on normal canine RBCs.
White blood cell count	x 10^9/L	5.5-19.5	6.0-17.0	Normal feline white blood cell counts may be a bit higher than those of dogs
Lymphocytes	x 10^9/L	1.5-7.0	1.0-4.8	Normal feline lymphocyte counts can be higher than those of dogs
Platelets	x 10^9/L	145-440	190-400	Cats have large platelets that tend to clump during collection. This can lead to an incorrect diagnosis of thrombocytopenia
Serum biochemistry †				
Alanine aminotransferase (ALT) Alkaline phosphatase (ALP) Total bilirubin	U/L U/L µmol/L	10-75 0-90 0-4	0-130 0-200 0-7	Normal feline ALT, ALP and bilirubin concentrations are lower than those of dogs. ALP is particularly important: modest elevations of ALP are much more significant in cats than in dogs
Cholesterol	mmol/L	1.5-6.0	2.74-9.5	Cholesterol is usually lower in cats than in dogs
Creatine kinase	U/L	0-580	0-460	CK values can be variable in both species, but particularly in cats
Creatinine	µmol/L	75-180	55-145	The normal range for creatinine extends higher in cats than in dogs
Phosphorus, Urea	mmol/L mmol/L	1.03-2.82 5.0-10.0	0.5-2.6 2.1-9.7	The normal range for phosphorus and urea extends lower in dogs than in cats
Urine analysis				
Random Specific Gravity	--	1.001-1.070	1.001-1.080	Maximal urine concentrating ability in normal cats is greater than in normal dogs
Bilirubin	--	Negative	Positive or negative	Normal cats do not have bilirubin in their urine. Normal dogs of both sexes may have bilirubin present (males more than females), particularly in concentrated urine.

* Reference values from Jain NC: Schalm's Veterinary Hematology 4th Edition, Lea & Febiger, Philadelphia, 1986.

† Reference values from Clinical Pathology Laboratory, Department of Pathology, Ontario Veterinary College; cited by Jacobs RM, Lumsden JH, Vernau W. Canine and feline reference values. In: John D. Bonagura (Ed): Kirk's Current Veterinary Therapy XII -- Small Animal Practice. W.B. Saunders Company, Philadelphia, 1995, pp. 1395-1417.

Age

Puppies and kittens differ from adult dogs and cats in some haematological, serum biochemical and urine analysis findings. Important differences are shown in Table 2. Most of the important differences are caused by rapid skeletal growth and continuing postnatal maturation of the haematopoietic, lymphatic and urinary systems. Skeletal growth is associated with physiological increases in serum total calcium and phosphorus concentrations. In puppies, the serum concentration of the bone isoenzyme of serum alkaline phosphatase (ALP) is increased, so that the total serum ALP concentration is two or three times that found in adults.

Puppies are born with serum total thyroxine concentrations similar to those of adults but by 3 weeks of age, levels are 2 to 5 times those in young adults. Concentrations decline to adult values by 12 weeks of age and continue to decline gradually thereafter.

Puppies and kittens are born with foetal erythrocytes, which are substantially larger than those produced post-natally. Foetal erythrocytes are gradually replaced over the first few months of life, so that the mean corpuscular volume (MCV) decreases over time. The rate of growth of the animal, and the rate of destruction of foetal erythrocytes exceeds the ability of bone marrow to produce adequate new red blood cells. A transient physiologic anaemia develops, and resolves over several months. The low concentration of iron in milk may also contribute to the development of this anaemia.

Renal maturation continues post-natally, so that puppies and kittens cannot concentrate urine to the same extent as adults. Transient glucosuria in the face of euglycaemia, caused by renal tubular functional immaturity, is a frequent finding in young puppies.

Table 2 The effects of age upon various clinical laboratory values

Laboratory Value	Age differences
Routine haematology	
Mean corpuscular volume (MCV)	Puppies and kittens are born with large foetal erythrocytes. The MCV at birth is about 90-100fl. Postnatal RBCs of smaller size replace foetal cells over the first few months of life.
Packed cell volume Haemoglobin (Hb) Red blood cell (RBC) count	Puppies and kittens are born with a slightly higher packed cell volume and Hb concentration than adults. These parameters decline over the first month of postnatal life to below adult values. This is because of destruction of foetal RBCs, rapid growth of the young animal and the low concentration of iron in mother's milk. From the beginning of the second month of postnatal life, these parameters increase. Puppies take about a year to reach adult levels.
White blood cell (WBC) count	In general, the WBC count is high in young animals, declines with age and increases again late in life. Differences are mainly due to changes in lymphocyte and neutrophil numbers. Superimposed on this general trend, there are fluctuations in neutrophil and lymphocyte counts in the first two months of life.
Serum biochemistry	
Calcium Phosphorus	Associated with active bone growth, serum calcium and phosphorus are higher in puppies than in adults. Phosphorus is usually more substantially elevated than calcium.
Alkaline phosphatase	Puppies have two to three fold higher serum alkaline phosphatase than adults throughout the period of skeletal growth. This is a consequence of the bone isoenzyme. Levels are even more impressive (20 to 25 fold elevation over adult levels) during the first few days of postnatal life. This may be because of intestinal absorption of intact alkaline phosphatase from colostrum.
Bilirubin	Slightly higher in very young puppies than in adults. It declines to the adult level by about two weeks of age.
Creatinine	Somewhat lower in young animals than adults, because of relatively low muscle mass.
Urea	Serum urea nitrogen concentration depends heavily on the length of the pre-sample fast and the protein content of food previously ingested. Making the rather artificial assumption of equal duration of fast, and identical food; urea would be somewhat lower in puppies than adult dogs.
Total protein	Concentration is lower in young animals than in adults because of low albumin and globulin levels. Albumin concentration reaches an adult level by about two months of age. Globulin takes longer.
Urine analysis	
Random specific gravity	On average, urine specific gravity will be lower in young animals than in adults. Puppies and kittens have immature renal tubular function. They are unable to concentrate or dilute urine to the same extent as adults.
Dipstick findings	Glucosuria in the face of normal blood glucose concentration is a common incidental finding in young puppies. It usually disappears by 3 weeks of age, as renal tubular function matures.

