COURSE CODE:	VPT 304
COURSE TITLE:	Clinical pathology
NUMBER OF UNITS:	3 Units
COURSE DURATION:	Three hours per week

COURSE DETAILS:

Course Coordinator: **Dr. Omotainse** *DVM., MVSc., PhD*
Email: omotains@yahoo.com
Office Location: **Department of Vet. Pathology, COLVET Building**
Other Lecturers: **Dr. Olaniyi, Moshood Olajire,** DVM, MVSC **&**
Dr. Ajayi, Olusola Lawrence, DVM, MVSC

COURSE CONTENT:

Role of clinical chemistry and haematology in the management of clinical problems. Determination of blood parameters including cell values (PCV, Hb content, RBC and WBC counts) and plasma/serum concentrations of proteins, enzymes, sugar, chemical ions and metabolites. Liver function tests, renal function tests. Examination of urine for glucose, proteins, ketone bodies, blood cells, acidity and bile pigments. Biopsy and exfoliative cytology procedures. Interpretation of clinical pathology data.

COURSE REQUIREMENTS:

This is a compulsory course for all DVM students and attendance of at least 75% is required to write the examination.

READING LIST:

1. Douglas J. Weiss and Jane K. Wardrop. *Schalm's Veterinary haematology*. 6th Ed. USA. Wiley-Blackwell, 2010.
2. Kaneko, J. J., Bruss, M. L. and Harvey J. W. *Clinical biochemistry of domestic animals*. 5th Ed. Academic Press, New York. 1997.
3. Kerr Morag G. *Veterinary Laboratory Medicine: Clinical biochemistry and haematology*. 2nd Ed. London, Blackwell Science Ltd. 2002
4. Benjamin M. Maxine. *Outline of Veterinary Clinical Pathology*. 3rd Ed. New Delhi. Kalyani Publishers. 2007
5. Nduka Nsirim. *Clinical biochemistry for students of pathology*. Longman Nigeria Plc. 1999.

LECTURE NOTES

INTRODUCTION:
Haematology
Clinical chemistry
Clinical microbiology & Parasitology
Urinalysis
Cytology/histology (biopsy)
GENERAL

In understanding the nature of a disease for effective treatment and control measures to be adopted, pathology must go beyond postmortem diagnosis to making use of changes in the structure and functions of organs/tissues in a living animal. Clinical pathology provides evidence regarding the physiological alterations resulting from pathological condition. Clinical tests make more meaning when considered in relation to the history of the patient.

Clinical chemistry-Application of chemistry and it's allied techniques for the elucidation of disease, diagnosis and management of patient.

Blood chemistry is of great value in some disease in conformation of diagnosis, prognosis and response to treatment {management of disease}. Interpretation of such analysis is done by comparing results with normal range values of blood constituents. Accuracy of such result depends on:

1) taking of appropriate samples in a proper manner.
2) availability of adequate information [history].

Clinical chemical analyses are mostly performed to determine parameters such as blood Glucose, body electrolytes and other metabolites, enzymes, proteins [including Ag & Ab] and renal functions.

Most clinical chemistry requires serum and occasionally plasma.

Blood for biochemical analysis will preferably require heparin as anticoagulant. This is because heparin is a natural anticoagulant produced by the liver and prevents the conversion of prothrombin to thrombin. Ammonium oxalate should not be used as anticoagulant if blood non-protein nitrogen or urea is to be determined. The oxalate and nitrates [eg Na-citrate] combine with Ca^{++} to prevent clotting. While serum/plasma could be frozen for a limited period of time, depending on the required analysis, whole blood should not be kept frozen to prevent lyses.

For general haematological examinations the anticoagulant of choice is EDTA because it best preserves the cellular components and integrity as well as prevents platelets aggregation. Haematological examination are mostly preformed in the following areas:

1) Microscopic examination of unstained preparation
2) Microscopic examination of stained smears
3) Haemoglobin estimation
4) Packed cell volume determination
5) Red blood cell count
6) Total and differential leucocyte counts
7) Platelet count.
8) Clotting time and any other

BLOOD SAMPLE COLLECTION

Sample are generally taken for either haematological or biochemistry or serology while haematological examination are performed with unclotted whole blood.

Serological examinations are made with serum from clotted blood, while collecting blood, animals should not be excited unnecessarily. The site for blood sampling depends on the purpose and volume of blood needed before taking blood, the site should be sanitized by shaving [if necessary] and wiping the exposed skin with alcohol or either and allowing it to dry.eg sites and animal species good for blood sampling.

*Marginal ear vein-rodents and ruminants, pigs
*Jugular vein –for large quantities of blood –ruminants, horses, dogs
*subcutaneous abdominal vein –lactating cattle (anterior mammary vein)
*Middle coccygeal vein-cattle, pig

*Cephalic (radial) vein-cat, dog
*Recurrent tarsal vein-cat, dog
*anterior vena cava-pig
*Cardiac puncture-Rodent

Samples should be collected in perfectly dry container to avoid haemolysis. The superficial vein generally need to be occluded to distend them by application of pressure with the fingers or a suitable tourniquet for a brief time usually not long[2mins]. This should not be too tight.

Sampling requires sharp and correct size of needle. Needles are inserted into the superficial vein at about 30 degrees to the skin.

Blood is withdrawn by gently applying negative pressure [traction] on the plunger of the syringe. Blood is delivered into the container after removing the needle. Selected anticoagulant is necessary in the container if unclotted blood is required. Adequate mixing is ensured by gently inverting the container several times immediately after sample is discharged into the container to ensure proper and uniform mixing. The choice of anticoagulant depends on the type of examination required.

Blood smears should be made as soon as possible, since anticoagulants have effect on the morphology of the cells (rbc & wbc) when exposed to them over a long time.

The rbc (biconcave cells) are produced in the bone marrow of adult animals with the regulatory influence of erythropoietin, Cu, vit B_{12} and proteins etc. Erythrocytes are generally/normally non-nucleated cells in the mammals, nucleated in fish, reptiles & birds. The main function of the rbc is to convey O_2 to the cells/tissues by making use of haemoglobin molecules it carries.

Haemoglobin is made up of 2 main units: Haeme and globin. The haeme which isc a unit with iron carries oxygen to tissues. Pathology of the rbc therefore is mainly in the area of production, morphology & number of the cells and the nature of haemoglobin, ie. Haemoglobinopathy.

Erythropoiesis is a continuous process. The lifespan of a normal RBC is about

140-150 Days in horse

80 Days in Cattle
52Days in Sheep
62-70 Days in Pigs
110-120 Days in Dogs
68-77 Days in Cats

Normally millions of RBC are being removed from circulation every minute due to old age. An old RBC [senescent RBC]are removed from circulation either by in tissue eg spleen or a small percentage get lysed in circulation. In both cases, the iron past of the haeme is removed for reuse by the bone marrow. These normal produres could also be disturbed in pathological cases.

Before being reused the iron moiety is locked in the macrophages as ferritin or haemosiderin when need they are released into circulation back to the BM.The remaining past of the haeme is converted in the liver to bilirubin.

Apart from the mature RBC which constitute about 95%/99% of the total erythrocytes there are few mature cells in circulation. These are reticulocytes (non-nucleated) but still with some RNA. Reticulocytes are better identified by special stains (supravital stains):

a) Brilliant cresyl blue in alcohol or saline for reticulocytes
b) New methylene blue

giving an orange coloured cell with purple network of RNA.
Normally, dogs , cats and pigs have little or reticulocytes in circulation; 0% in ungulates. Presence of reticulocytes in noticeable number in peripheral circulation is therefore a regenerative reaction of the bone marrow.

DETERMINATION OF PACKED CELL VOLUME [HAEMATOCRIT]

This is generally to determine the proportion in volume of the red blood cells corpuscles in relation to the total volume of blood in circulation.
It provides a good evaluation of the RBC status ie. information about the erythrocytes and haemoglobin in the circulatory system. This is b/c the volume of the rbc in normal blood is directly proportional to their no. and to the Hbg. Value.

General method:

A plane capillary tube is filled with anticoagulated blood eg. Blood with EDTA. Alternatively, freshly collected blood could be filled into heparinized (heparin-coated) capillary tubes, which are available commercially. Likewise heparinized capillary tubes can be used to directly collect blood from the animal eg. from the ear vein. The capillary tubes are filled to about ¾ or ⅞ of the length. The tubes are sealed at one end either with plastiseal or the use of Bunsen burner. The tubes are arranged in a special microhaematocrit centrifuge which is fitted with a head for carrying up to 24 capillary tubes. The capillary tubes are arranged in a circular manner with the sealed end outward (centrifugal) and the open end towards the centre (centripetal). The properly covered m-centrifuge is set to rotate for 5 minutes at 12,000 rpm. PCV is read by the use of special microhaematocrit Reader.
Buffy coat is a band of leucocytes and thrombocytes immediately above the packed red cells.

Inferences:

-Anaemia-------Low PCV value
-Normal PCV
-Haemoconcentration-----High PCV value

Total Red Blood Cell Count.

Because of the large population of rbc in the blood, examination is only possible after reasonable dilution has been made with suitable diluting fluid.

Examples of diluting fluids:

a. Hayem's diluting fluid:
   -1g. of Sodium chloride
   -5g of Sodium sulphate
   -0.5g of mercuric chloride
   -200ml distilled water.
   Filter if necessary.
b. Dacies' fluid:
   -99ml of 3% aq. Solution of Sodium citrate

-1ml of 40% formaldehyde

Preferred for it keeps and preserves the cells better.

c. Physiological saline- where clumping occurs as a result of the above fluids.

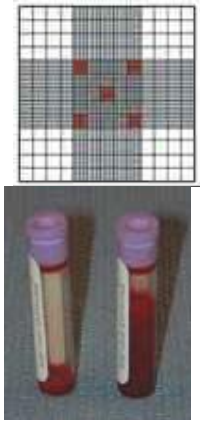
The basic equipment required in rbc total count are: microscope and haemocytometer with the diluting pipettes. Blood sample is initially drawn into the red dil. pipette held in horizontal position to the 0.5 mark on the stem. The open end can be wiped off and can be used to withdraw the overdrawn blood by the use of cotton wool or filter paper. In a vertical position in the dil. fluid, draw the volume by gentle suction and rotation into the pipette beyond the bulb to the 101mark . The pipette is further gently mixed for about 2 minutes with the thumb and a finger at both ends in a horizontal position.

2 chambers separated by Central Moat

25 groups of 16 squares each =400 small squares.

It is recommended to select the 4 corner groups and the central group of 16 smallest squares, giving

1. Count only those erythrocytes which touch the left hand end and upper lines of any square
2. Disregard those touching the right-hand and bottom lines.



R=Average of two(2) fields

Area of a smallest Sq=1/400mm2

Dept =1/10mm

Volume =1/4000mm3 for a Sq.

80 Squares =80/4000=1/50mm3

Dilution factor =200

Actual =200×50×R cells

=10,000R

eg R=(661+685)÷2=673

POLYCYTHAEMIA VERA

Primary –dehydration etc

Secondary – diseases-respiratory problem

ANAEMIA

Below normal range of the RBC and or haemoglobin value

-haemorrhage

-haemolytic
-Dyshaemopoietic
-Hypoplastic
(*)-congestive heart failure
-chronic respiratory diseases
-Pulmonary &mediastinal neoplasia

There is tissue hypoxia which leads to increase in circulating RBC.

Evaluation of RBC parameter

Determination of Hb value:

1) Tallquist method: this is an indirect method. Comparing the colour of whole blood with a colour standard. The method is not sensitive and could be of upto 40% error
   Here, a drop of blood on blotting paper is allowed to dry. The colour is compared with a colour scale graduate upto 100% = 13.8 g/100ml. the method is best used for screening purpose only
2) oxyHaemoglobin method : a direct method used to measure the oxyhaemoglobin by high absorption with the help green filter in spencer haemoglobinometer. A drop of blood is lysed in a glass chamber the RBC are lysed by the used of applicator stick to obtain a clear fluid , which is allowed unto the haemoglobinbinometer. The Hb is read off
3) Haematin method: acid haematin or Sahli's method. Use of a special haemoglobinometer and pipette. Mix fresh blood with N/10HCL in a pipette to a standard level to for acid haematin. The colour is diluted to be at par with colour of a standard tinted glass within 30min the content of the pipette is there by recorded on the graduated scale.(defect in the microvasculature through which RBC circulate)
4) Cyanmethaemoglobin method: 0.02ml of blood is added to modified Drabkin's solution 9put cyanide 0.05g , K-ferrocyanide 0.20g, dist H2O TO 1L) cyanmethaemoglobin is read in a photoelectric colorimeter with suitable filter (yellow-Green)
5) Carboxyhaemoglobin

DETERMINATION OF MCV

Mean corpuscular volume. This is the cubical volume of the erythrocyte
MCV=(PCV×10)/RBC Fl or
PVC/RBC x 10
MEAN CORPUSCULAR HAEMOGLOBIN (MCH)
The amount of haemogobin expressed in each erythrocyte
Hb/RBC×10 pg

MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION (MCHC)
Hb/PVC *100

CLASSIFICATION OF ANAEMIA
Base on Aetiological, morphological, responsiveness.
a). Based on Aetiology

1) Haemorrhagic anaemia
   -acute blood loss (profuse blood loss eg. traumatic cut);

-chronic blood loss
*chronic haemorrhages
-GIT ulcers
-Enteritis
-Coccidiosis
-Neoplasm
-Haemophilia[dogs &foals]
-Surgical operation
-Hypersplenism

Causes
Vit C & K deficiency
Parasites eg. *Haemonchus contortus*, Hook worms, ectoparasites
Plant poisioning eg. warfarin, sweet clover, bracken fern in cattle
-Traumatic injuries
-Idiopathic thrombocytopenia purpura

2) Haemolytic anaemia- caused by lyses of RBC within the vessels
Causes-Intrinsic
-Extrinsic
Clinical Feactures:
Splenomegaly
Lymphnodepathy
Jaundice
Lethargy
Headache
I) Intrinsic causes
-RBC membrane defect eg. hereditary spherocytosis
-abnormal haemoglobinopathy eg. sickle cell
-here metabolic defect as in pyruvate kinase deficiency
II) Extrinsic causes-Acquired haemolytic anaemia eg. Idiopathic auto-immune haemolytic anaemia
-Iso-immune hereditary disorders eg. haemolytic disease of the new born.[piglets, foals, puppies, Man(children)]
-incompatible transfusion
III) Infections-eg *Babesia sp, Anaplasmosis* in ruminants, haemobatonellosis, bacillary haemoglobinuria (*Cl. haemolyticum*) in cattle & horses
IV) Drugs, Chemical poisions-Cu in [sheeps], Pb, Phenothiezine, mercury in ruminants and pigs

3) Dyshaemopoietic anaemia- selective depression of erythrogenesis.
Causes :
a)nutritional deficiency –Cu , Co, Fe, Protein, Vit. Deficiency
b)parasitic diseases eg some worm infestation
c)chronic infectious diseases eg. chronic infectious nephritis in dogs, FeLV, Ehrlichiosis
d)hypothyroidism
e) Neoplasm

4) Hypoplastic (or aplastic) anaemia
Due to generalized bone marrow depression affect all other cells and platelets
This can be caused by:
-plant food poisioning eg. bracken fern in cattle, trichloro-ethylene from soya bean meal in calf
-irradiation
-sulphonamide poisoning.