Breed

There are some recognised breed variations in reference values for canine laboratory tests. Most concern normal haematocrit, which varies substantially among canine breeds. It is very likely that further breed variations will be recognised as our profession's collective clinical experience grows. Table 3 lists

various breeds and known breed-associated variations. Perhaps the most striking breed variation concerns the Akita. Typically, these Japanese working dogs have unusually small red blood cells (MCV 52 - 60 fl) compared with members of other canine breeds. In addition, they may have an unusually high ionic concentration of potassium in the cytoplasm of their erythrocytes. Consequently haemolysis caused at the time of venipuncture, or afterwards, can lead to pseudohyperkalaemia. This phenomenon, thought to be a consequence of retained sodium-potassium ATPase activity in Akita erythrocyte plasma membranes, can lead uninformed clinicians to misdiagnose hypoadrenocorticism, acute renal failure, or other disorders that are associated with hyperkalaemia.

Table 3 Known breed-related variations in clinical laboratory data of normal dogs

Breed	Variation
Akita	Some have microcytic RBCs (typical MCV 55-65fl), high RBC count, and normal PCV; some have high potassium concentration inside their RBCs, leading to pseudohyperkalaemia with haemolysis
Beagle, Boxer, Chihuahua, Dachshund	Higher RBC count, PCV and Hb values than most other breeds of dog
Cavalier King Charles Spaniel	Unusually large platelets. This can lead to an erroneous diagnosis of thrombocytopenia because auto-analysers fail to count the large platelets
Dalmatian	Urate crystals are commonly observed on urine sediment examination. In other breeds, an equivalent number of crystals might indicate liver disease or a portosystemic vascular shunt
German Shepherd Dog	Higher RBC count, PCV, Hb values and eosinophil count than most other breeds of dog
Greyhound	Higher RBC count, PCV and Hb values and lower total thyroxine concentration than most other breeds of dog.
Miniature Poodle	Higher RBC count, PCV and Hb values; macrocytic RBCs in some individuals (MCV 80 to 100fl)

Gender and Pregnancy

Little has been published recently concerning the effects of gender or neutering status on companion animal clinical laboratory data. Early studies of Beagles suggested that males have slightly higher blood haemoglobin concentration than females. More recent studies did not support the earlier findings. Any minor effects of gender or neutering status on routine haematology and serum biochemistry are not likely to lead to diagnostic errors. However, in the case of some specialised tests, errors can be made if the gender of the patient is not taken into account. Sex hormone assays must obviously be interpreted with the age, sex and neutering status of the patient borne in mind. In intact females, the stage of the oestrus cycle must also be considered. Less obviously, it has recently been shown that there are significant differences in the serum concentration of alpha-1-antitrypsin that depend upon gender and neutering status. Among healthy dogs, the serum alpha-1-antitrypsin concentration was significantly higher in sexually intact females than in spayed females, sexually intact males and castrated males. Measurement of serum alpha-1-antitrypsin concentration may in future prove useful in the diagnosis of pulmonary emphysema, hepatic failure, pancreatitis and other disorders.

Pregnancy in cats and dogs has been shown to be associated with a decline in haematocrit. In one study, the mean packed cell volume (PCV) of a group of Beagles decreased from 0.53 L/L before pregnancy to a nadir of 0.32 L/L at term. After parturition, the mean haematocrit increased, returning to normal by 9 weeks post-partum. Similar effects should be expected in other canine breeds and cats. The anaemia is thought to be a consequence of fluid retention and increased plasma volume. Other expected effects of fluid retention, such as hypoalbuminaemia, are less well characterised in dogs.

During dioestrus and pregnancy, serum total thyroxine concentration is increased due to changes in protein binding.

Drugs

Numerous drugs can alter the results of routine haematology, serum biochemistry, urine analysis and more specialised laboratory tests. Such alterations may be a consequence of physiological effects of the drug, or may merely reflect interference with the methodology used in a particular test. Some of the altered results are caused by drug toxicity; others do not indicate detrimental effects in the patient, but may lead to misdiagnosis. Important examples of some of these drug effects are discussed below.

Glucocorticoids

Serum activities of some liver enzymes may be elevated in dogs that have recently received glucocorticoid medication, though the magnitude of the effect is unpredictable. The enzymes usually affected are ALP, gamma glutamyltransferase (GGT) and, to a lesser extent, alanine transaminase (ALT). Parenteral, oral and topical routes of glucocorticoid administration can all be associated with this effect. Therefore, the clinician should inquire about the use of topical medications when investigating a dog with raised liver enzymes. Increased serum activities of ALP and GGT (which may be dramatic) are in part due to glucocorticoid-induced *de novo* synthesis of these liver enzymes. Glucocorticoid-induced hepatocellular swelling and consequent cholestasis may also play a role. Elevation of serum ALT concentration is thought to be a consequence of increased hepatocellular membrane permeability and enzyme leakage secondary to hepatocellular swelling. A recent publication by Rutgers *et al.* (1995) described in detail the biochemical and sub-cellular pathologic features of glucocorticoid-induced hepatopathy in dogs.

An indistinguishable profile of liver enzyme elevations may be found in dogs that have received glucocorticoid medication, patients with cholestasis (both intrahepatic and posthepatic) and patients with true hyperadrenocorticism (Cushing's syndrome). Therefore, when faced with elevated liver enzyme test results, it is essential to consider the possibility that administered glucocorticoid, rather than cholestasis or Cushing's syndrome is responsible for the result. The owner should be asked whether glucocorticoid has recently be administered *by any route*. Questions concerning aural, cutaneous and ophthalmic medications should not be forgotten.