Based on Morphological classification:
Based on the size of the RBC and the content of the haemoglobin eg.
a)macrocytic, normocytic ,microcytic
b)hypochromic or normochromic
combine a)&b)
-microcytic hypo chromic
-macrocytic normochromic(megaloblastic anaemia)
-macrocytic hypochromic

c) Regeneration or non-regenerative anaemia
regenerative anaemia are those that the bone marrow is responding adequately(actively) by increasing the production and release of erythrocytes into the circulation. Here one can see immature RBC in circulation[reticulocytes]. Such anaemia is characterized by the presence of polychromasia. Special stains will desmonstrate reticulocytosis.
Regenerative anaemia is a feature of haemorrhagic and haemolytic anaemias.
Non-regenerative anaemia shows that there are problems with the BM which could be either nutritional or toxic.

Morphology of RBC in stained Blood Smears

1) Polychromatophilic cells –are those that pick up uniformly blue stain with Romanowsky stain or Wright stain. The presence of such RBC in a film is referred to as polychromasia.

2) Erythroblasts –with nuclei (nucleated RBC). These cells will disappear normally after few weeks of age in circulation

3) Cells with eccentrically placed inclusion bodies known as Howell-Jolly bodies eg. in cats and horses. They are regratile bodies when stained with NMB. These H-J bodies (nuclear reminants indicate chemical or drug toxicity eg . phenothiazine in horse

4) Anisocytosis: this describes the presence of variation in the size of RBC

5) Poikilocytosis: this describes the presence of variation in the shape of the RBC not due to handling or making of the smear

6) Schistocytes-fragments of RBC eg. in DIC(disseminated intravascular coagulation)
7) Spherocytes –small rounded cells that are deeply stained without central parlar eg. in immune-mediated haemolytic anaemia.
8) Heinz-Bodies-pieces of oxidized haemoglobin appearing as protrusion (nose-like projection) from the side of RBC. They appear as pale circular structures common in haemolytic anaemia caused by toxic materials like onion, benzocaine copper which inflict oxidative injury on the RBC
9) Distemper inclusions- viral nucleocapsids which stain pink or blue
10) Reticulocytes (inmatured RBC)will show strips network or short chains of ribosomes in the RBC. The appear as pale yellow cell with basophilic precipitates of RNA. Best viewed at 100X. increased reticulocyte-reticulocytosis
11) Anisocytosis and poikilocytosis result from the marked presence of macrocytic cells,spherocytes , schistocytes ,microcytes and acanthocytes.

12) Acanthrocytes- RBC with 2-10 blunt elongated fingerlike surface projections. It is associated with haemangiosarcoma in the liver.

13) Elliptocytes – oval shaped RBC
14) Dacryocytes- tear drop shaped RBC
15) Stomatocytes –cup shaped RBC
16) ECCENTROCYTES (blister cells) –RBC with haemoglobin concentration at one pole with an unstained area at the other end.eg. in haemolytic diseases, membrane defect
17) Borr-cells(Echino elliptocytes)-elongated RBC with ruffled margins
18) Crenation : presence of RBC covered by short spiky surface projections. Most are artifact and can be confused with ecanthocytes. It can be differentiated from ture poikilocytes for it is uniformly affecting almost all RBC in a given film area as opposed to scatted RBC on the blood film. Staining of wet blood film will produce REFRACTORY BUBBLES on the surface of the cells.

Parasite like haemobartonella should not be confused with stain aggregates which are ppts. of stains along the sides of the RBCs. The aggregate do not resemble any of the parasites and vary in size. They appear as refractile(jewel) when focused at 100x

Haemolytic Anaemia:

Results from defect on the RBC (inherent & acquired) or defect in microvasculature through which the RBCs circulate.

Intravascular haemolysis- within the vessels

Extravascular haemolysis- outside the vessels eg by macrophages in the spleen & liver.

Some haemolysis occur both intravascularly and extravascularly.eg. haemobartonellosis, Babesiosis, pyruvate kinase def. Pyruvate kinase(PK) is an enzyme in the glycolytic pathway essential for the production of ATP. ATP is essential to maintaining RBC membrane stability. PK def. is a hereditary disease in Dogs.

Microangiopathic haemolysis-mechanical intravascular sharing of RBC while passing through abnormally tortuous capillary beds eg in glomerulonephritis, DIC and haemangiosarcoma.

Turbulent flow of blood- large vessels eg in heartworm disease and TRAUMATIC disruption of red cell in heart disease.

Some characterics features seen in Anaemias

-Usually accompanied by mild pancytopenia
-Anaemia appear generally as Normocytic Normochonic to macrocytic normochronic
-presence of occasional megaloblasts in blood films
-presence of occasional giant but fully haemoglobinized RBC
-presence of occasional RBC with bizarre and or multiple nuclear fragments (inclusion bodies)
Ineffective erythropoiesis[Maturation Defect Anaemia(MD)]
a) Nuclear defect (megaloblastic anaemia)
-Failure of the precursor nuclei to divide and mature at the same rate as the cytoplasm.
Asynchrony of nuclear/cytoplasmic maturation.

c) Cytoplasmic defect
-iron defect
-lead defect
-Vit. B6
-Large cells with immature pale nuclei with irregular chromatin clumps
-cytoplasm is too haemoglobinized for the degree of nuclear maturaration
Cytoplasmic Defect: failure of haemoglobin formation. The RBC continues to divide, in the presence of mature nucleus.
Hypercellular red cell Bone marrow (BM). Small metarubricytes.
The RBC continue to divide to smaller cells because they never acquire full complement of haemoglobin.
Normal reticulocytes produce/release at slow rate.
Blood film=microcytic,hypochromic anaemia
b/c of small RBC with increased area of central pallor
-fragile RBC=poikilocytosis
- low polychromesia

Normocytic normochronic anaemia

1) Anaemia due to inflammatory disease and neoplasia. The anaemia is accompanied with hyperplasia of the white blood cells. BM (bone marrow) smear will be with erythroid hypoplasia, granulocytic hyperplasia.
2) Anaemia with selective Erythroid Hypoplasia
   eg. -low erythropoietin production
   -Hypothyroidism (reduced oxygen demand by peripheral tissue)
   - Selective RBC precursor cells destruction as in toxic or immune-mediated mechanism.
   In general bone marrow hypolasia(aplastic anaemia)
   -severe anaemia

-severe leucopenia
- thrombocytopenia may be present or absent
Aetiology –toxic or immune-mediated destruction of precursor cells
-myelophthisic anaemias
Toxic materials:- infection agents-FeLV, ehrlichiosis
-Toxic chemicals- Estrogen , drugs [griseofulvin in cat]
-Ionizing radiation

Myelophthisic anaemia (occupation of the Bone marrow (BM) by other abnormal cells & connective tissues)

a) Neoplastic diseases
b) Non-neoplastic diseases

1) Most common are:
-haematopoietic
-lymphoid leukemia, lymphosarcoma
-Granulocytic leukemia.

2) myelofibrosis [conn. tissue replacement of BM]
by -estrogen toxicity
-ionizing radiation
Blood Films:
i-non regenerative anaemia
ii-presence of poikilocytes

Polycythaemic –increased in circulation of RBC is characterized by increase in PVC, Hb, RBC values than normal range values. Polycythaemia can be:

a) Relative: When there is a decrease in plasma volume due to dehydration.
b) Transient: Here polycythaemia is due to excitement and is usually just for about an hour after which RBC values return to normal.
c) Absolute: due to increased BM[bone marrow] production of RBC.

- VETERINARY CLINICAL PATHOLOGY
- BY Dr. AJAYI, O.L.
- Veterinary Clinical Biochemistry
- PREAMBLES
- Variables affecting chemistry test results
- Many factors other than disease influence the results of chemistry tests. These factors may be pre-analytical, analytical and post-analytical.
  Preanalytical variables are variables associated with the patient, sample collection and sample handling. These generally affect the composition of the body fluid before analysis.
  Analytical variables are factors which influence the analytical procedure.
  Post-analytical variables involve the different ways data from the laboratory is presented, stored and transferred to the clinician.
  Whenever possible, these variables should be controlled in order to minimize their effect on test outcome.
- PRE-ANALYTIC VARIABLES
- Preanalytical variables affect the composition of the sample (and hence sample quality) before the sample is analyzed in the laboratory. These variables are the most important and common sources of error in laboratory analysis and are often

overlooked when interpreting test data. Control of preanalytical variability involves proper sample collection, preparation and handling by the veterinarian or technician. When such factors cannot be controlled, they must be recognized and considered when evaluating results for disease.

- Preanalytical variables include biological (patient) and non-biological (sample) factors
- Preanalytical variables contd.
- BIOLOGICAL OR PATIENT VARIABLES Biological variables are associated with the patient. There are factors that are inherent to the patient, such as breed, age and sex, which cannot be controlled, but must be considered when evaluating test results. Other biological variables involve those factors which can be controlled by you, the veterinarian, when drawing the blood sample, such as ensuring the animal is fasted for 12 hours before sample collection.
- Preanalytical variables contd.
- INHERENT PATIENT OR BIOLOGICAL VARIABLES These variables are inherent to the patient and cannot be controlled. They must be considered when interpreting test results.
- Species: There are species-specific differences in the source of analytes e.g. alanine aminotransferase is a liver specific enzyme in small but not large animal species.
- Breed: e.g. healthy Draught horses typically have higher creatine kinase values than Thoroughbreds
-
- Preanalytical variables contd.
- Age: Many chemistry analytes change with age, i.e. young animals e.g. elevated alkaline phosphatase in all species
- Gender: Physiological states associated with pregnancy or lactation in female animals can affect analyte.
- Controllable Biological or Patient Variables
- Standardization of collection techniques can minimize the effect of these variables on chemistry results. If these variables are unavoidable or desirable (e.g. sample collection post-exercise to detect rhabdomyolysis in horses), they must be considered during test interpretation.
- Recent food ingestion: This will produce a post-prandial Lipemia. Lipemia may affect the analytical methods used to measure certain plasma constituents, thus producing invalid results.
- Stress: Stress (secondary to animal handling or underlying disease) may have profound effects on laboratory results, due to endogenous corticosteroid and/or adrenaline release.
- Exercise: The effect of exercise on plasma constituents is dependent on both the species and the intensity of the exercise. In general, blood samples should be collected from animals prior to exercise.
- Controllable Biological or Patient Variables contd.
- Drugs: The technique of drug administration, e.g. intramuscular, can directly affect analyte results. Which analytes may be affected by intramuscular drug administration?

  Drugs can also interfere with measurement of the analyte. Drug interference can be grouped into two general categories:

  1)) Physiological (in vivo) effects of the drug or metabolites on the analyte to be measured

  2) In vitro effects due to some physical or chemical property of the drug or its metabolites, which interfere with the actual assay procedure.

  This markedly interferes with both hematologic, chemistry and urinalysis results. Interference with chemistry tests is dependent on the method and instrumentation used.

- Non-biological or Sample Variables
- Non-biological variables involve sample collection, handling and transport to the laboratory. All of these variables can be controlled to minimize the effect they have on laboratory results.
- Sample Collection
- Strict attention should be paid to sample collection to minimize artefacts induced from poor sample collection.
- Venipuncture: Clean venipuncture is essential to minimize artefactual changes in the results. Poor venipuncture technique may cause hemolysis, which alters chemistry results in a variety of ways.
- Anticoagulant: The preferred samples for chemistry tests are heparinized plasma or serum (no anticoagulant). Fluoride-oxalate anticoagulant can be used for glucose. Citrate and EDTA anticoagulants should not be used for chemistry tests.
- Sample Handling
- After sample collection, strict attention should be paid to sample handling to minimize the effects of these variables on results.
- Separation: Whenever possible, especially when there is to be a delay between sample collection and submission to a laboratory, serum or plasma should be separated from cells as soon as possible after sample collection.
- Labeling: All body fluid samples taken from a patient should be correctly labeled with the patient name or identification and the type of specimen (e.g. serum, plasma, synovial fluid, peritoneal fluid).
- Storage: Most veterinarians do not have the advantage of having a clinical pathology laboratory within walking distance of their clinic. There is usually at least a 24-hour delay between sample collection and delivery to the laboratory under the latter circumstances.
- ANALYTICAL VARIABLES
- Analytical variables affect the procedure by which the analyte is measured by the instrument. Analytical variables are caused by features inherent to the sample, e.g. interferences such as lipemia, or by features inherent to the analyzer. The latter are minimized by using quality assurance procedures within the laboratory
-
- Interferences

- The degree to which endogenous substances interfere with chemistry analyzers depends on the type of analyzer, the methods used to detect the analytes and the amount of interfering substances in the sample (i.e. laboratory-specific).
-
- Causes of interferences
- Lipemia: Lipemia is caused by increased triglycerides (as chylomicrons or very low density lipoproteins). Lipemia interferes with chemistry tests by the following mechanisms.
  1) Light scattering: Results in falsely increased absorbance readings of some analytes, e.g. total bilirubin, hemoglobin.
  2) Volume displacement/solvent exclusion : This falsely decreases values of some analytes, e.g. electrolytes (mostly sodium and chloride, but also potassium to a lesser extent).
  3) Hemolysis: Hemolysis of erythrocytes is enhanced in the presence of lipemia.

- 5) Water release: Release of red blood cell water dilutes analytes.
- Icterus: Bilirubin interference arises from its spectral properties and its ability to react chemically with other reagents (resulting in decreased analyte values). This particularly affects creatinine concentrations.

- Hemolysis: Hemolysis is usually an in vitro artefact due to poor venipuncture technique, lipemia, freezing of whole blood samples, delayed separation of serum or plasma from cells, or delayed sample submission. However, hemolysis can occur in vivo with certain types of hemolytic anemias
  Hemolysis interferes with chemistry tests by the following mechanisms:
  1) Increased absorbance: Released hemoglobin increases absorbance in the hemoglobin spectral range.
  2) Inhibition of reactions: Released hemoglobin can directly inhibit chemical reactions.
  3) Analyte release: Release of analytes found in high concentrations in red blood cells will falsely elevating the values of these analytes .
  4) Enzyme release: Release of enzymes which participate in chemical reactions, e.g. adenylate kinase

- .
- Hyperproteinemia: Monoclonal immunoglobulins (paraproteins), particularly when present in high concentration, interfere with chemistry analysis by the following mechanisms:
  1) Hyperviscosity: This affects sample volume and is dependent on the class of immunoglobulin (which immunoglobulins produce hyperviscosity and why?).
  2) Binding to analytes: Immunoglobulin binding to some analytes producing increased or decreased analyte values, e.g. hyperphosphatemia has been reported in a dog with chronic lymphocytic leukemia and an IgM monoclonal gammopathy.
  3) Volume displacement: This has effects similar to lipemia.
- Drugs: There are a number of different methodology-related drug interferences that may bias results. One of the most common drug interferences seen in veterinary medicine is the artefactual elevation of chloride values in samples from dogs on bromide therapy (an anticonvulsant). It is always wise to inform the laboratory of any medications in a particular animal, so that drug influences on chemistry tests can be minimized (but usually not eliminated).
-
- Quality Control, Test Validity and Reference Values
- Laboratory Quality Control
- Valid laboratory test results can be among the most valuable components of the diagnostic process, likewise invalid test results can be among the most dangerous.
- The essence of a good quality control is to defect potential source of error in laboratory valves.
- A good quality control programme must include the following:
- (1) Systematic and periodic monitoring of proper operation of equipment such as spectrophotometer, electronic cell counters e.t.c.
- (2) A system of monitoring reagents inventory to ensure that reagents are in this their expiration dates.
- (3) Use of control with known ranges of acceptable results for each test performed in the laboratory.
- (4) Written records to monitor each of the above components and document that they are being executed.
- Quality control should also be a daily practice.
-
- Quality Control, Test Validity and Reference Values cont.
- Laboratory works performed in private practices is rarely performed in such an optimal setting; therefore, the likelihood of serious error is much higher.
- II Validity of laboratory test results

- (A). Assay validation
- Because reagents and instrument used to perform laboratory tests on animals specimens are often marketed solely for testing human specimens, one cannot assume that a known procedure will produce valid results on samples from other species.
- Quality Control, Test Validity and Reference Values cont.
- Typical components of a validation procedure include assessment of:
- (a) Analytical specificity: Which is the ability of the assay to measure the analyte in question in the presence of potentially interfering substances.
- (b) Analytical sensitivity: Which is the lower limit of detection of the analyte.
- © Accuracy: This is the degree of agreement between the results and some standard or true value.
- (d) Precision: The precision of a laboratory method is its reproducibility. A test can have a high degree of precision but a low degree of accuracy. Factors influencing precision are assay methodology, instrumentation and skill of the technician.
- A common means of measuring and reporting the precision of a quantitative laboratory method is the coefficient of variation (cv).
- The cv is expressed as a percentage and can be determined by running the same sample repeatedly. The mean and standard deviation of the set of results is calculated, and the cv is expressed as follows:
- Cv = 100 (stand deviation ÷ mean)

Quality Control, Test Validity and Reference Values cont.

- Accuracy: This is monitored in the following ways.
- (a) Regular use of control materials for which values have been determined in outside reference laboratories.
- (b) Regular participation in laboratory proficiency programs in which the laboratory period really receives samples of an known composition to be assayed.
-
-
- (2) Precision
- (a) Graphic plots and rule based facilitate the evaluation of accuracy and precision using control data.
- III Reference values are usually a set of values from a group of normal healthy animals which conform to a group of stated, selected and known criteria.
- Needed to provides a basis for comparison with values obtained from sick animals.
- Quality Control, Test Validity and Reference Values cont.
- The stated selection criteria may include.
- (1) Clear definition of the clinical parameters used to select a clinically normal or health animal.
- (2) Population parameter.
- i Species (PCV higher in dogs than cats)
- ii Breed (Active breeds have higher pcv).
- iii Age (young animal have higher protein concentration and lymphocyte
- iv Sex .

Quality Control, Test Validity and Reference Values cont.

- 3) Environment and physiological conditions.
- (a) Diet (animals on high protein diet have higher BUN)

- (b) Fasting (B/d glucose and bile acids are lower following fasting)
- (c) Pregnancy
- (d) Level of excitement (pcv and lymphocyte are higher in exercise cats).
- (e) Body condition (Creatinine is higher in well-muscled animals).
- (f) Altitude (RBC of cattle is higher at high altitude).
- (g) Medications (corticosteroids the Neutrophil counts and ALP are higher)

Quality Control, Test Validity and Reference Values cont.

- (4) Specimen collection and handling
  - (a) Collection site
  - (b) Anticoagulant
  - © Sampling time
  - (d) Collections interval before testing. .
  - (e) Storage condition.
- (5) Analytical method (including instrumentation reagents, specific calibration standard and calculation method.
- (6) Statistical method used to derive reference intervals.
- The more diverse the population parameters, the higher the reference interval. e.g. A population of dogs of any age, seize or breed, fasted or nonfasted varying in health.
- Reference values should not be transferred from one laboratory to the other unless analytical method is the same.

Quality Control, Test Validity and Reference Values cont.

- Reference interval determination
- Clinical Relevance of Test Results
- - Test values that fall within the reference interval are considered negative for a specified disease condition. If the disease condition is absent, it is a true negative (TN), and if the disease is present, it is false Negative (FN).
- - Test values that fall outside the reference interval are considered abnormal and positive for the disease condition. If the disease is present it is a true positive (TP) and if the disease is absent, it is false positive (FP).
- Test vary in their FP and FN rates for various disease and are defined by their sensitivity, specificity and predictive value for the particular disease.

Quality Control, Test Validity and Reference Values cont.

- Sensitivity
- Sensitivity is the frequency of a positive or abnormal test result (e.g. one outside the reference interval) when a disease is present. It is the ability of the test to identify disease subjects.
- Sensitivity = [TP ÷ (TP + FN)] x 100
- The more sensitivity a test, the more the FP, and less the FN. Therefore, when sensitivity increases, specificity decrease. Tests are seldom highly sensitivity and specific at the same time.
- Narrowing the Reference interval will increase sensitivity at the expense of specificity. (FPs are increase and FNs are decrease). Screaming tests need a high degree of sensitivity becomes FN results are more unacceptable than FP

results.

- 2. Specificity
- This is the frequency of a negative or normal test result when a disease is absent (i.e. the percentage of TN results). It is the ability to identify patient, that do not have a particular disease.
- Specificity = [TP ÷ (TP + FN)] x 100
- Widening the reference interval will increase specificity at the expense of sensitivity (FPs are decrease and FNs are increased).

- Predictive value of a positive test (P+)
- PV+ is the % of patient with a positive or abnormal test result that are diseased.
- PV+ = [TP ÷ (TP + FP)] x 100
- Pv+ is affected by sensitivity, specificity and prevalence of the disease in question.
- The PV+ of a tests is higher when applied to a population with a high incidence of the disease; a positive test is more likely correct. This occurs when a population is selected because clinical signs or physical examination suggest a particular disease. The use of multiple tests will increase the PV+. Tests in series ↑s the Pv+. Specificity is ↑ at the expense of sensitivity becos there will be fewer FPs but more FNs when compared to using single test.

- Predictive value of a negative test (Pv-)
- PV- is the % of patients with a positive or abnormal tests results that are free of disease.
- PV+ = [TN ÷ (TN + FN)] x 100
- Doing multiple tests in parallel (biochemical profiles or batteries of tests) increase the Pv-. Sensitivity is increased at the expensed specificity. There are fewer FNs but more FPs when compare with a single test. The greater the number of tests in the profile the greater the chance of FPs.

REFERENCE RANGES

- Reference ranges vary considerably from one laboratory to another, and are dependent on the methodology and instrumentation utilized. This is especially true for serum enzymes, where methods may vary in pH, temperature, specific cofactors and substrate used in reaction. As a consequence, published "normal" values may not be valid for results generated by your lab. Reference ranges should be established by each laboratory, as they are instrumentation and reagent dependent. You should not compare one laboratory's results to another laboratory's reference intervals.
  Reference ranges are usually determined from a population of healthy adult animals. There are 2 general methods for determining reference ranges, based on the distribution of the data from these healthy animals. The resulting range then will include 95% of normal samples, regardless of the method. As a result, up to 5% of normal animals may fall slightly outside the reference range for a given test.
- When numerous tests are run on the same animal, the chances of obtaining one or more slightly "abnormal" results on an animal that is actually normal rises (p = 1 - 0.95). For 12 tests, p = 0.46; for 21 tests, p = 0.66.

- Gaussian distribution: This is when the data is normally distributed, i.e. distributed symmetrically around the mean as illustrated to the right. The reference range is calculated as the mean ± S, which encompasses 95% of the observations in healthy animals. The top 2.5% and bottom 2.5% results from healthy animals will fall outside an established range.
- Non-Gaussian distribution: For data that is non-gaussian (i.e. skewed), the data can be mathematically transformed, e.g. to logarithms. If this yields a normal or Gaussian distribution, the geometric mean ± 2 SD can be used for reference range determination (geometric means are based on the log-transformed data). However, in most instances, percentiles are used to create reference ranges from non-Gaussian data.
-
- CHEMISTRY PATTERNS
- Chemistry tests can be grouped together on the basis of body system or physiologic process. Grouping tests into common parameters is the best way to interpret chemistry data as it enables pattern recognition. Patterns of change within and among these groups can provide useful diagnostic information about disease involvement of various organ systems. Grouping tests together can also help you select certain tests to identify disease processes in certain body systems. Test selection is important if cost is a factor in laboratory testing.

- The following is one way of grouping routine chemistry tests:
- Electrolytes: Sodium, potassium and chloride.
- Acid/base parameters: Bicarbonate, anion gap.
  Note: Acid base status is dependent on electrolytes, so these should be interpreted together.
- Minerals: Calcium, phosphate and magnesium.
  Note: These are often influenced by kidney function, so kidney parameters should be evaluated concurrently. Also, many bovine practitioners order mineral and electrolyte panels.
- Protein parameters: Total protein, albumin, globulin, A/G ratio.
- Kidney parameters: Urea nitrogen, Creatinine.
  Note: Urine specific gravity should be concurrently evaluated when assessing renal function, especially on initial presentation of the animal. In addition, kidney function affects proteins, minerals, electrolytes and acid-base balance, as well as hematopoiesis. (Can you think of how renal disease alters hematopoiesis?)
- Liver parameters:
  Hepatocellular leakage enzymes: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Sorbitol dehydrogenase (SDH), Lactate dehydrogenase (LDH).
  Cholestatic enzymes: Alkaline phosphatase (AP), gamma glutamyl transferase (GGT).
  Liver function tests: Total bilirubin, direct bilirubin, indirect bilirubin, bile acids, ammonia.
- Pancreatic parameters: Amylase, lipase, trypsin-like immunoreactivity.
- Carbohydrate parameters: Glucose, fructosamine, glycosylated hemoglobin.
- Lipid parameters: Cholesterol, triglycerides.
  Note: Lipid metabolism is altered by disease processes affecting the pancreas and glucose homeostasis.
- Muscle parameters: Creatine kinase (CK), AST, ALT, LDH.
- Iron parameters: Serum/plasma iron, total iron binding capacity (TIBC), % iron saturation of transferrin.
- Electrolytes
- In general, electrolyte levels in blood are influenced by changes in free water and by changes in electrolytes themselves, namely rate of intake, excretion/loss, and

translocation within the body. Translocation can occur via movement into or out of cells or into specific fluid compartments, such as a distended abomasum.

Sodium

- Interpretation of Serum Sodium Results
- Na+ concentration is inextricably linked with extracellular fluid (ECF) concentration, therefore interpretation of sodium levels should always include consideration of the hydration status of the patient (and therefore, changes in free water). Sodium is the major extracellular cation and is a primary determinant of plasma osmolarity and ECF volume. The body attempts to maintain a constant ECF volume, as major changes in ECF volume
- Sodium cont.
- can have profound effects on the cell. The kidney plays a critical role in maintenance of ECF volume, via sodium and water retention. Regulation of body water is accomplished by monitoring of plasma osmolality (determined primarily by sodium concentration) and blood volume. This is achieved by osmoreceptors and baroreceptors
- Sodium cont.
- Osmoreceptors sense changes in osmolality. With hyperosmolality (hypernatremia), osmoreceptors stimulate vasopressin or ADH secretion from the pituitary gland and stimulate thirst. Thirst is stimulated by as little as a 1-2% decrease in osmolality. The end result is water retention by the kidney and increased water intake. Increases in free water will thus reduce sodium concentration. Opposite changes occur with hypoosmolality.
- Baroreceptors are sensitive to changes in effective circulating volume (ECV). The ECV is that part of the extracellular fluid that is in the arterial system and is effectively perfusing the tissues. It usually varies directly with ECF volume. With hypovolemia (decreased ECV), baroreceptors stimulate the renin-angiotensin system, the end result being mineralocorticoid (aldosterone) release from the adrenal cortex. Aldosterone stimulates increased absorption of NaCl and promotes the excretion of potassium and hydrogen in the distal tubules of the nephron. NaCl retention promotes water resorption, thus correcting the hypovolemia. Hypovolemia also stimulates thirst (a decrease in ECV of 7-10% is required for thirst stimulation). Opposite changes occur with hypervolemia.
-
- Hyponatremia

Hyponatremia results from retention of free water or excess losses of sodium from the body. Hyponatremia usually (but not always) indicates a hyposmolal state. It is important to correct severe hyponatremia gradually to prevent this fatal complication.

Causes of hyponatremia

- Pseudohyponatremia: Artefactual decreases in sodium occur with flame photometry or indirect potentiometry in hyperlipemic and hyperproteinemic states. Sodium concentrations can also be reduced in hyperosmolar states, such as hyperglycemia or mannitol therapy.
- Sodium cont.
- Gain of free water

1) Excessive water intake: This will result in increased GFR and decreased sodium absorption with natriuresis. This occurs with psychogenic polydipsia (has been reported in large breed dogs), the syndrome of inappropriate ADH release (ADH release without appropriate osmotic or volemic stimuli - has been reported in dogs secondary to heartworm infection and neoplasia), antidiuretics and hypotonic fluid administration. In these situations, animals are normovolemic.

2) Perceived decreased ECV: Inappropriate water retention occurs when the body perceives a decrease in ECV and stimulates non-osmotic ADH release (often due to hypoalbuminemia). This occurs in congestive heart failure, liver disease, nephrotic syndrome and advanced renal failure (in this condition, there are reduced numbers of nephrons to appropriately excrete the excess water from polydipsia). In these situations, animals are hypervolemic.

- Loss of sodium in excess of water (hypertonic fluid losses)

Sodium can be lost in excess of water via renal or non-renal mechanisms. This usually results in hypovolemia.

1) Renal losses: This occurs due to lack of aldosterone (necessary for sodium absorption in DCT of the kidney), proximal renal tubule dysfunction (resulting in reduced sodium absorption) in renal disease (especially in horses and cattle), osmotic losses due to polyuria (diabetes mellitus), and diuretic therapy. Cattle with renal failure have a consistent moderate to marked hyponatremia.

2) Non-renal losses: This includes gastrointestinal losses (diarrhea in cattle), third space losses (ruptured or obstructed urinary tract, pancreatitis, peritonitis, chylothorax) and cutaneous losses (sweating in horses).

Horses and cattle with severe diarrhea are very likely to be moderately or markedly hyponatremic and dehydrated. Hyponatremia is exacerbated by decreased intake of sodium in this situation. Dogs and cats with vomiting and diarrhea are less likely to be hyponatremic, unless there are other causes of sodium loss.

- Common causes of hyponatremia in small animals include gastrointestinal losses of sodium, diabetes mellitus (although concurrent dehydration may normalize sodium values), congestive heart failure (with or without diuretics), third space losses, addison's disease, hypotonic fluid administration, liver disease and diuretic administration. Common causes in large animals include diarrhea, sweating (horses), drainage of fluid, and sequestration within third spaces.

- Normonatremia
- Serum Na+ concentration within the reference range can still indicate an abnormal state if body water is abnormally high or low.
- Animals that are normonatremic but dehydrated have proportional deficits in body water and body Na+. Vomiting, diarrhea, and renal disease are common conditions in which normonatremia and dehydration are found.
- Normonatremic animals with increased extracellular fluid have increased total body Na+.