As previously mentioned, cats are relatively resistant to the effects of glucocorticoids on serum biochemical parameters and they do not produce a glucocorticoid-induced isoenzyme of ALP.

Glucocorticoids have powerful effects on leukocyte trafficking and consequently affect the results of routine haematological tests. Shortly after an oral dose of prednisone, mature neutrophilia, lymphopenia and eosinopenia develop in dogs. Neutrophilia is a consequence of transient increased release from marrow, reduced margination and reduced egress of cells from the circulating pool. Fewer cells leave the circulation to traffic to sites of inflammation. Lymphopenia is a consequence of apoptosis and the fact that some circulating T-lymphocytes are redistributed to extravascular lymphoid tissues, such as lymph nodes, spleen and bone marrow. The mechanisms leading to eosinopenia in glucocorticoid-treated animals are less well understood. Egress of eosinophils from blood and reduced release from marrow may be involved. Glucocorticoids inhibit survival of human eosinophils *in vitro*. In dogs, but not usually in cats, glucocorticoid administration can cause monocytosis. This is rather unusual because in several other species glucocorticoids cause monocytopenia. Glucocorticoid-induced changes in the leukogram may be misinterpreted as evidence of infection. This can lead to unnecessary further laboratory tests and inappropriate, empirical anti-microbial therapy.

Administered glucocorticoids tend to suppress the hypothalamic-pituitary-adrenal axis. As a consequence of suppressed adrenocorticotropin (ACTH) secretion, endogenous cortisol secretion is decreased. Over time, the adrenal cortices atrophy. If a test of adrenal function is done after several days of oral glucocorticoid administration; or several days after a patient has received an injection of long-lasting glucocorticoid medication; a false low result may be obtained. The ACTH stimulation test is one such adrenal function test that is substantially affected by administration of glucocorticoid medications. Although performance of this test after *known* glucocorticoid administration is not usually recommended, the ACTH stimulation test is an excellent means of distinguishing iatrogenic from true hyperadrenocorticism in a patient showing unexplained clinical signs of hypercortisolism. The plasma cortisol concentration in a patient that has recently been receiving exogenous glucocorticoid medication will be low and if treatment has been sufficiently prolonged, cortisol will fail to rise much in response to ACTH. In contrast, a patient with true hyperadrenocorticism will usually have an exaggerated increase in plasma cortisol concentration in response to ACTH.

To further complicate matters, some steroid medications (hydrocortisone, prednisone, prednisolone) interfere with the measurement of cortisol in assays used to test the hypothalamic-pituitary-adrenal axis. These medications appear as cortisol in the assay, leading to falsely elevated values. For this reason, such medications should be discontinued at least 24 hours before blood is drawn for cortisol measurement. Another glucocorticoid (dexamethasone) does not interfere in cortisol assays and can therefore be used in tests of adrenal function, such as the low dose dexamethasone suppression test (LDDST). Dexamethasone can be given before or during an ACTH stimulation test without adversely affecting the test result. This knowledge can be helpful when dealing with a suspected Addisonian crisis (hypoadrenocortical crisis). It is not necessary to withhold potentially life-saving glucocorticoid therapy while carrying out this diagnostic test, so long as dexamethasone is used in preference to prednisolone, or other short-acting glucocorticoids.

Other effects of glucocorticoids upon clinical laboratory parameters include lowering of urine specific gravity and lowering of serum thyroxine concentration. The clinician should inquire about the use of glucocorticoids when investigating polydipsia and when dealing with patients with suspected hypothyroidism.

Anticonvulsant drugs

Phenobarbitone, phenytoin and primidone can increase serum liver enzyme activities. Enzyme induction and, to a lesser extent, hepatocellular swelling and secondary cholestasis are contributing factors. At conventional doses, these anticonvulsant drugs cause less marked elevation of serum ALP and GGT activities than do glucocorticoids. These anticonvulsants may also lower serum thyroxine concentration, perhaps by increasing its metabolism and excretion.

Many owners of dogs receiving phenobarbitone or primidone notice that their animals become polydipsic and sometimes polyphagic following administration of these drugs. The polydipsia is thought to occur secondary to inhibition of antidiuretic hormone secretion by the hypothalamus. Urine specific gravity of dogs on these drugs may be somewhat lower than expected for their hydration status.

Potassium bromide, which has recently undergone renaissance as an anticonvulsant, interferes with the measurement of serum chloride concentration. An artificially high result is obtained because bromide

appears as chloride in commonly used assays. Bromide also interferes with the measurement of serum cholesterol concentration.

Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs can cause gastrointestinal ulceration and haemorrhage. This is mild and clinically insignificant in most patients, but may be sufficient to cause a spurious positive faecal occult blood test result. In other patients, NSAID-induced gastrointestinal haemorrhage may be a severe problem. Clinical judgement must therefore be exercised when interpreting tests results for faecal blood in patients receiving NSAIDs.

NSAIDs have many other pathophysiological effects that may genuinely alter clinical laboratory data. Prolongation of bleeding time is routine with aspirin use; since the drug causes thrombocytopathia. This effect may be beneficial or detriment to the patient, depending on clinical circumstances. Other potential adverse consequences of NSAID use include hepatocellular injury leading to increased serum ALT activity, and renal vasoconstriction with consequent azotaemia. These are more likely to develop if pre-existing liver or renal disease is present.

Antibiotics

Many antibacterial drugs are highly concentrated in urine. This is helpful in the management of urinary tract infections but can interfere with some laboratory tests that are applied to urine.

If copper reduction methods are used to measure urine glucose, high-dose penicillins, cephalosporins and nalidixic acid can give false negative results. These drugs do not interfere with the assessment of urine glucose concentration by the glucose oxidase method.