- Hypernatremia
- This can develop if water is lost in excess or if Na+ is ingested in excess of water. The first mechanism is the most common one. Hypernatremia is always associated with hyperosmolality and results in CNS signs due to cellular dehydration.

Causes of hypernatremia

- Pseudohypernatremia: This occurs with dehydration.
- Water deficit

Animals are usually normovolemic.

1) Excessive water losses: Panting (hyperthermia, fever, heat stroke) or losses through the kidney from diabetes insipidus (lack of ADH). Animals will not usually develop hypernatremia in these situations unless access to water is restricted concurrently.

2) Inadequate intake: Lack of access to water, primary adipsia/hypodipsia

- Hypotonic fluid losses: These can be renal or non-renal. Renal losses can occur with osmotic or chemical diuresis or renal failure (e.g. polyuric renal failure in horses and cattle). Non-renal losses include gastrointestinal losses (vomiting, diarrhea), third space losses, cutaneous losses (burns). Animals will not usually develop hypernatremia in these situations unless access to water is restricted concurrently.

- Salt gain
  Increased sodium intake (with restricted water access, e.g. salt poisoning in calves), hypertonic fluid administration, and increased sodium retention by the kidneys, such as in hyperaldosteronism.
- Hypernatremia is relatively uncommon in small animals. It can be seen with diabetes insipidus (if water deprived), panting and hyperaldosteronism. In large animals, it is often due to water restriction and salt poisoning, water deprivation, renal disease and hypertonic saline administration
- Potassium

- Interpretation of Serum Potassium Results
- Potassium is the major intracellular cation (intracellular K+ concentration is approximately 140 mEq/L) and is important for maintaining resting membrane potential of cells. 60-75% of total body potassium is found within muscle cells, with the remainder in bone. Only 5% of potassium is located in the ECF, therefore potassium concentration in blood is not always a reflection of total body potassium levels. Plasma (ECF) K+ concentration is tightly regulated; fairly small changes can have marked effects on organ function.
  Regulation of potassium
  Ingested K+ is absorbed non-selectively in the stomach and small intestine. Regulation of plasma K+ is by renal excretion and movement of K+ from extracellular fluid to intracellular fluid. If these mechanisms are functioning normally, the amount of K+ ingested has little effect on plasma K+. However, if one or more of the regulatory mechanisms is faulty, then the amount of K+ ingested can exacerbate abnormalities in plasma K+.

- Urinary excretion of K+ is largely by secretion of K+ into the urine by the distal tubules. 70% of filtered K+ is absorbed in the PCT of the kidney regardless of K+ balance. 20% is absorbed in the ascending limb of the loop of Henle and the remaining 10% is delivered to the distal nephron. Principle cells in the distal nephron secrete K+ and absorb Na+ under the influence of aldosterone. Intercalated cells in the distal nephron absorb K+ in exchange for H+. K+ is also excreted in the colon, which is also influenced by aldosterone.
  Translocation of K+ is largely dependent on insulin and catecholamines. Shifts of K+ in and out of cells can also occur with changes in the pH of ECF.
  Severe abnormalities of plasma K+ are life-threatening situations.
-
- Hypokalemia
- Hypokalemia increases the resting membrane potential of cells, resulting in muscle weakness, impaired urine concentrating ability, polydipsia and arrythmias. It is usually due to gastrointestinal or renal losses of potassium. Remember that blood K+ values are not always a reflection of total body K+ stores; K+ values can be normal in blood, despite severe deficits in total body K+.

  Causes of hypokalemia
- Pseudohypokalemia: Severe lipemia will result in a mild hypokalemia.

- Decreased intake: This occurs with anorexia in large animals, including horses (especially foals), camelids and cows. Decreased intake in small animals rarely results in hypokalemia unless there are additional losses of potassium. Hypokalemia can be seen in cats fed low K+ diets.
- Transcellular shifts: Shifting of K+ from ECF to ICF occurs with metabolic alkalosis resulting in alkalemia, insulin release or administration, and catecholamine release (from adrenaline stimulating beta2-adrenergic receptors and activating the sodium-potassium [Na/K] ATPase pump in muscle). Similarly,. endotoxemia may also result in hypokalemia because endotoxins also stimulate the Na/K ATPase pump in muscle cells.
-
-
- Increased loss

  The potassium deficit will be enhanced if intake of potassium is decreased.

  1) Gastrointestinal losses: Causes include vomiting of gastric contents (the loss of chloride enhances K+ excretion in the kidneys, promoting the hypokalemia), vagal indigestion, and diarrhea. Diarrhea in horses and cattle often produces a hypokalemia. Severe diarrhea and vomiting in dogs and cats can also result in hypokalemia. Saliva is potassium-rich and disorders such as choke in horses and cattle can result in hypokalemia.

  2) Third space losses/sequestration: Accumulation of fluid in body cavities (e.g. peritonitis) or distended gastrointestinal system (e.g. volvulus, ileus) can result in hypokalemia. This may be dilutional from perceived volume depletion due to losses of fluid from the intravascular space (with secretion of ADH and stimulation of water intake).

-
- 3) Cutaneous losses: Sweating (horses).

  4) Renal losses: Renal losses of potassium can occur via several mechanisms, the main one being aldosterone, which stimulates sodium absorption in exchange for potassium excretion in the principle cells of the collecting ducts. Aldosterone is stimulated by the renin-angiotensin system in response to hypovolemia and decreased delivery of chloride (hypochloremia) to the macula densa.. Potassium excretion is also enhanced by increased distal tubule flow rates (any cause of polyuria, e.g. glucosuria, post-obstructive diuresis), increased lumen electronegativity (high concentrations of unadsorbable anions in the renal tubule lumen, e.g. penicillin, ketones), increased distal delivery of sodium and metabolic alkalosis.
-
-
- Hyperkalemia
- Hyperkalemia decreases the resting membrane potential, predisposing the cell to excitability. This results in cardiac arrythmias.

  Causes of hyperkalemia

  Hyperkalemia is usually due to decreased renal excretion of potassium, due to renal failure, urinary tract obstruction or rupture, or hypoaldosteronism.
- Pseudohyperkalemia

  1) Serum: Serum K+ is higher than plasma K+ due to release of K+ from platelets during clotting. The difference between serum and plasma K+ in dogs with normal platelet counts can be as high as 0.6 mEq/L. This difference is greater with higher platelet counts.

  2) Hemolysis: This will increase K+ in animals with high K+ in their erythrocytes, including horses, pigs, cattle and cats. Remember that certain dog breeds have high K+ in their mature red blood cells, such as Akitas and other Japanese

breeds. All dogs have high K+ in their reticulocytes, so K+ can be artefactually elevated in hemolysed samples (or samples with delayed separation from cells) with high reticulocyte counts from any dog breed.

- 3) Leukocytosis: Very high leukocyte counts (> 100,000/uL) can result in hyperkalemia due to leakage of intracellular K+ from cells.
  4) Age: Potassium is higher in foals < 5 months of age than adult horses. Foals < 1 week old have higher K+ than foals > 1 week old.
  5) K+ EDTA: Contamination of serum/plasma sample with K+ EDTA will result in very high (non-physiologic) K+ values (>20 mEq/L) as well as marked hypocalcemia and hypomagnesiumia (due to chelation).
- Increased intake: This is usually iatrogenic and does not usually result in hyperkalemia if kidney function is normal, e.g. KCl administration.
- Transcellular shifts: Shifting of K+ from ICF to ECF occurs with tissue necrosis, exercise (this occurs especially in horses and is due to release of K+ from muscles - K+ is a local vasodilator for muscle cells), uroperitoneum (especially in foals), hypertonicity (e.g. diabetes mellitus - occurs due to solvent drag) and hyperchloremic metabolic acidosis. A high anion gap metabolic acidosis (titration acidosis) does not usually result in hyperkalemia as the organic anion moves into the cell with H+. Hyperkalemia in animals with a titration acidosis is not due to translocation, but due to decreased renal excretion of K+.
-
-
- Increased intake: This is usually iatrogenic and does not usually result in hyperkalemia if kidney function is normal, e.g. KCl administration.
- Transcellular shifts: Shifting of K+ from ICF to ECF occurs with tissue necrosis, exercise (this occurs especially in horses and is due to release of K+ from muscles - K+ is a local vasodilator for muscle cells), uroperitoneum (especially in foals), hypertonicity (e.g. diabetes mellitus - occurs due to solvent drag) and hyperchloremic metabolic acidosis. A high anion gap metabolic acidosis (titration acidosis) does not usually result in hyperkalemia as the organic anion moves into the cell with H+. Hyperkalemia in animals with a titration acidosis is not due to translocation, but due to decreased renal excretion of K+.
-
-
- Inherited causes
  Hyperkalemic polymyopathy of horses: This is due to a genetic defect in the alpha subunit of the sodium channel of muscle cells (the sodium channels remain perpetually open) observed in Quarterhorses and other heavily muscled breeds like Appaloosas and Paints. The condition appears to be clinically worse in males. It is characterized by intermittent episodes of muscle fasciculation and weakness concurrent with increases in serum K+ values.
- Drug-induced hyperkalemia: Trimethoprim induces hyperkalemia by inhibiting sodium resorption in the cortical collecting ducts of the kidney.
-
- Chloride
-
- Hypochloremia
- Hypochloremia is always associated with a metabolic alkalosis and often results in a paradoxic aciduria. The low Cl results in sodium avidity in the kidney. Sodium is absorbed in exchange for H+ and K+ as Cl cannot be absorbed with the Na+ to maintain electroneutrality.
- Causes of hypochloremia
- Loss of chloride > sodium: Vomiting of gastric contents, ptyalism and gastric reflux

in horses, sweating (horses), diarrhea in horses (especially due to large intestinal problems as chloride is absorbed in the ileum and colon of horses), renal disease (especially in cattle), administration of high Na+ fluids.

- Sequestration of chloride-rich fluids: Displaced abomasum, vagus indigestion, gastric rupture, gastric dilation-volvulus, gastrointestinal ulcers (horses).
-
-
- Hyperchloremia: This is associated with a tendency towards acidosis (HCO3 loss).

Causes of hyperchloremia

- Pseudohyperchloremia: Bromide administration - measured as chloride by ion-specific electrodes.
- Drug administration: diuretics, administration of Cl-containing fluids.
- Chloride retention: Renal failure, renal tubular acidosis, Addison's disease, diabetic ketoacidosis, chronic respiratory alkalosis.
-
- Acid-base status
- In health, blood pH (which is taken as the same as ECF pH) is maintained within a narrow range of approximately 7.4 to 7.5. Traditional interpretation of acid-base status involves the Henderson-Hasselbach equation, where pH is determined by the ratio of bicarbonate to carbon dioxide.

  The major extracellular buffer of acids in the body is bicarbonate followed by plasma proteins and bone. Intracellular buffers include proteins, organic phosphate, inorganic phosphate and hemoglobin (in erythrocytes). Regulation of acid-base involves chemical buffering with intra- and extra-cellular buffers, control of partial pressure of carbon dioxide by altering respiration and control of bicarbonate and hydrogen excretion by the kidneys.

- The kidney is central to acid-base regulation. Filtered bicarbonate is absorbed in the PCT of the kidney and regenerates the bicarbonate lost in buffering acids produced by normal body metabolism. Hydrogen is excreted by the PCT and DCT of the kidney. Excretion of H+ by the DCT is dependent on sodium resorption and exchanges for K+.

  There are four primary types of acid-base disorders: metabolic acidosis, respiratory acidosis, metabolic alkalosis, and respiratory alkalosis. The majority of patients with acid-base imbalance have either metabolic acidosis or metabolic alkalosis or a mixed disorder of both.
-

  Acidosis

- Acidosis can be primary metabolic (decreased HC03) or respiratory (hypercapnea) or secondary in compensation for a primary alkalosis. Acidosis has profound effects on the body, resulting in arrythmias, decreased cardiac output, depression, and bone demineralization.
- Primary metabolic acidosis: This can be due to loss of bicarbonate (hyperchloremic metabolic acidosis) or titration of bicarbonate (high anion gap metabolic acidosis).
-
-
- Primary respiratory acidosis: This is due to increased pCO2 from decreased effective alveolar hypoventilation. Any disorder interfering with normal alveolar ventilation can produce a respiratory acidosis. The most common causes are

primary pulmonary disease, ranging from upper airway obstruction to pneumonia. Diseases or drugs that inhibit the medullary respiratory center also produce a profound respiratory acidosis, e.g. general anesthesia.

- Alkalosis

- Alkalosis can be primary metabolic (increased HCO3) or respiratory (hypocapnea) or secondary in compensation for a primary acidosis. Usually respiratory alkalosis is the compensatory mechanism for a primary metabolic acidosis. Alkalosis results in tetany and convulsions, weakness, polydipsia and polyuria.
- Primary metabolic alkalosis: This is due to loss of hydrogen (usually with chloride) from the gastrointestinal or urinary tracts. Hypochloremia is a consistent feature and indicator of metabolic alkalosis. HCO3 is generated on an equimolar basis to the amount of hydrogen lost.
-
- Primary respiratory alkalosis: This is due to hyperventilation and is associated with decreased pCO2. Hyperventilation is usually stimulated by hypoxia associated with pulmonary disease, congestive heart failure, or severe anemia. Psychogenic disturbances, neurologic disorders, or drugs that stimulate the medullary respiratory center, will also stimulate hyperventilation
- Compensation
- In general, changes in bicarbonate produce compensatory changes in carbon dioxide and vice versa. Compensation causes parallel changes in pCO2 and bicarbonate.
- Primary metabolic acidosis: The primary abnormality is a decrease in HCO3. The compensatory response includes extarcellular buffering by bicarbonate, intracellular and bone buffering (phosphate, proteins, bone carbonate), respiratory compensation and renal hydrogen excretion Metabolic acidosis stimulates central and peripheral chemoreceptors, thus stimulating alveolar ventilation (and producing a secondary respiratory alkalosis or reduced pCO2), e.g. dogs with lactate acidosis from hypovolemia often hyperventilate (called Kussmaul's respiration).
- Decrease in pCO2 = 1.5 [HCO3) + 8
-
- Primary respiratory acidosis: The primary abnormality is an increase in pC02. The compensatory response is intracellular buffering of hydrogen (such as by hemoglobin) and renal retention of bicarbonate (a secondary metabolic alkalosis), which takes several days to occur.
- Primary metabolic alkalosis: The primary abnormality is an increased HCO3. This is initially buffered by hydrogen from extracellular (mostly) and intracellular buffers (such as plasma proteins and lactate). Chemoreceptors in the respiratory center sense the alkalosis and trigger hypoventilation, resulting in increased pC02 or a compensatory respiratory acidosis. Naturally, the extent of respiratory compensation will be limited by the development of hypoxia with continued alveolar hypoventilation. In addition to respiratory compensation, the kidneys excrete the excess bicarbonate (due to increased filtered bicarbonate and by active HCO3 secretion by a subpopulation of intercalated cells in the collecting tubules). However, this takes several days to occur.
-
-
- Primary respiratory alkalosis: The primary abnormality is a decreased pC02. The compensatory response to a respiratory alkalosis is initially a release of hydrogen from extra- and (mostly) intracellular buffers. This is followed by reduced hydrogen excretion (mostly as ammonium phosphate) by the kidneys. This results in decreased plasma bicarbonate which is balanced by an increase in chloride (to

maintain electroneutrality), thus producing a secondary hyperchloremic metabolic acidosis. The pH can revert to normal from compensation in chronic respiratory alkalosis.

- Remember these rules for compensation:
- Compensation does not produce a normal pH (except in a chronic respiratory alkalosis, in which compensatory metabolic acidosis can correct the pH).
- Overcompensation does not occur.
- Sufficient time must elapse for compensation to reach steady-state, approximately 24 hours.
-
- Primary respiratory alkalosis: The primary abnormality is a decreased pC02. The compensatory response to a respiratory alkalosis is initially a release of hydrogen from extra- and (mostly) intracellular buffers. This is followed by reduced hydrogen excretion (mostly as ammonium phosphate) by the kidneys. This results in decreased plasma bicarbonate which is balanced by an increase in chloride (to maintain electroneutrality), thus producing a secondary hyperchloremic metabolic acidosis. The pH can revert to normal from compensation in chronic respiratory alkalosis.
- Remember these rules for compensation:
- Compensation does not produce a normal pH (except in a chronic respiratory alkalosis, in which compensatory metabolic acidosis can correct the pH).
- Overcompensation does not occur.
- Sufficient time must elapse for compensation to reach steady-state, approximately 24 hours.
- Characteristic findings in the different primary acid-based disorders with appropriate compensatory changes are illustrated in the table below.
-
- The following formulas can be used to determine if compensation is occurring in an acid-base disturbance (in these formulae, N = midpoint of the reference range; obs = measured value):

  For a primary metabolic disturbance, the expected respiratory compensation is:
- $pCO_2(expected) = pCO_2(N) + [(HCO_3(obs) - HCO_3(N)) \times 0.7] +/- X$;

  where X = 2 for metabolic alkalosis, and X = 3 for metabolic acidosis
-
  For a primary respiratory disturbance, the expected metabolic compensation is:
- $HCO_3 (expected) = HCO_3 (N) + [(pCO_2 (obs) - pCO_2 (N)) \times X]$;

  where X = 0.15 for acute respiratory acidosis, X = 0.35 for chronic respiratory acidosis,
  X = 0.25 for acute respiratory alkalosis, and X = 0.55 for chronic respiratory alkalosis
-
- Mixed acid-base disturbances
- These are quite common and can be detected by non-parallel changes in HCO_3 and the anion gap, chloride and pCO_2. The following features give you an indication that a mixed acid-base disturbance is present:
- The pH is normal but there is an abnormal pCO_2 and/or bicarbonate. (Remember that compensation rarely results in a normal pH.)
- Changes in pCO_2 and bicarbonate occur in opposing directions. (Remember that with compensation, changes in pCO_2 and bicarbonate parallel each other.)
- Change in pH is opposite to that predicted from the pCO_2 and HCO_3.
- Compensation exceeds upper or lower limits.
-

- High anion gap

In an uncomplicated high anion gap acidosis, the change in AG is equivalent to the change in bicarbonate. If the change in anion gap is greater or less (usually by 2:1) than the change in bicarbonate, a mixed acid-base disturbance is present.

- If the decrease in bicarbonate is greater than the increase in anion gap, this indicates that there is a mixed disturbance, with something lowering the bicarbonate greater than expected. In this instance, this is compatible with a mixed high anion gap and hyperchloremic (normal anion gap) acidosis,e.g. renal failure, resolving diabetic ketoacidosis, diarrhea with a high anion gap acidosis (e.g. lactic acidosis).
- If the decrease in bicarbonate is less than the increase in anion gap, this indicates that there is a mixed disturbance, with something preventing the bicarbonate from being as low as it should be. This is compatible with a mixed high anion gap acidosis and metabolic alkalosis, e.g.lactic acidosis with sequestration of chloride-rich fluid, renal failure with vomiting/diuretics, vomiting and diarrhea/diabetic ketoacidosis/lactic acidosis. In this case, the bicarbonate and chloride will be low and the anion gap will be high.
- Normal anion gap acidosis or alkalosis

In an uncomplicated normal anion gap acidosis or a metabolic alkalosis, the change in chloride is equivalent to the change in bicarbonate. If the change in chloride is greater or less (usually by 2:1) than the change in bicarbonate, a mixed acid-base disturbance is present.

- If the decrease in chloride is greater than the increase in bicarbonate, this indicates that there is a mixed disturbance, with something decreasing the bicarbonate. In this instance, this is compatible with a mixed normal anion gap acidosis and a metabolic alkalosis. This can occur in renal failure with vomiting/diuretics, vomiting and diarrhea, and liver disease.
- If the increase in chloride is less than the decrease in bicarbonate, this indicates that there is a mixed disturbance, with something enhancing the decrease in bicarbonate. This is compatible with a mixed high anion gap and normal anion gap acidosis
- Summary
- High anion gap with change in anion gap > change in bicarbonate. This indicates:
- 1) Mixed high anion gap metabolic acidosis and metabolic alkalosis. Most common causes include renal failure and vomiting, renal failure and diuretics, diabetic ketoacidosis and vomiting, lactic acidosis and vomiting.
- 2) Non acidotic high anion gap with normal or increased bicarbonate. This occurs in a pure metabolic alkalosis, carbenicillin therapy and dehydration (increased albumin).
- 3) Mixed high anion gap metabolic acidosis plus respiratory acidosis, e.g. cardiopulmonary arrest.
- High anion gap with change in anion gap < change in bicarbonate. This indicates:
- Mixed high anion gap and normal anion gap acidosis.
- High anion gap acidosis masked by low anion gap (decreased albumin, paraproteins).
- Combined high anion gap acidosis and chronic respiratory alkalosis.

- Anion Gap
- The anion gap (AG) is calculated from measured results by the following formula:
- AG = (Na++K+)-(Cl-+ HCO3-)
- Na+ and K+ account for about 95% of total serum cations while Cl- and HCO3 account for about 85% of the total serum anions in a healthy individual. The "normal" anion gap is due to the presence of various unmeasured anions, e.g. plasma proteins, phosphate, and sulfate. An increased anion gap indicates the presence of excessive amounts of one or more of these anions or of some other anion.

The formula shown above is derived from the following equations:

- To maintain electrical neutrality, anions must equal cations. This can be expressed as
- Na+ +K+ +UC = Cl- +HCO3- +UA
-
- Unmeasured cations (UC): Calcium, magnesium, and gamma globulins. These are rarely ever increased enough to decrease the anion gap.
  Unmeasured anions (UA) : Lactate, phosphate, sulfate, ketones, metabolites of some poisons, such as ethylene glycol, plasma proteins.
- Rearrangement of the equation above gives -
- UA-UC = (Na++K+)-(Cl-+HCO3-)
  Anion gap = (UA-UC)
- Since UC cannot change enough to affect the anion gap and still be compatible with life, UC can be dropped from the equation. Thus,
- Anion gap = UA = (Na++K+)-(Cl-+HCO3-)
- As can be seen from these equations, the AG is a figure that indicates the amount of unmeasured anions in serum.

Increased anion gap

- Titration acidosis: Most common cause of an increased anion gap.
- Alkalemia: loss of protons from plasma proteins (unmeasured anions) increases their negative charge. Alkalemia also stimulates lactic acid production. The increase in AG is very mild.
- Dehydration: Will increase plasma protein concentration (especially albumin).
- Sodium containing drugs: Sodium salts, penicillin.
- Age: Anion gap is higher in foals than adult horses.
- Decreased cations: magnesium and calcium. Have minimal affect on the anion gap because of their low concentrations.
- Decreased anion gap
- Acidemia: Causes dissociation of protons from plasma proteins, decreasing their negative charge.
- Decreased albumin: A very common cause of a lower than expected or decreased anion gap.
- Assay artefacts: Artefactually elevated chloride, e.g. bromide therapy.
- Dilution: Dilutes plasma proteins.
- Increased unmeasured cations: Calcium, magnesium, gamma globulins, lithium. (These rarely cause an increased anion gap as most increases are incompatible with life. It is unusual to see a low anion gap in multiple myeloma.)
- Mineral group
- Calcium-phosphate homeostasis involves interrelated actions of parathyroid hormone (PTH), vitamin D metabolites, and calcitonin on kidneys, bone, and intestines.

Parathyroid hormone
PTH is secreted by chief cells of the parathyroid gland and results in increased calcium and decreased phosphate in serum. Secretion is stimulated by decreased ionized calcium, decreased calcitriol, magnesium, dopamine, PGE2. Secretion is inhibited by increased ionized calcium and increased calcitriol.
Parathyroid hormone has 2 primary sites of action, the kidney and the bone. In the kidney, PTH enhances renal resorption of calcium in the distal tubules, collecting ducts and ascending limb of the loop of Henle and promotes excretion of phosphate in the proximal renal tubules. It also activates alpha1-hydroxylase in the kidney, which converts vitamin D to its active form, 1,25 (OH)2D. Parathyroid hormone acts on osteoblasts in bone, stimulating secretion of osteoclastogenic cytokines, IL-6, IL-11 and PGE2 .

- Parathyroid hormone related protein (PTHrP)

Parathyroid hormone related protein is produced by several different cell types including lymphocytes, squamous epithelium, endocrine glands, bone, skeletal and smooth muscle and the kidney. The precise role of the protein is not known, but it is thought to be important for cartilage formation, mammary gland production and movement of calcium across the placenta and mammary gland. It is not involved in calcium homeostasis in physiologic states. PTHrP has a similar amino-terminal end to PTH and binds to PTH receptors. Therefore, PTHrP has a similar effect on calcium and phosphate as PTH. PTHrP is secreted by apocrine anal sac adenocarcinomas, some lymphomas and squamous cell carcinomas. It is responsible for the paraneoplastic hypercalcemia seen in these disorders. Specific assays for PTHrP are available.

- Vitamin D

Vitamin D is ingested as vitamin D3 or produced in the skin under the influence of UV light. It is transported to the liver via vitamin D binding proteins where it is converted (with the enzyme 25 hydroxylase) to calcidiol or 25(OH)D. This is then transported to the kidney, where it is converted to its active form, calcitriol or 1,25 (OH)2D by the enzyme, alpha1-hydroxylase in the proximal renal tubules. This enzyme is stimulated by PTH, decreased calcium or phosphate. It is inhibited by calcitriol, increased calcium or phosphate.
Calcitriol stimulates calcium and phosphate absorption in the intestine. In the kidney, calcitriol promotes renal phosphate resorption in the proximal convoluted tubule and calcium resorption in the distal convoluted tubule. In bone, calcitriol facilitates the action of PTH on osteoblasts. Calcitriol can be produced by tumor, macrophages and lymphoma cells, and is responsible for the hypercalcemia of malignancy seen in some dogs with lymphoma

- Calcitonin

Calcitonin is produced by parafollicular cells in the thyroid gland (C cells). Production is stimulated by an increase in ionized calcium. beta-adrenergic stimulation, dopamine, estrogen, gastrin and glucagon.
Calcitonin counters the calcium- raising effects of vitamin D and PTH by inhibiting osteolysis and stimulation of renal excretion of calcium.

- Calcium (Total)
-
- Total serum calcium comprises three major forms: ionized calcium (about 50% of total), protein bound (about 40% of total), and calcium complexed with anions such as bicarbonate, citrate, lactate, and phosphate (about 10% of total).
- Hypocalcemia

- Clinical signs of hypocalcemia in dogs include muscle tremors, convulsions, ataxia, and weakness. In horses, hypocalcemia is associated with synchronous diaphragmatic flutter and signs of tetany including stilted gait, muscle tremors, flared nostrils, inability to chew, recumbency, convulsions, and cardiac arrhythmias. In cows, hypocalcemia is usually manifested as weakness and recumbency. Signs of hypocalcemia develop when ionized calcium is too low for normal muscle and nerve function. Because of factors that influence ionized and protein-bound calcium fractions, the total calcium result does not necessarily correlate with ionized calcium and is not by itself always a reliable indicator of clinical hypocalcemia.
  A mild hypocalcemia in the presence of hypoalbuminemia usually does not indicate a disorder of calcium
-
- Causes of hypocalcemia

- Spurious. Hypocalcemia can be due to hypoalbuminemia.
  Calcium can be measured reliably in heparinized plasma and the results are comparable to serum Ca results. However, exposure of blood to anticoagulants such as EDTA, citrate, and oxalate (the anticoagulant in the sodium fluoride tube) reduces calcium to an unmeasurable level. Since Ca < 2 mg/dl is not compatible with life, exposure to agents that chelate calcium are indicated by such a result.
- Hypoparathyroidism. This has been reported in dogs, cats and one horse. Clinical signs include seizures, ataxia, and lens cataracts. It is characterized by hypocalcemia, normal or increased phosphate and normal magnesium. Low concentration of parathyroid hormone confirms primary hypoparathyroidism.
- Nutritional secondary hyperparathyroidism. This is so-called bran disease of horses (it occurs with all grain diets or grass diets high in oxalates) but can occur in all species with excess phosphate, reduced calcium or lack of vitamin D in the diet. Ionized calcium values are low, stimulating PTH with bone resorption.
-
-
- Renal secondary hyperparathyroidism. This occurs especially in dogs, but also in cats with chronic renal failure. PTH is stimulated from low ionized calcium (due to excess phosphate), vitamin D production is impaired due to renal insufficiency.
- Milk fever. This is seen in highly producing dairy cows and results in paresis. They have low calcium and phosphate. Magnesium is often normal. Eclampsia can also be seen in dogs, cats, ewes, sows, mares and goats and produces tetany in these breeds.
- Pancreatitis. Mild hypocalcemia, usually without clinical signs referable to hypocalcemia, is fairly common in acute pancreatitis. The mechanism is unknown.
- Idiopathic hypocalcemia in foals. This is seen in young foals, from 4 days to 5 weeks old. They display tachycardia, sweating, muscle rigidity, recumbancy, seizures and opisthotonus.
- Hypercalcitonism: C cell tumors in dogs, horses and cattle.
- Toxicosis: Sodium phosphate enemas, blister beetle (canthradin) toxicosis in horses, ethylene glycol toxicity (hypocalcemia is a common finding in the chemistry panel of dogs and cats poisoned with antifreeze
- Hypercalcemia