Beta-lactam antibiotics (penicillins and cephalosporins) can also falsely elevate serum creatinine, depending on the particular assay used.

Certain antibiotic drugs can produce false positive results in tests for proteinuria. Commonly used urine 'dipstick' tests and the sulphosalicylic acid precipitation test for urine protein can be affected. Sulfisoxazole, penicillins and cephalosporins will interfere with the sulphosalicylic acid precipitation test leading to a false positive test result.

Sulphonamide crystals can be seen in concentrated urine, particularly if the urine is acidic. These unusual crystals can sometimes cause confusion, although they are readily recognised by experienced observers. If sulphonamide crystals are found on urine sediment examination, this should prompt the clinician to exercise caution with further therapy since tubular damage has been associated with sulphonamide crystalluria. The patient may need to be encouraged to consume more water, or an alternative antibiotic drug may be chosen.

Potentiated sulphonamides have been reported to cause reversible, iatrogenic hypothyroidism when used at high doses. However, no effect on thyroid function test results was noted when one product (trimethoprim-sulphadiazine, Tribrissen®) was used at the manufacturer's recommended dose. Sulpha drugs can also raise serum liver enzyme activities by enzyme induction, or by causing an idiosyncratic hepatopathy.

Other drugs

Renally excreted radiographic contrast agents can falsely increase urine specific gravity and give a false positive 'dipstick' protein reaction. These agents may crystallise in acid urine, producing unusual urine sediment examination findings for several days after their use.

Reducing agents such as ascorbic acid (vitamin C) and aspirin falsely lower serum glucose concentration measured by the glucose oxidase method and can produce a false negative result for urine glucose when a reagent strip is used to test urine from a diabetic patient. Ascorbic acid can also falsely increase serum creatinine concentration.

Hydration and nutritional status

Dehydration increases the concentration of solutes and cellular elements in blood. Diagnostic errors can be made if dehydration is missed during physical examination and clinical laboratory data is evaluated uncritically. In animals with normal urine concentrating ability, dehydration is associated with high urine

specific gravity. Elevated serum creatinine and urea nitrogen should always be interpreted in light of physical examination findings and urine specific gravity. In the presence of concentrated urine, azotaemia is most likely to be prerenal, rather than renal or postrenal in origin. Prerenal azotaemia is usually corrected promptly after rehydration is achieved. As a matter of routine, urine should be obtained *before* fluid therapy in dehydrated patients.

Hyperalbuminaemia is strong evidence of dehydration, because the only well recognised cause of this particular abnormality is dehydration. (Although, recently, primary liver tumours have been reported to cause hyperalbuminaemia in dogs). Other possible findings in dehydrated patients include hyperglobulinaemia, increased serum electrolyte concentrations (hypernatraemia, hyperchloraemia, hyperphosphataemia, hyperkalaemia, hypercalcaemia), reduced total CO_2 (metabolic acidosis due to tissue hypoperfusion), and raised haematocrit. Occasionally, significant anaemia and hypoproteinaemia may be masked by dehydration. The clinician should critically evaluate the packed cell volume and total plasma protein in light of the estimated hydration deficit in severely dehydrated animals. Does it seem likely that rehydration of the patient in question will unmask anaemia or hypoproteinaemia? If so, further monitoring steps and possible therapeutic interventions, such as the administration of a plasma expander or blood transfusion may be appropriate.

Fasting, or feeding of a protein-restricted diet can lower the serum urea concentration. Serum creatinine concentration is relatively unaffected, so that the urea:creatinine ratio tends to decrease. Feeding of a high protein diet, or gastrointestinal haemorrhage, have the opposite effect. Prolonged starvation or protein malnutrition can also lead to hypoalbuminaemia, hypokalaemia and raised serum liver enzyme activities. Maximal urine concentrating ability is reduced in patients with low serum urea.

Eating a normal meal tends to raise the blood concentrations of glucose, cholesterol, triglycerides, trypsin-like immunoreactivity (TLI), total bile acids and ammonia. The effects of feeding on serum total bile acids and blood ammonia are well known, and are useful in diagnostic tests of liver function that may include pre- and post-prandial blood sampling. Serum TLI is also raised by feeding; in this case making it more difficult to diagnose exocrine pancreatic insufficiency. Blood for TLI measurement should therefore be drawn after a 12 hour fast. The magnitude of glucose elevation after feeding is modest, and is unlikely to contribute to diagnostic errors. Patients with suspected borderline diabetes mellitus should be evaluated with serial blood glucose measurements, rather than a single measurement. The time of the most recent meal should be taken into consideration when evaluating blood glucose values. Post-prandial hypertriglyceridaemia, on the other hand, can be substantial. Sufficient lipaemia may be present in a post-prandial serum specimen to cause turbidity and consequently to alter the results of several other laboratory tests. Lipaemia can also cause *in vitro* haemolysis, worsening the confusion (see the following section on specimen factors that can lead to misinterpretation of clinical laboratory data). Post-prandial hypercholesterolaemia does not usually lead to diagnostic errors. To avoid the confounding effects of lipaemia and other post-prandial vagaries, it is best to obtain blood samples for most purposes after a 10-12 hour fast.

Obesity causes a mild increase in serum total thyroxine concentration.

Stress and intercurrent illness

Stress and illness produce physiological effects that may manifest in routine clinical laboratory data. The stress of venipuncture is sufficient to raise blood glucose concentration in many cats. This 'excitement' associated hyperglycaemia can occasionally hinder the diagnosis of borderline diabetes mellitus in this species. In stressed or ill dogs, the "stress leukogram" (consisting of neutrophilia, lymphopenia, eosinopenia and monocytosis) is a familiar haematological finding that may be mistaken for evidence of inflammation. The "stress leukogram" is thought to be a consequence of glucocorticoid and adrenaline release.