- Hypercalcemia is not common in any species but is encountered more often in dogs and horses than in cats and cows. Evaluation of a patient with Ca result above or at the top of the reference range should be done with consideration of albumin concentration and evidence of acid-base imbalance
-
- Causes of hypercalcemia
- Physiologic. Young, growing dogs, especially large breeds, often have Ca slightly higher than the reference range for adult dogs.
- Primary hyperparathyroidism. This has been reported in both dogs and cats and is due to chief cell neoplasia (adenoma or carcinoma) or hyperplasia. In dogs, it is familial in Keeshonds and inherited in German Shepherd dogs. Primary hyperparathyroidism is diagnosed by identifying a parathyroid adenoma by surgical exploration and biopsy and/or by measuring high or normal PTH concentration in conjunction with high ionized calcium values.
- Humoral hypercalcemia of malignancy. Hypercalcemia is a paraneoplastic syndrome in domestic animals and is a great tumor marker. Lymphoid neoplasms are the most common of the tumors to cause hypercalcemia. The second is adenocarcinoma of the apocrine glands of the anal sac. Primary or metastatic bone tumors occasionally cause hypercalcemia. In horses, hypercalcemia has been seen with lymphoma, ameloblastoma, gastric SCC and an adrenal cortical carcinoma. In most instances, the hypercalcemia is due to elaboration of PTHrP, although other cytokines (IL-6, IL-1) and vitamin D are involved.
-
-
- Addison's disease. The hypercalcemia is thought to be due to enhanced absorption of calcium in the gastrointestinal tract, hemoconcentration, decreased GFR from volume contraction and increased complexing and protein binding of calcium. Replacement therapy with corticosteroids returns the calcium to normal within a few days.
- Vitamin D toxicity. Until recently, hypervitaminosis D was a rare cause of hypercalcemia. Rodenticides containing cholecalciferol are now widely available. Ingestion of these rodenticides produces marked hypercalcemia within 24 hours. Other causes of hypervitaminosis D are excessive dietary supplementation and ingestion of plants whose leaves contain cholecalciferol (Cestrus diurnum or Solanum malacoxylon, and Trisetum flavescens).
- Chronic renal failure. Hypercalcemia should be attributed to renal failure only after other causes of hypercalcemia have been considered and ruled out.
-
-
- Measurement of intact parathyroid hormone (iPTH), ionized calcium (ICa), and 25-hydroxyvitamin D can usually discriminate between the various causes of hypercalcemia in dogs. Guidelines for interpretation of these
- tests in combination are shown in the table at right.
-
- Phosphate
- Phosphate is absorbed in the small intestine (especially the jejunum) by both paracellular and active transport. Absorption is decreased by low vitamin D, high calcium and low phosphate levels in the diet and other compounds such as iron and aluminium.
- Hypophosphatemia
- Phosphate is an essential component of ATP, the energy source of the cell. Mild to moderate decreases in phosphate are common and are of minimal significance. Severe hypophosphatemia can produce the following clinical signs:
- Hematologic effects: Hemolysis is the most severe. ATP is required for normal red blood cell membrane integrity. This is an important complication (life-threatening)

of therapy for diabetic mellitus. Hypophosphatemia also decreases neutrophil function and platelet survival.

- CNS: Low phosphate causes seizures, coma and ataxia.
- Muscular: Ileus in the gastrointestinal system and cardiomyopathy may result.
- Kidney: Metabolic acidosis due to impaired bicarbonate absorption and calciuria result (with bone lysis).
-
- Causes of hyperphosphatemia
- Laboratory error: This can be due to high bilirubin, mannitol, and oxalate, EDTA and citrate anticoagulants.
- Decreased absorption

  1) Postprandial alkaline tide

  2) Enteral nutrition: Phosphate decreases 12-24 hours after tube feeding in cats and may induce intravascular hemolysis.

  3) Miscelllaneous: Malabsorption, vitamin D deficiency, Ph-binding antacids, starvation, vomiting and diarrhea.
- Transcellular shifting

  1) Respiratory alkalosis: This causes a decrease in blood pCO_2 which increases intracellular pH. The increase in pH stimulates phosphofructokinase which enhances glycolysis, causing phosphate to move into cells to supply the enhanced phosphorylation that results. Respiratory alkalosis occurs with hyperventilation such as secondary to sepsis, heat stroke, CNS problems, hepatic coma, salicylate toxicity and fear.

  2) Insulin or glucose administration

  3) Hypothermia: This results in low phosphate, high calcium, and glucose, low potassium, acidosis and azotemia.
-
- Increased renal loss

  1) Hyperparathyroidism, pseudohyperparathyroidism: Phosphate may be normal or elevated if there is a concurrent decrease in GFR.

  2) Renal disease: renal tubular acidosis, chronic renal failure in horses.

  3) Osmotic diuresis: Polyuria from osmotic diuresis will result in renal phosphate losses, e.g. diabetes mellitus.

  4) Inhibitors of renal resorption: Tumoral osteomalacia results when some tumors, especially mesenchymal tumors, release inhibitors of phosphate reabsorption. These inhibitors are called phosphatonin. Impaired renal phosphate absorption also occurs with Cushings syndrome, volume expansion, drug administration (diuretics, corticosteroids, sodium bicarbonate), and metabolic acidosis.
- Unknown mechanisms: Hepatic lipidosis in cats, oxalate toxicity in large animals.
-
- Hyperphosphatemia
- Spurious Hyperphosphatemia

  1) Hemolysis: Hemolysis or prolonged contact of serum with cells in the blood sample causes movement of phosphate from the red cells into serum and can raise the Pi result.

  2) Post-prandial: A mild increase occurs after eating.

  3) Monoclonal gammopathy: Hyperphosphatemia can be observed in monoclonal gammopathies, due to binding of phosphate to the monoclonal protein.
- Decreased excretion

  1) Decreased GFR: This is the most common cause of hyperphosphatemia. Many animals that are azotemic are also hyperphosphatemic. Acute and severe reduction in GFR, as in acute renal failure or severe hypovolemia, is more likely to result in hyperphosphatemia than is chronic renal failure.

  2) Hypoparathyroidism: Phosphate is retained whilst calcium is lost in the urine due to lack of PTH.

3) Acromegaly: Growth hormone promotes retention of phosphate.
4) Hyperthyroidism: Phosphate is increased in up to 21% of hyperthyroid cats.

- Increased absorption
  1) Hypervitaminosis D: This produces hyperphosphatemia as well as hypercalcemia.
  2) Increased intake: Ingestion of excess phosphate (nutritional hyperparathyroidism) or administration of phosphate containing fluids or compounds.
- Transcellular shifts
  1) Acute tumor lysis syndrome: This results in high phosphate, high potassium, high uric acid and low calcium. Animals often die of acute oliguric renal failure.
  2) Severe soft tissue trauma: This can also result in increased phosphate as phosphate is higher intracellularly than extracellularly, e.g. rhabdomyolysis
- Urea nitrogen and Creatinine
- These tests are used as indicators of glomerular filtration rate (GFR). Neither is perfect in this regard, but they are clinically useful nonetheless.

Decreases in GFR are generally due to one of two main causes:

- decreased renal perfusion due to hypovolemia or cardiac dysfunction (prerenal causes)
- loss of functional nephrons (renal causes)
- combination of the two above causes
- Azotemia
- Azotemia is defined as an increase in urea nitrogen (UN) and creatinine and can result form a variety of disorders including, but not limited to, renal failure. Uremia is the term for the clinical syndrome of renal failure with azotemia and multisystemic problems such as polyuria, polydipsia, mild non-regenerative anemia (in chronic renal failure), vomiting, weight loss, depression, and other sequelae of inadequate renal function.
- Azotemia can be due to prerenal, renal or post-renal causes. Differentiation of the causes of azotemia requires urinalysis (especially assessment of urine specific gravity), evaluation of clinical signs and results of other diagnostic tests (e.g. radiographic evidence of urinary tract obstruction).
- Prerenal azotemia
-

Prerenal azotemia is due to a decrease in GFR from circulatory disturbances causing decreased renal perfusion, such as hypovolemia (shock, hemorrhage, Addison's disease, vomiting), cardiac disease or renal vasoconstriction. Prerenal azotemia can usually be distinguished from renal azotemia by clinical signs (evidence of dehydration or hypovolemia), urinalysis (urine should be concentrated, i.e. > 1.030 in the dog, > 1.035 in the cat, > 1.025 in large animals; and there should be no other evidence of renal tubule dysfunction such as proteinuria, cylindriuria) and response to therapy. Urine specific gravity may be decreased (despite a prerenal azotemia) if there are other factors reducing the concentrating ability of the kidney (see urine specific gravity).

- Therefore, often a response to therapy (fluid administration) is required to differentiate between a primary renal and prerenal azotemia (the azotemia should correct with appropriate fluid therapy within 24-48 hours in a pre-renal azotemia) . Note that many causes of a prerenal azotemia will result in renal hypoxia and ischemia. If this is severe or chronic enough, a primary renal azotemia may result, and may co-exist with a renal azotemia.
  As UN levels in blood are dependent on flow rate through the renal tubules

(decreased flow rate in prerenal azotemia enhances renal absorption of UN, and increases UN levels in blood), UN may increase without any increase in creatinine in early pre-renal azotemias.

- Renal azotemia

Renal azotemia results from a decreased GFR when more than 3/4 of the nephrons are non-functional. Renal azotemia may be due to primary intrinsic renal disease (glomerulonephritis, ethylene glycol toxicity) or may be secondary to renal ischemia from prerenal causes or from kidney damage from urinary tract obstruction (post-renal azotemia). Loss of 3/4 of kidney function usually follows concentrating defects (requires loss of 2/3 of the kidney), therefore isosthenuric urine (usg 1.008-1.012) is common in renal azotemia. In addition, there may be other evidence of renal tubular dysfunction in the urinalysis, such as proteinuria, granular or cellular casts, and glucosuria without hyperglycemia (these features are not always present in urine from animals with a renal azotemia)..

Azotemia with a urine specific gravity less than those values stated above is presumptive evidence of renal azotemia or renal failure UNLESS there is also evidence of other diseases or conditions affecting urine concentrating ability independently of renal failure. The greatest difficulty in differentiating renal from prerenal azotemia is encountered in those cases with a urine specific gravity greater than isosthenuric (1.012), but < 1.030 in the dog, < 1.035 in the cat and < 1.025 in large animals

- Note that in cats, primary glomerular disease may occur without loss of renal concentrating ability (so the cat may have renal azotemia with concentrated urine). In horses and cattle, increases in UN are modest in renal azotemia due to excretion of UN into the gastrointestinal system (the urea is broken down into amino acids in the cecum and rumen, respectively). Therefore, creatinine is a more reliable indicator of GFR in these species.
  As mentioned above, a high anion gap metabolic acidosis is common in all species with renal failure. Hypermagnesemia and hyperkalemia are features of oliguric or anuric renal failure in all species
- Post-renal azotemia

- Post-renal azotemia results from obstruction (urolithiasis) or rupture (uroabdomen) of urinary outflow tracts. This is best diagnosed by clinical signs (e.g. frequent attempts to urinate without success or presence of peritoneal fluid due to uroabdomen) and ancillary diagnostic tests (e.g. inability to pass a urinary catheter) as urine specific gravity results are quite variable. Animals with post-renal azotemia are markedly hyperkalemic and hypermagnesemic. Uroperitoneum can be confirmed by comparing the concentration of creatinine in the fluid to that in serum or plasma; leakage of urine is indicated by a higher creatinine in fluid than in serum. Post-renal azotemia can result in primary renal azotemia (failure) due to tubule dysfunction from impaired renal flow.

- Urea Nitrogen

- Measurement of urea concentration in serum is included in chemistry profiles mainly to screen for decreased glomerular filtration rate (GFR). Urea concentration is measured as urea nitrogen, and the test is usually called blood urea nitrogen (BUN) or serum urea nitrogen (SUN). Concentrations of UN are expressed in mg/dL.

- Urea is synthesized by hepatocytes from ammonia generated by catabolism of amino acids derived either from digestion of proteins in the intestines or from endogenous tissue proteins. Urea is excreted by the kidneys, intestine (high in horses), saliva and sweat. In ruminants, urea is excreted into the gastrointestinal system where it is converted to amino acids and ammonia which are then used for protein production (remember urea is added as a supplement to many bovine diets).
  Concentrations of UN are dependent upon:
- Hepatic urea production: The rate of urea production is dependent on hepatic function and digestion and catabolism of protein, i.e. urea formation is decreased in liver disease (e.g. portosystemic shunts) and increased with protein catabolism or increased protein digestion in the intestine.
- Renal tubular flow rate: Urea is freely filtered through the glomerulus and passively diffuses out of the tubules at a rate dependent on flow rate through the tubules; the remainder of the filtered urea is excreted in urine. At high flow rates, approximately 40% of filtered urea is reabsorbed. At low flow rates, as happens in hypovolemic individuals, approximately 60% of filtered urea is reabsorbed and added back to the blood urea concentration. This explains the high UN levels seen with decreased GFR of any cause.
- Causes of increased UN

- Increased protein catabolism: Fever, burns, corticosteroid administration, starvation, exercise.
- Increased protein digestion: Hemorrhage into the gastrointestinal system, high protein diets.
- Decreased GFR: Due to pre-renal, renal or post-renal causes.
- Causes of decreased UN

- Decreased protein intake or protein anabolism: Dietary restriction of protein, young animals (high anabolic rate).
- Increased excretion: Any cause of polyuria, e.g. hyperadrenocorticism, diabetes mellitus.
- Decreased production: Liver disease.

- Creatinine
- Creatinine is produced as the result of normal muscle metabolism.
- Phosphocreatine, an energy-storing molecule in muscle, undergoes spontaneous cyclization to form creatine and inorganic phosphorous.
- Creatine then decomposes to creatinine. Production of endogenous creatinine is quite stable.
- An additional and relatively minor source is creatinine ingested during consumption of animal tissue and absorbed from the intestines.
- Creatinine is filtered freely through the glomerulus and is not reabsorbed in the tubules.
- Therefore, creatinine is a more reliable measure of GFR, compared to UN, in all species as it is not influenced by diet or protein catabolism.

- Causes of increased creatinine

- Artifact: When measured by the Jaffe technique (which is based on color production and is used by the chemistry analyzer at Cornell University), both creatinine and non-creatinine chromogens react with the reagent. Non-creatinine chromogens include acetoacetate, glucose, vitamin C, uric acid, pyruvate, cephalosporins and amino acids. When present in high concentrations, these can artefactually elevate creatinine values.
- Physiologic: Higher in foals (up to 8 mg/dL; thought to be due to defective placental transfer) and heavily muscled horses (up to 2.5 mg/dL).
- Decreased GFR: Due to prerenal, renal or post-renal causes. In ruminants and horses, creatinine is the best indicator of GFR.
- Increased production: A mild increase (< 1 mg/dL) may be seen after ingestion of a recent meat meal. Acute myositis does not consistently increase creatinine itself (although severe myositis or myopathy can produce renal azotemia from myoglobinuric nephrosis).
-

- Causes of decreased creatinine

-

- Artifact: With the Jaffe reaction, severe icterus (total bilirubin > 10 mg/dL or an icteric index > 10 units) may artefactually decrease creatinine concentrations (this is based on data in humans and may not occur in animals or in every icteric animal). This effect can be minimized by utilizing a reaction blank.
- Decreased production: Loss of muscle mass, young puppies (low muscle mass). Severe liver disease from cirrhosis may result in decreased creatinine values from decreased creatine production.
- Increased GFR: This occurs in animals with portosystemic shunts and during pregnancy (due to increased cardiac output).
-

- Total Protein
-
-

  Refractometry: This method is used for estimating plasma protein (including fibrinogen) in EDTA plasma. It measures the refractive index of a sample relative to the refractive index of water.