Stress and non-adrenal illnesses may raise plasma cortisol levels sufficiently to complicate adrenal function testing. Since stress can lead to false positive results in tests for hyperadrenocorticism, physical restraint and other stressful procedures should be avoided as much as possible during the course of these adrenal function tests. Hypoglycaemia is another potential cause of false positive results in these tests, because low blood glucose is a potent stimulus for glucocorticoid secretion. It is important to ascertain that patients (particularly those with diabetes mellitus) are not hypoglycaemic during the course of low dose dexamethasone suppression tests (LDDSTs) and ACTH stimulation tests. In dogs, stress- or illness-

induced plasma cortisol elevation may also lead to increased serum ALP activity, because of steroid-induced isoenzyme synthesis. This combination of findings may lead the unwary clinician to misdiagnose hyperadrenocorticism. A patient with raised serum ALP activity and an adrenal function test result suggestive of hyperadrenocorticism should be evaluated critically. Does other clinical information (*e.g.,* history, physical examination findings, imaging studies) support a diagnosis of hyperadrenocorticism? If not, a false positive result is a distinct possibility in a patient with non-adrenal illness. Repetition of adrenal function testing, ultrasound imaging of the liver and adrenal glands, ACTH assay and test therapy with a non-adrenolytic drug (such as ketoconazole) can be used in combination to resolve this potential diagnostic dilemma. Ideally, repeat adrenal function testing should be done with minimal stress after any known non-adrenal illness has been successfully treated.

As stated previously, in dogs raised serum ALP activity may be a consequence of stress. Occasionally this finding will lead to misdiagnosis of cholestatic liver disease. To avoid this mistake, the clinician should assess the patient for evidence of cholestasis by other means. Abdominal ultrasound examination, pre- and post-prandial serum bile acid quantitation, and assessment of bilirubin concentration in urine and blood can be used to help rule in or rule out cholestasis. Separation and quantitation of the component ALP isoenzymes that make up the total serum ALP activity is possible. This turns out to be of modest value when attempting to distinguish cholestasis from other causes of raised ALP. When the serum activity of the glucocorticoid-induced isoenzyme of ALP is markedly elevated (>2000 U/L), or when it forms a substantial fraction of total ALP (>90% of total ALP), a diagnosis of hyperadrenocorticism is highly likely. Although this does not rule out cholestasis absolutely, it may help the clinician who chooses to apply careful diagnostic parsimony to the patient with raised serum ALP.

Non-thyroidal illnesses can temporarily lower serum thyroid hormone concentrations, potentially leading to misdiagnosis of hypothyroidism. The term ‘euthyroid sick syndrome’ has been used to describe this situation. Thyroid hormones circulate in free and protein-bound forms. The free hormones are physiologically active. Illnesses leading to decreased binding protein concentration, reduced binding affinity, or circulating inhibitors of binding will be associated with decreased total thyroid hormone concentration, but free hormone concentrations will not necessarily be affected. To further complicate matters some debilitating illnesses; such as congestive heart failure, diabetic ketoacidosis, hyperadrenocorticism, hypoadrenocorticism, renal failure and severe liver disease; may be associated with decreased circulating *free* hormone as well as total thyroid hormone. Nevertheless, thyroxine (T_4) supplementation is not considered appropriate for patients with euthyroid sick syndrome, even if free T4 is low, since, at least in humans, it does not improve survival. Identification and treatment of the underlying disease is what is recommended.

As you can see, distinguishing true hypothyroidism from euthyroid sick syndrome can be problematic in ill animals. Total thyroxine concentration (TT_4), as measured and reported by most clinical pathology laboratories, cannot be relied upon. Free thyroxine (fT_4) can now be measured accurately by many veterinary clinical pathology laboratories using equilibrium dialysis and provides a more accurate indicator of thyroid status. However, the test is expensive and not available everywhere. Assay of canine endogenous TSH (cTSH) can sometimes be helpful in making the distinction between true hypothyroidism and euthyroid sick syndrome. An elevated cTSH in the face of low TT_4 is 92 to 100% specific for hypothyroidism. Unfortunately the combined test is not as sensitive: only 63 to 87% of truly hypothyroid dogs have an elevated cTSH and low TT_4. Most patients with euthyroid sick syndrome can be distinguished from patients with true hypothyroidism on the basis of a thyroid-stimulating hormone (TSH) response test. Unfortunately, bovine TSH is expensive and sometimes difficult to obtain.

Some illnesses cause alterations in clinical laboratory data that, at first glance, seem paradoxical or indicative of completely different illnesses. For example, immune-mediated haemolytic anaemia (IMHA) is frequently associated with marked neutrophilic leukocytosis and a left shift. This so-called ‘leukemoid response’ is often misinterpreted as evidence of a serious infection or marrow neoplasia. If unaware of this possible feature of IMHA, the clinician may be inclined empirically to institute potent antibiotic therapy and to delay immunosuppressive therapy. This would be inappropriate, since the leukemoid response does not indicate an infectious process. Rather, it is thought to be a corollary of bone marrow hyperactivity consequent to haemolysis. In some cases, it may reflect an inflammatory underlying cause for the immune-mediated disease. The specific cytokines or other mechanism(s) responsible for the leukemoid response in canine IMHA are yet to be elucidated.

Neoplastic illnesses frequently cause potentially-confusing alterations in clinical laboratory data, termed 'paraneoplastic syndromes'. Paraneoplastic effects are caused by humoral factors secreted by tumour cells. Secretion of such humoral factors is generally inappropriate, or unexpected, given the neoplastic tissue of origin. For example, hypercalcaemia is found in about 80% of patients with apocrine gland adenocarcinoma of the anal sac. This particular form of paraneoplastic hypercalcaemia is caused by tumour secretion of parathyroid hormone-related peptide (PTHrP). Clinicians unaware of this disease association might fail to carry out (or repeat) a thorough rectal examination and might expend resources pursuing other causes of hypercalcaemia, such as lymphoma or multiple myeloma. Some further examples of potentially-confusing alterations in clinical laboratory data caused by neoplastic and non-neoplastic illnesses are shown in Table 4.

Table 4 Potentially confusing effects of selected disorders on clinical laboratory data

Abnormal Laboratory Value	Increase/ Decrease	Usual disease associations	**Potentially confusing associations**
Routine haematology			
Mean corpuscular volume	Decrease	Blood loss anaemia	Portosystemic shunts may be associated with microcytosis or microcytic anaemia.
Packed Cell Volume Haemoglobin concentration Red Blood Cell count	Increase	Dehydration Breed variation Exposure to high altitude Chronic hypoxaemia Polycythaemia rubra vera	Polycythaemia may be caused by renal tumours and some other expansile renal lesions because of increased renal erythropoietin production. Haematocrit may be higher than 70%, and may lead to seizures or other signs of blood hyperviscosity. Dehydration may be misdiagnosed if the physical examination findings and the total plasma protein are not taken into consideration. Paraneoplastic erythrocytosis may also occasionally be caused by other tumours.
Neutrophil count	Increase	Infection Inflammation	Immune-mediated haemolytic anaemia frequently causes a marked neutrophilic leukocytosis with a left shift. The mechanism is incompletely understood. This 'leukemoid response' may be misinterpreted as evidence of infection, unnecessarily delaying immunosuppressive therapy. Paraneoplastic neutrophilia is occasionally reported with some tumours.
	Decrease	Toxic bone marrow suppression Primary bone marrow disorder	A severe inflammatory focus may cause transient neutropenia as PMNs marginate and leave the circulation in large numbers. A 'degeneratve left shift' with more young, band neutrophils than mature, segmented neutrophils in the peripheral blood may develop.
Platelet count	Increase	Acute blood loss Inflammation Primary (essential) thrombocytosis	Chronic gastrointestinal blood loss may cause marked thrombocytosis in addition to microcytic, hypochromic anaemia. The high platelet count can be a useful marker of chronic gastrointestinal bleeding, but can also lead to misdiagnosis of a primary bone marrow disorder.
Serum biochemistry			
Calcium	Increase	Skeletal immaturity Lymphoproliferative disorders Hypervitaminosis D	Apocrine adenocarcinoma of the anal sac is associated with hypercalcaemia in about 80% of cases. If careful rectal examination is not done, hypercalcaemia may be incorrectly attributed to lymphoma, myeloma or some other cause.
Glucose	Decrease	Artifact Neonatal and juvenile hypoglycaemia Insulinoma	Sepsis, leukaemia, large liver tumours, and other tumours are less well-recognised causes of hypoglycaemia

Specimen factors that can lead to misinterpretation of laboratory data

Sample collection and handling

Venipuncture

In an ideal world, every blood sample would be obtained rapidly by uncomplicated venipuncture. In practice, problems with patient restraint, patient conformation and technical expertise often slow or disrupt the procedure. Difficulties encountered during or immediately after venipuncture often lead to unwanted blood coagulation, haemolysis and other problems:

- If there is a sufficient delay in collecting blood from the vein and transferring it to the anticoagulant blood tube, blood may begin to clot in the syringe or vacuum tube.
- If blood is inadequately mixed with anticoagulant once it is collected, it will clot in the tube.
- If undetected, partial coagulation will lead to errors in routine haematological testing. In particular, platelet counts will be artificially low.
- Blood vessel walls may be punctured several times during difficult venipuncture. Damaged blood vessel wall components and tissue fluid may be aspirated into the syringe or vacuum tube, accelerating clotting and compromising the results of coagulation and other tests.
- Haemolysis may result from use of excessive negative pressure while drawing blood, or from forcing blood through a small diameter needle into the blood tube. Haemolysis interferes with several haematogical and serum biochemical tests.
- If an insufficient volume of blood is obtained during difficult venipuncture and is then mixed with a relative excess of anticoagulant, artifactual alterations in erythrocyte indices will occur. For example, ethylenediaminetetraacetic acid (EDTA) excess causes shrinkage of red blood cells, falsely lowering PCV and MCV, but raising mean corpuscular haemoglobin concentration (MCHC).
- If the vein is raised for an excessive period of time, the tourniquet effect may slightly alter the results of several haematological and biochemical tests (*e.g.*, ammonia and lactate quantitation).

Cystocentesis

Potential technical problems with cystocentesis are comparable to those experienced with venipuncture. In most cases, urine samples intended for bacterial or fungal culture are ideally obtained by cystocentesis. This is because urine aspirated directly from the bladder, through surgically-prepared skin, is free of contaminating organisms. Urine obtained by catheter, or by free-catch, is contaminated to a lesser or greater extent. Technical difficulties experienced during attempted cystocentesis most commonly lead to failure to obtain a urine specimen. Sometimes a blood-contaminated specimen is obtained. Rarely in ascitic patients, the urine specimen may be contaminated with ascitic fluid; or ascitic fluid may be obtained instead of urine. Even more rarely, the colon is punctured and the specimen may be contaminated with faeces.

Factors that complicate cystocentesis include:-

- Degree of fullness of the bladder—cystocentesis is more difficult if the bladder is nearly empty. Patients with lower urinary tract disease are usually candidates for cystocentesis; unfortunately, their illness causes them to empty their bladder frequently. Sometimes to permit cystocentesis fluid therapy must be given to encourage filling of the bladder.
- Struggling of the patient—cystocentesis is best carried out on a well-restrained, relaxed patient.
- Tenseness of the abdominal wall—nervous patients, or patients with abdominal pain may tense their abdominal muscles making palpation of the bladder difficult.
- Obesity of the patient—palpation or immobilisation of the bladder is more difficult in a grossly obese dog.