  Biuret Method: This is the colorimetric method used on the automated chemistry analyzer. It detects all proteins and is accurate for the range of 1-10 g/dl.
  Turbidometric methods: Quantitative of protein in CSF, urine and other low-protein fluids requires more sensitive techniques than either the Biuret or refractometer method.
- Albumin
-
-

  Albumin is a globular protein with a MW of 69,000 daltons.
- It is synthesized in the liver and catabolized by all metabolically active tissues.
- Albumin makes a large contribution to plasma colloid osmotic pressure due to its small size and abundance (35-50% of total plasma proteins by weight).
- It also serves as a carrier protein for many insoluble organic substances (e.g., unconjugated bilirubin).

  Hyperalbuminemia
- Overproduction of albumin is not known to occur.
- Relative: Hyperalbuminemia is a relative change seen with dehydration. Globulins will also increase in this situation, resulting in hyperproteinemia with no change in A:G ratio.

- Drugs: Increases in albumin are reported in experimental studies in dogs administered corticosteroids. It is not clear if this is due to increased production of corticosteroids or dehydration secondary to free water losses from corticosteroid-induced polyuria.
- Laboratory error: Albumin values can be artifactually elevated in severely lipemic or hemolyzed samples, but this is analyzer- and method-dependent. Albumin is also higher in heparinized plasma than serum (due to non-specificity of bromcresol green which also binds to globulins, including fibrinogen), however newer procedures have been developed to minimize this phenomenon.
-
- Hypoalbuminemia

- Physiologic: Excessive fluid administration (overdilution).
- Decreased production

  1) Decreased production can occur if there are
  - insufficient amino acids available for hepatic
  - production of albumin. This occurs in cases of chronic
  - severe malnutrition due to dietary deficiency) or
  - starvation.

  2) The liver is the main site of albumin production.
  - Chronic hepatic disease will result in
  - hypoalbuminemia when there is a > 80% reduction in
  - functional mass.

-
-
- 3) Acute phase reactions stimulate downregulation of albumin production. An acute phase reactant response is initiated in response to trauma, inflammation, neoplasia, etc and involves release of cytokines (IL-1, IL-6, TNF) from macrophages.
- These cytokines act on regulatory elements in hepatocyte genes, resulting in upregulation of transcription of acute phase reactant proteins (fibrinogen, serum amyloid A, ceruloplasmin, haptoglobin) and downregulation of transcription of other proteins, including albumin and transferrin (so-called "negative acute phase reactants").
- Increased degradation of albumin may also play a role in the hypoalbuminemia in this reaction.
- In this case, the A:G is decreased due to the combination of low albumin and high globulins.

-
- Increased loss of albumin

  This occurs with the following:

  1) Protein-losing glomerulopathy: This can result in nephrotic syndrome which is characterized by proteinuria, hypoalbuminemia, hypercholestorelemia and edema. In these conditions, albumin is lost, but globulin levels are usually maintained, resulting in a low A:G.

  2) Severe hemorrhage: Both albumin and globulins are lost, resulting in a normal A:G.

  3) Protein-losing enteropathies. In these conditions, albumin and globulins are usually lost concurrently, thereby maintaining a normal A:G.

-
- 4) Severe exudative dermatopathies. This may also associated with concommitant

albumin and globulin loss (A:G tends to remain normal).

- Sequestration: Hypoalbuminemia can be due to sequestration of albumin within body cavities, e.g. peritonitis.
- Catabolism: This is not a well-characterized mechanism for low albumin. Increased albumin catabolism may occur with negative energy balance or protein malnutrition (e.g. chronic infections, neoplasia, trauma) and , potentially, as part of an acute phase response (see decreased production above).
-
-
- Globulins
- Globulins can be divided into three fractions based on their electrophoretic mobility. Most of the alpha and beta globulins are synthesized by the liver, whereas gamma globulins are produced by lymphocytes and plasma cells in lymphoid tissue.
- Alpha globulins: consist of alpha-1 and alpha-2 globulins.
  Alpha-1 globulins include alpha-1 antitrypsin, alpha-1 antichymotrypsin, orosomucoid (acid glycoprotein), serum amyloid A, and alpha-1 lipoprotein (HDL).
  Alpha-2 globulins include alpha-2 macroglobulin (protease inhibitor), haptoglobin (binds free hemoglobin), protein C (inhibitor of activated coagulation factors FVIII and FV), ceruloplasmin (carrier of copper) and alpha-2 lipoprotein (VLDL).
- Beta globulins: consist of beta-1 and beta-2 globulins.
  Beta-1 globulins include transferrin (binds iron) and hemopexin.
  Beta-2 globulins include complement factors 3 and 4, C-reactive protein, plasminogen, beta-2 lipoprotein (LDL), beta-2 microglobulin and some proportion of IgA (especially) and IgM. Fibrinogen also migrates in this region.
-
- Globulins contd.
- Gamma globulins: consists of the immunoglobulins: IgM, IgA, IgG.
- For the routine chemistry profile, total globulins are calculated as follows:
- TP - albumin = globulin
-

  Globulins can also be measured quantitively and qualitatively with electrophoresis. Radial immunodiffusion is used for accurate quantification of immunoglobulins and has also replaced immunoelectophoresis for determining the immunoglobulin comprising a monoclonal gammopathy.
- Hypoglobulinemia
-
- Decreases in alpha and beta globulins are not significant. Decreased gamma globulins are seen when there is a deficiency of immunoglobulins (dependent on class of Ig involved and severity of the decrease). Radial immunodiffusion (RID) is the best method for pursuing these diagnoses.

  Decreases in globulins of all fractions may be seen in protein-losing enteropathies, exudative dermatopathies, and hemorrhage. Concomitant loss of albumin in these conditions tends to maintain a normal A:G ratio with a low total protein.
- Inherited hypogammaglobulinemia
-

  A variety of inherited immunodeficient syndromes have been reported. Although some involve cell-mediated immunity (e.g. PSCID), they often have concurrent gamma globulin deficiencies due to impaired helper T cell function.
- Primary severe combined immunodeficiency: This has been reported in Arabians horses (full and crosses). It is characterized by a lymphopenia, decreased IgM in a presuckle foal, absent IgM and IgA post-suckling. IgM, IgG and IgA are all low after 3 months of age as maternally-derived antibodies are degraded. Animals

have thymic and lymph node atrophy and die at a young age (usually when maternal antibodies disappear) of opportunistic infections, e.g. Pneumocystis carinii, adenovirus, cryptosporidiosis.

- Inherited hypogammaglobulinemia contd.
- Agammaglobulinemia: This has been reported in foals. They have no B cells and lack Igs by 3 months of age. T cell function is normal as are lymphocyte counts. They die of repeated infections, with a poor response to therapy, by 12-18 months of age.
- IgM deficiency: Selective IgM deficiency has been reported in horses (Arabians, Paso Fino, quarterhorses and thoroughbreds) and Dobermans. Horses usually die of fatal pneumonia, arthritis and enteritis. Dogs usually have no clinical signs as long as IgG and IgA levels are normal.
- IgA deficiency: This has been reported in various dog breeds, including Sharpeis, Beagles, and German Shepherd Dogs. They suffer from recurrent infections involving the urinary tract, respiratory tract, and skin.
- Transient hypogammaglobulinemia: This has been reported in Arabian horses and dogs. They have a delayed onset of post-natal immunoglobulin synthesis and are susceptible to adenoviral and bacterial infections.
- Acquired immunodeficiencies
- These are, by far, more common than inherited immunodeficiencies.
- Failure of passive transfer (FPT): Animals are dependent upon ingestion of colostrum for passive immunity as immunoglobulins do not cross the placenta as they do in human beings. FPT results when neonates fail to suckle or if dams leak colostrum pre-parturition. For diagnosis of FPT, determination of IgG is recommended within 24 to 48 hours of birth.

- Infectious diseases

  1) Viruses: Feline leukemia virus and feline immunodeficiency virus are known causes for acquired immunodeficiencies in cats. Canine distemper virus causes immunodeficiency in dogs. Bovine viral diarrhea causes immunodeficiency in cattle and Aleutian mink disease virus (a parvovirus) causes immunosuppression in ferrets.

  2) Parasites: Toxoplasmosis and Theileria cause immunodeficiency. Generalized infection with Demodex canis is often found in immunodeficient dogs, however it may be a result of immunodeficiency and not its cause. Eperythrozoon wenyonii infection in cattle is associated with reduced humoral immunity.

  3) Johne's disease causes decreased T cell function.
- Acquired immunodeficiencies contd.
- Neoplasia: Lymphoma in cattle and horses is associated with immunosuppression. Very low IgM levels are often observed in horses with lymphoma and can be a valuable non-invasive tumor marker if there is a high clinical index of suspicion for lymphoma.
- Idiopathic: Idiopathic immunodeficiency has been reported in young llamas with failure to gain weight, ill-thrift and recurrent infections. Many of these llamas have concurrent Eperythrozoon infections.
- Hyperglobulinemia
- Increases in total globulins can result from increases in any or all of the fractions as determined by electrophoresis.

Alpha globulins

- Acute phase reactant response: This usually results in increased alpha (especially

alpha-2) globulins. Acute phase reactants are a diverse group of proteins that increase in serum very rapidly (within 12-24 hours) following tissue injury of any cause (inflammation, acute bacterial and viral infections, necrosis, neoplasia, trauma). Raised serum levels are the result of increased hepatic synthesis mediated by cytokines (IL-1, IL-6, TNF). They also tend to remain elevated in chronic inflammatory conditions.

- Nephrotic syndrome: A dramatic increase in alpha-2 globulins is often seen (due to VLDL and alpha-2 macroglobulin).
- Drugs: In dogs, corticosteroid administration results in an increase in alpha-2 globulins.
-
- Hyperglobulinemia contd.
- Beta Globulins
- Inflammation (acute and chronic): increased beta globulins often accompanies increases in gamma globulins (response to antigenic stimulation).
- Active liver disease and suppurative dermatopathies (both of which are associated with elevated IgM).
- Nephrotic syndrome (associated with an increase in transferrin).
-
- Gamma Globulins
-

Increases in this fraction occur most commonly in conditions in which there is an active immune response to antigenic stimulation usually resulting in a polyclonal gammopathy. Neoplasms of immunoglobulin-producing cells (plasma cells, B-lymphocytes) can also be responsible for monoclonal increases in this fraction.

- Polyclonal gammopathy
  This is seen as a broad-based peak in the beta and/or gamma region. Some common causes include various chronic inflammatory diseases (infectious, immune-mediated), liver disease, FIP (alpha-2 globulins are often concurrently elevated - see adjacent ELP tracing), occult heartworm disease, and Ehrlichiosis. Beta-gamma bridging occurs in disorders with increased IgA and IgM such as lymphoma, heartworm disease and chronic active hepatitis.
-
- Gamma Globulins contd.
- Monoclonal gammopathy
  This is seen as a sharp spike in the beta or gamma region. The peak can be compared to the albumin peak - a monoclonal gammopathy has a peak as narrow as that of albumin. Both neoplastic and non-neoplastic disorders can produce a monoclonal gammopathy.
  1) Neoplasia: Multiple myeloma is the most common cause (producing an IgG or IgA monoclonal). Other neoplastic disorders associated with a monoclonal gammopathy include lymphoma (IgM or IgG) and chronic lymphocytic leukemia (usually IgG). Extramedullary plasmacytomas are solid tumors composed of plasma cells that are usually found in the skin of dogs. They have also been reported in the gastrointestinal tract and liver of cats and dogs. They can be associated with a monoclonal gammopathy, or even a biclonal gammopathy (if there are multiple tumors).
- Gamma Globulins contd.
- An increase in IgM is called macroglobulinemia. Waldenstrom's macroglobulinemia is a neoplasm of B-cells (lymphoma) that has a different presentation from multiple myeloma. Patients usually have splenomegaly and/or hepatomegaly and lack osteolytic lesions. In contrast, multiple myeloma is a disorder of plasma cells that have undergone antigenic stimulation in peripheral lymph nodes and then home in on the bone marrow (the marrow produces

appropriate growth factors that support growth of myeloma cells). Therefore, myeloma is characterized as a bone marrow disorder, with osteolytic bone lesions (in 50% of canine cases) and Bence-Jones proteinuria.

- Gamma Globulins contd.
- 2) Non-neoplastic disorders: Monoclonal gammopathies (usually IgG) have been reported with occult heartworm disease, FIPV (rarely), Ehrlichia canis, lymphoplasmacytic enteritis, lymphoplasmacytic dermatitis and amyloidosis. These causes should be ruled out before a diagnosis of multiple myeloma is made in a patient with an IgG monoclonal gammopathy.
-
- A:G Ratio
-
- This is the ratio of albumin present in serum in relation to the amount of globulin. The ratio can be interpreted only in light of the total protein concentration. Very generally speaking, the normal ratio in most species approximates 1:1.

  For example, high total protein with a normal A:G ratio suggests dehydration, while the same protein with a low A:G ratio would indicate hyperglobulinemia
- Blood Glucose
- Glucose is derived from digestion of dietary carbohydrates, breakdown of glycogen in the liver (glycogenolysis) and production of glucose from amino acid precursors in the liver (gluconeogenesis). In ruminants, the main source of glucose is gluconeogenesis from volatile fatty acids (prioponate) absorbed from rumen by bacterial fermentation. Glucose is the principal source of energy for mammalian cells. Uptake is mediated by a group of membrane transport proteins, called glucose transporters (GLU), some of which are insulin-dependent, e.g. GLU-4.
- The blood glucose concentration is influenced by hormones which facilitate its entry into or removal from the circulation. The hormones affect glucose concentrations by modifying glucose uptake by cells (for energy production), promoting or inhibiting gluconeogenesis, or affecting glycogenesis (glycogen production) and glycogenolysis. The most important hormone involved in glucose metabolism is insulin.
- Hormonal influences on blood glucose
- Insulin: Insulin enables energy use and storage and decreases blood glucose concentration. Insulin is produced by beta cells in the pancreatic islets. Insulin release is stimulated by glucose, amino acids and hormones (e.g. glucagon, growth hormone, adrenaline). Release is inhibited by hypoglycemia, somatostatin, and noradrenaline.
- Insulin decreases blood glucose by promoting glucose uptake through GLU-4 and its use in metabolism (e.g. energy production, protein production) by liver, muscle and other tissue cells. Insulin also inhibits glucose production by inhibiting gluconeogenesis and glycogenolysis.
- Insulin increases fatty acid and triglyceride synthesis (through stimulation of endothelial lipoprotein lipase), thus increasing fat stores (adipogenesis), and enhances glycogen synthesis and storage in the liver.
- Insulin induces the cellular uptake of K+, phosphate and Mg+.
-
- Hormonal influences on blood glucose contd.
- Several hormones oppose the action of insulin and, therefore, will increase blood glucose. The main hormones that mediate this effect are glucagon, growth hormone, catecholamines, and corticosteroids. The increase in blood glucose can occur through inhibition of insulin release, stimulation of glucose-yielding

pathways (glycogenolysis, gluconeogenesis), or decrease of glucose uptake or use by tissues. Collectively, increases in these hormones can induce a state of insulin resistance. Insulin resistance can also be mediated by inflammatory cytokines (TNF-alpha), obesity and pregnancy. Inflammatory cytokines are thought to be responsible for insulin resistance observed in sepsis. Pregnancy-associated hormones may also contribute to insulin resistance and hyperlipidemic syndromes in pregnant horses, ponies and camelids.