- Location of the bladder—cystocentesis is usually more difficult if the patient has an intrapelvic bladder or a retroflexed bladder in a perineal herneal sac.

Sample Handling and Storage

Once a specimen of blood or urine is collected it must be handled appropriately. This is more critical for some analyses than others.

Urine

Urine intended for bacterial culture should be processed within 30 minutes or promptly refrigerated at 4°C. Failure to do this may permit bacterial replication in the urine specimen and may lead to misdiagnosis of urinary tract infection. This is more likely to happen if catheter-obtained or free-catch specimens are mishandled, because the number of bacteria is greater in specimens obtained by these routes. Refrigerated specimens may be stored for up to 6 hours without a significant increase in bacterial numbers; however fastidious organisms may die during storage. Refrigeration of urine chemically preserved using a mixture of boric acid, glycerol and formate is an option if culture cannot be started within 6 hours. Bacterial viability has been reported to be up to 72 hours using this technique.

Urine sediment examination is best carried out on fresh urine specimens. Cellular elements and tubular casts degenerate in stored specimens, particularly if urine is stored at room temperature. Crystals may form *in vitro* in refrigerated urine. Urine should be warmed to room temperature before analysis.

Urine should not be exposed to fluorescent light for a long period of time before the reagent strip tests for urobilinogen and bilirubin are applied. Bilirubin is rapidly oxidised to biliverdin and urobilinogen is converted to urobilin upon exposure to ultraviolet light. The urine takes on a green colour and the strip tests may yield false negative results.

Glucose present in infected urine that is handled improperly may be consumed by bacteria, leading to a false negative dipstick result.

Blood

After blood collection, serum or plasma should ideally be separated from blood cells within 20 to 30 minutes. This will prevent consumption of glucose by cells in the specimen and may reduce the amount of *in vitro* haemolysis. The specimen should then be stored either refrigerated at 4°C or frozen at -20°C until analysis. Most analytes in serum or plasma are stable in a refrigerator at 4°C for 24 hours or more; however there are important exceptions. Handling of blood specimens intended for quantitation of glucose, ammonia, ionised calcium and blood gases requires special care because these analytes are particularly labile.

Storage of whole blood at room temperature leads to a decrease in blood glucose concentration of about 0.39 mmol/L/hour, or about 10% per hour·. The rate of *in vitro* glucose consumption is dependent upon the number of cells per unit volume in the sample and may be more rapid in specimens from patients with polycythaemia, leukocytosis or thrombocytosis. If serum or plasma cannot be separated promptly from blood cells to avoid artifactual hypoglycaemia, blood can be collected into a sodium fluoride tube. Sodium fluoride is both an anticoagulant and a glucose preservative. Certain enzymatic methods for glucose quantitation cannot be used with fluoride-containing specimens, because the enzyme used in the assay is 'poisoned' by fluoride. If in doubt, the clinician should contact the clinical laboratory to discuss methods for optimising blood glucose measurement.

Blood ammonia determination is sometimes useful in the diagnosis of severe liver disease, particularly if hepatic encephalopathy is present. Used in this setting, the test has high specificity but low sensitivity. Blood for ammonia determination is usually collected into heparinised tubes. For obvious reasons ammonium heparin tubes should not be used; sodium or potassium heparin tubes are suitable. After collection, blood should immediately be placed on ice and transported to the clinical laboratory. Plasma should be harvested in a pre-cooled centrifuge and ideally the analysis should be carried out within an hour of blood collection. Storage of canine whole blood or plasma intended for ammonia quantification, even in a -20°C freezer, leads to erroneous results.

Blood ionised calcium measurement provides a more accurate assessment of calcium homeostasis than does total calcium because ionised calcium comprises the physiologically active fraction. Homeostatic

mechanisms regulate blood ionised calcium concentration, not total calcium. An increasing number of veterinary clinical laboratories are offering to measure blood or serum ionised calcium. The astute clinician should be aware of some potential pitfalls when handling specimens for ionised calcium measurement.

pH changes may occur after collection of a whole blood specimen and alter the ionised calcium concentration. Glycolysis (with consequent formation of lactic acid) causes a decrease in pH and a rise in the concentration of ionised calcium in the sample. Blood cells should therefore be separated from serum or plasma if the specimen cannot be promptly analysed. A study has shown that heparinised venous whole blood can be stored at 4°C for up to 3 hours after collection without significant alteration in ionised calcium concentration.

The same study showed that serum and plasma can be collected and stored *anaerobically* at 4°C for much longer (up to 240 hours) without significant alteration in ionised calcium concentration.

Ionised calcium is substantially bound by all standard anticoagulants, including heparin. Therefore ionised calcium concentration is a little lower in plasma than in serum taken from the same patient at the same time. The assay is very sensitive to the concentration of anticoagulant used, therefore this should be standardised. Calcium-titrated heparin (S 4500 Heparin for ionised calcium analysis, Radiometer, Copenhagen) is the preferred anticoagulant, if plasma rather than serum is to be submitted to the laboratory. The use of serum to avoid these chelation problems is an acceptable alternative.

Specimens for blood gas analysis should be collected and transported to the laboratory anaerobically on ice. The rectal temperature of the patient and the blood haemoglobin concentration (or at least the PCV) should be measured at the time that the blood gas specimen is drawn. The laboratory usually needs to be informed of these values to permit correct measurement of blood gases.

Interfering substances

Ideally, each veterinary clinical laboratory would be aware of the effects of commonly-encountered interfering substances on their own laboratory test results and would be in a position to provide the conscientious clinician with a set of interferograms upon request. Interferograms are simple graphs that show the effects of increasing amounts of lipid, bilirubin and haemoglobin upon test results. In practice, it can be quite difficult to get hold of a set of interferograms, even in a university veterinary hospital. A useful, but less complete alternative is provided by some clinical laboratories. Test results from specimens that were found by the laboratory to contain interfering substances are “rubber stamped” with a list of known effects of the interfering substance.

Lipaemia

Lipid droplets in canine plasma or serum may cause visible turbidity of what is normally a clear, straw-coloured fluid. Lesser degrees of lipaemia may be inapparent on examination of the specimen, or may cause a slight haze. Marked lipaemia of whole blood may give the specimen the appearance of a “strawberry milk shake”. Lactescent plasma or serum is routinely encountered when post-prandial blood specimens are centrifuged for analysis. If encountered in a true fasting specimen, lipaemia may be indicative of hypothyroidism, hyperadrenocorticism, pancreatitis, diabetes mellitus, or a primary hyperlipidaemia.

The scattering of light caused by fat droplets affects certain laboratory tests that depend upon absorption or refraction of light. In general, the new analytical methods that employ dry reagent technology are less affected by lipaemia than are conventional wet chemical methods. Spectrophotometric and flame photometric determinations are affected. Refractometry is substantially affected, so that artificially high results for total plasma protein are obtained, compared with the results obtained using biochemical methods. The total plasma protein value obtained by refractometry of specimens from healthy, lipaemic animals may exceed 130 g/L. Electrolytes measured by flame photometry (*e.g.* sodium and potassium) will be decreased; values obtained using ion selective electrodes are unaffected by lipaemia. Serum bile acids (SBAs) are artificially elevated by lipaemia. This frequently causes problems in interpreting post-prandial SBAs. A modest amount of food should therefore be fed during the test. The objective is to provide sufficient food to cause emptying of the gall bladder and no more.

Lipaemia can enhance *in vitro* haemolysis. Therefore the interferences mentioned below in the section concerning haemolysis can be a consequence of both lipaemia and haemolysis.

Haemolysis

Lysis of erythrocytes *in vitro* causes interference with a number of clinical laboratory tests. Obviously the PCV is decreased. Usually, the MCHC is increased. This can be conceived in terms of the analyser attempting to put all of the haemoglobin it finds (some of it free in solution) into the remaining, non-haemolysed erythrocytes that it finds.

Some spectrophotometric tests that depend upon transmission (or absorption) of light may be substantially affected by haemolysis. ALT, albumin, total bilirubin, calcium, cholesterol, glucose and phosphorus are artificially elevated by haemolysis in many spectrophotometric assay systems. Newer analytical systems are less sensitive to this sort of interference.

Lysed erythrocytes release their constituents into solution. Canine erythrocytes are not particularly rich in potassium, so pseudohyperkalaemia is not a common sequel to *in vitro* haemolysis, except in specimens from Akitas. Erythrocytes are rich in lactate dehydrogenase (LDH) and aspartate aminotransferase (AST). Serum concentrations of these enzymes increase after haemolysis.

Hyperbilirubinaemia

Hyperbilirubinaemia may artificially increase the concentrations of albumin, cholesterol, glucose and total protein; depending on the biochemical methods used for the determination of each of these analytes. The concentration of serum creatinine may be artificially decreased if the Jaffe method is used for analysis.

Timing of specimen collection

Blood samples for certain purposes must be collected at specific times relative to feeding or administered medications. Examples include post-prandial SBAs (2 hours after food); serum trypsin-like immunoreactivity (after a 12 hour fast); ACTH stimulation test (before and 1 or 2 hours after ACTH administration); and serum T_4 for assessment of adequacy of thyroid medication (about 4 to 6 hours post pill). If specimens are inadvertently collected at an incorrect time, or if the clinician is unaware of the necessity for careful timing, diagnostic errors may be made.

Human factors that can lead to misinterpretation of laboratory data

Human beings have a natural proclivity to err; we tend to cut corners, jump to conclusions, and overlook things. Veterinarians are far from being immune to these tendencies. Yet, despite the complexity of our work, development and maintenance of a habit of thoroughness can help considerably in the avoidance of diagnostic errors. In the approach to clinical laboratory data, it is useful to follow a set of guidelines, similar to the one offered here.

- First assess specimen quality. Look for comments concerning unwanted coagulation, lipaemia, haemolysis, or hyperbilirubinaemia. These can cause spurious, marked alterations in some parameters. If you are uncertain of the possible effects of an interfering substance, make enquiries of your clinical laboratory.
- Look at the reported laboratory values and identify abnormal values. Classify the degree of abnormality of each as mild, moderate, or marked. This should go beyond simple numerical considerations. For some analytes (e.g. serum calcium or sodium) a small percentage increase is highly significant. For others (e.g. ALP) a 10-fold increase is not overwhelmingly impressive. When faced with an unfamiliar analyte, avoid errors concerning units of measurement by meticulous examination of results.
- Consider whether or not any of the abnormalities might be normal for the particular patient under study (e.g. anaemia of late pregnancy; puppy hypercalcaemia; raised serum ALP activity in a patient receiving glucocorticoid therapy).
- Consider whether inadequate patient preparation, or inappropriate timing of specimen collection might be responsible for an abnormal result (e.g. post-prandial hypercholesterolaemia; failure to

fast a patient adequately before TLI quantitation; collection of blood for T_4 measurement at an inappropriate time post pill).

- Rank the problems identified from the clinical laboratory data in order of importance, taking into consideration both the degree of abnormality and the physiological relevance of the analyte. Consider possible hierarchical relationships among the problems. Group problems together loosely, in an interchangeable format. Consider differential diagnoses for individual problems, or for groups of problems.
- Decide what further investigations, if any, are required to rule in or rule out your differential diagnoses. Discuss the costs, risks and benefits of these investigations with the owner of the patient. If deemed appropriate, carry out the investigations needed to permit accurate prognostication and formulation of an optimal therapeutic plan.