- Hormonal influences on blood glucose contd.
- Glucagon: Glucagon causes an increase in blood glucose, by stimulating gluconeogenesis and glycogenolysis and facilitating glucose release from hepatocytes. Low blood glucose is the main stimulus for glucagon release from alpha cells in pancreatic islets.
- Catecholamines (epinephrine/norepinephrine): Epinephrine from the adrenal medulla acts via beta-adrenergic receptors, whereas norepinpherine is released from nerve endings and acts on alpha2-adrenergic receptors. Norepinephrine and epinephrine have somewhat opposing effects on insulin release (norepinephrine inhibits, epinephrine stimulates), but the net effect of both is increased blood glucose. This occurs via stimulation of glycogenolysis and release of glucose from hepatocytes (epinephrine), and indirectly through inhibition of insulin release (norepinephrine), and release of growth hormone (epinephrine) and ACTH (which increases cortisol). The increase in glucose in response to catecholamines is usually transient (primarily due to intermittent release of catecholamines) and can be quite pronounced in cats, cattle and camelids.
-
- Hormonal influences on blood glucose contd.
- Growth hormone (GH): This increases blood glucose by inhibiting glucose uptake by cells. It also promotes glycogenolysis in muscle tissue. Progesterone may cause insulin resistance by stimulating secretion of GH. Growth hormone is released from the pituitary by growth hormone-releasing hormone, which is secreted by the hypothalamus usually in response to low blood glucose and epinephrine.
- Corticosteroids: These increase blood glucose by inducing glucose release from hepatocytes and inhibiting glucose uptake by cells (through decreasing GLU-4). Corticosteroids also stimulate gluconeogenesis and glucagon secretion (which also increases blood glucose).
-
-
- The table below summarizes the effects of these different major hormones on physiologic processes that affect blood glucose concentrations
-
- Sample considerations
- Serum - serum glucose values decrease rapidly in samples that have not been separated from the cellular constituents of blood. Glucose values decrease by 10% per hour if serum is left in contact with cells. Note that the decrease in glucose is enhanced in patients with increased leukocyte or platelet counts, even if collected into fluoride oxalate tubes.
- Sodium fluoride (NaF) at concentrations of 10 mg/dl blood will inhibit glucolysis by erythrocytes, leukocytes and platelets. However, sodium fluoride is hypertonic and causes lysis of red blood cells. This releases intra-erythrocyte water which dilutes the glucose concentration. Glucose concentrations in sodium fluoride samples are consequently lower than in promptly separated serum samples (by approximately 7-12%).
- Lipemia and hemolysis may interfere with methodology
-
- Hypoglycemia

- Lack of glucose produces seizures as the brain relies entirely on glucose for its energy source. Neonatal animals are predisposed to hypoglycemia due to immature hepatic gluconeogenic pathways, low fat stores and muscle mass and rapid glycogen depletion. Hypoglycemia can be due to decreased production (e.g. inherited disorders, liver disease) or increased use (e.g. insulinomas, sepsis).
- Hypoglycemia contd.
- Artifact: Improper sample handling - e.g., mailing serum on clot; high doses of vitamin C can inhibit some assays used to measure glucose.
- Decreased production

  1) Inherited defects: Hypoglycemia is a feature of certain glycogen storage diseases, namely deficiencies of alpha 1-4 glucosidase (Pompe disease) and glucose-6-phosphatase (von Gierke's disease).

  2) Idiopathic: Juvenile hypoglycemia (usually affects small breed dogs).

  3) Liver disease: Severe liver disease and portosystemic shunts can produce hypoglycemia. More than 70% of the functional liver mass must be lost before hypoglycemia ensues.

  4) Decreased intake: Starvation, malabsorption. In horses, glucose decreases if they are fed a high grain diet, with little roughage.
-
- Hypoglycemia contd.
- Increased utilization

  1) Idiopathic: Hypoglycemia of hunting dogs and endurance horses.

  2) Neoplasia: Insulin-secreting tumors (insulinoma) or tumors secreting insulin-like growth factors (mesenchymal tumors, hepatic tumors).

  3) Sepsis: Hypoglycemia occurs due to liver dysfunction, impairment of insulin degradation and enhanced glucose utilization.
- Hypoglycemia contd.
- 4) Bovine ketosis, ovine pregnancy toxemia: During pregnancy, there are increased glucose demands from the fetus and the mammary glands (plasma glucose is the source of lactose). Ruminants are predisposed to hypoglycemia in pregnancy or lactation as they rely on gluconeogenesis for glucose production. Bovine ketosis results in anorexia, depression, decreased mild production, ketonemia, ketolactia, ketonuria and hypoglycemia. It usually occurs in dairy cows in the first 1-2 months of lactation due to increased demands for glucose by the mammary gland. It is initiated by poor diets or inappetance from other diseases. Alimentary ketosis results from feeding cattle spoiled silage with excess butyric acid. A spontaneous ketosis also occurs in cows in peak lactation despite abundant, good-quality feed. The animls are not acidotic and often recover spontaneously (despite decreased milk production). Beef cows can also develop ketosis, especially in the last 2 months of pregnancy, when carrying twins.
  Ovine pregnancy toxemia occurs in sheep, carrying more than 1 fetus, that are calorically deprived or stressed. They, like beef cows, develop fatty liver and acidosis and may die of liver dysfunction.
  Ketosis has also been reported rarely in lactating dairy goats and dogs.

  5) Addison's disease: Hypoglycemia occurs due to decreased gluconeogenesis and increased insulin-mediated glucose uptake by skeletal muscle.
-
-

Hyperglycemia

- Sustained hyperglycemia causes glycosylation of protein groups. The first change is the nonenzymatic addition of glucose to protein amino groups to form Amadori products. These reach a steady state over time and do not accumulate further. Any cause of hyperglycemia (transient or sustained) may result in glucosuria if

glucose concentrations are high enough to exceed the renal threshold. The renal threshold for glucose is species-dependent and is reported to be 180-220 mg/dL in dogs, 280-290 mg/dL in cats (lower thresholds may occur in diabetic cats), and 150 mg/dL in horses and cattle.

- Physiologic: Physiologic hyperglycemia occurs post-prandially and in response to stress in all species. This can be mediated by epinephrine (and is transient, lasting 4-6 hours) or corticosteroids (results in a more sustained increase in glucose). Cats and cattle tend to produce marked stress hyperglycemias. In cattle, a very high glucose (> 500 mg/dL) is a poor prognostic indicator. In liver disease, a prolonged postprandial hyperglycemia may be observed.
- Therapeutic agents: Glucocorticoids, dextrose-containing fluids, thyroxine, xylazine, megesterol acetate etc.
-
- Hyperglycemia contd.
- Disease

  Sustained increases in glucose can be seen with insulin deficiency (type II diabetes mellitus) or insulin resistance. Insulin resistance can be a result of increased concentrations of hormones (e.g. glucocorticoids, growth hormone, progesterone) or inflammatory cytokines (TNF-a) that oppose insulin release or the action of insulin on peripheral tissues. Obesity is also associated with insulin resistance, particularly in cats and likely in horses. Adipose tissue is now known to be an endocrine organ and can produce specific hormones (e.g. leptin) as well as inflammatory cytokines (TNF-a).

  1) Diabetes mellitus: This is inherited in Keeshonds and Golden Retrievers. It has been associated with BVD infection in cattle and paramyxovirus infection in llamas. Cats are prone to non-insulin dependent diabetes mellitus. This is thought to be associated with deposition of pancreatic amyloid (from amylin protein), which is related to pancreatic islet dysfunction. When islet destruction is widespread, cats do become insulin-dependent.

- Hyperglycemia contd.
- 2) Hyperadrenocorticism: In dogs and horses, hyperglycemia is due to insulin resistance.

  3) Acromegaly: Hyperglycemia is due to insulin resistance.

  4) Hyperglucagonemia: Hyperglycemia is due to insulin resistance.

  All the above produce prolonged or sustained hyperglycemia.

  5) Hyperthyroidism in cats: Increases in glucose are often transient. The exact mechanism is unknown (? defective insulin secretion, ? enhanced sensitivity to catecholamines).

  6) Acute pancreatitis: Hyperglycemia, which is usually transient, may occur due to stress, glucagon secretion and decreased insulin production.
- Hepatocellular Leakage Enzymes
-
- The hepatocellular leakage enzymes are useful in detecting injury to liver parenchymal cells. Generally, increased serum activity represents enzyme leakage from cells through damaged cell membranes.
- AST: Used in small and large animals. Present liver as well as skeletal muscle and erythrocytes.
- ALT: Used in small animals only. Largely liver-specific, but can also increase in severe myopathies (release of muscle enzyme) and hemolysis.
- SDH: Liver specific in nearly all species. Used in large animals in place of ALT (which is not a good marker of liver disease in large animals).
- GLDH: Liver specific in all species. Used in large animal panels concurrently with

SDH (due to superior storage stability) and on exotic (non-mammalian) panels as a marker of liver injury.

- Disease effects
- Serum activity of the leakage enzymes increases when a sufficient number of hepatocytes experience increased membrane permeability. Serum levels depend on both the number of cells affected and the severity of injury to individual cells. Serum levels do not correlate with reversibility. Diffuse hypoxia (reversible injury) may result in greater serum activity than end-stage cirrhosis (irreversible injury). Increases are not specific with regard to the nature of the injury
- "Primary" hepatic disorders
- inflammation: Viral, bacterial, fungal, immune-mediated, idiopathic. Inflammation can be suppurative or non-suppurative. Note that primary bile duct obstruction may result in secondary hepatocellular injury as accumulated bile acids are toxic to cells. Leakage enzyme levels in end-stage liver disease or portosystemic shunts may be normal or only mildly increased due to reduced number of cells and minimal active injury.
- intoxications: Drugs, chemicals, plants. These can cause very high enzyme activity if associated with diffuse necrosis.
- neoplasms: Hepatocellular and bile duct carcinomas, metastatic neoplasia. Variable increases are possible depending on the extent of active hepatocellular injury.
- Disorders with secondary hepatic effects
- circulatory: Heart failure, shock, severe anemia, portosystemic shunts, septicemia, gastrointestinal disease in horses (displaced colon, acute colitis).

metabolic: Endocrine disease (producing fatty liver, e.g. diabetes mellitus, Cushing's disease, and idiopathic lipidosis), hyperthyroidism, acute pancreatitis.

- Aspartate aminotransferase - AST
  Glutamic oxaloacetic transaminase, GOT

Aspartate amino transferase catalyzes the transfer of the alpha amino group of aspartic acid to alpha-ketoglutaric acid, resulting in the formation of oxaloacetic acid and glutamic acid. AST is useful as an indicator of liver and/or muscle injury in large and small animals.

- Causes of increased AST
- Artifact: Hemolysis or leakage from cells can cause erroneously high values (enzyme is present in RBC).
- Drug effects: Anticonvulsants may cause an increase in AST, which is thought to be secondary to hepatocellular injury in dogs. Corticosteroids generally do not result in increased AST levels, unless they result in a steroid hepatopathy (in dogs).
- Physiologic effects: In horses, exercise can increase serum activity as much as 30%. In early training, resting levels are 50-100% greater than resting levels of horses not in training.
- Disease effects

1) Myopathies: Muscle trauma (including "down" animals), rhabdomyolysis, white muscle disease (vitamin E-selenium deficiency), and infectious myositis (black leg or Clostridial myositis), and muscular dystrophy may result in marked increases.

Serum CK activity will also increased. Note that as AST has a longer half life than CK, increases in AST persist for longer than increases in CK. Therefore, in chronic muscle disease, AST may be elevated, whilst CK levels may be normal. When there is active muscle disease, both CK and AST are elevated (and CK will decline more rapidly as the injury resolves

- 2) Liver disease: AST will increase in liver disease with the same causes as for ALT. Increased levels seen with hepatocellular injury often aren't as high as those seen with muscle damage. CK levels are normal unless there is concomitant muscle disease. Other liver specific enzymes (SDH) would also be increased. In cats, AST appears to be a more sensitive marker of liver injury (values are often mildly increased with normal ALT in conditions such as pyogranulomatous hepatitis secondary to feline infectious peritonitis virus infection).

- Alanine aminotransferase - ALT
- Alanine amino transferase catalyzes the transfer of the alpha amino group of alanine to alpha- ketoglutaric acid, resulting in the formation of pyruvic and glutamic acid.
  Serum half-life is 59 hours in dogs and < 24 hours in cats. Following acute hepatic injury, serum enzyme activity peaks at about 48 hours and then begins to decrease.
  Increases in the enzyme occur due to cell damage (increased membrane permeability or necrosis) and induction (increased synthesis).

  Organ specificity

  ALT is virtually liver specific in dogs, cats, rabbits, rats and primates. Some increases are possible in severe muscle diseases of the dog and cat due to release of enzyme from this tissue. ALT is found in the liver, muscle (cardiac and skeletal), kidneys, and erythocytes.

  Causes of increased ALT
- Artifact: Hemolysis will cause increased levels in the cat. Cats have a high RBC to plasma ALT ratio. Hemolysis has a minimal effect on ALT in cattle, horses, and dogs.
- Drugs: Anticonvulsants (primidone, phenobarbitone, dilantin) increase ALT 4 x normal. Although these drugs may induce ALT synthesis, increases in ALT are thought to be secondary to hepatocellular necrosis.

- Disease effects
  1) Liver disease: Both primary and secondary hepatic disease can cause increased ALT levels, if altered cell membrane permeability or necrosis occur. Usually ALT values exceed AST values in liver disease. Hepatic neoplasia can result in marked increases in ALT in dogs, although increases in AST are often higher than the increases in ALT. Bile duct obstruction will increase ALT (and AST) due to the toxic effects of retained bile salts on hepatocytes. Trauma will often increase ALT levels, even without morphologic evidence of cell injury.

  2) Muscle disease: In large animals, ALT will increase with muscle injury but is not more useful than AST in this regard so it is not included on large animal chemistry panels. In small animals with severe muscle injury (ischemic myopathy in cats, muscular dystrophy in dogs), ALT will increase with CK and AST. However, the increases in ALT are usually less than increases in AST in primary muscle disease and SDH values should be normal (unless there is concurrent liver injury).

- Sorbitol dehydrogenase - SDH
  Iditol dehydrogenase (ID)
-

  Sorbitol dehydrogenase is found in highest concentration in the liver. It is a cytoplasmic enzyme with a short half life (<4 hours in the dog). It catalyzes the conversion of fructose to sorbitol. It is a very specific indicator of liver disease in all species, although increases can occur with primary or secondary liver disease (e.g. many horses with inflammatory gastrointestinal disease will have high SDH values). Increases occur within 24 hours of liver injury. SDH is the enzyme of choice for detecting hepatocellular injury in horse and cattle

  SDH is not a stable enzyme. In cattle, serum samples are only stable for 5 hours at room temperature, 24 hours at 4 C and several days frozen. In horses, it has similar stability.
-
- Serum Alkaline Phosphatase
  Gamma Glutamyl Transpeptidase

- The primary utility of these two enzymes is to serve as indicators of cholestasis. Cholestasis implies impairment of bile flow, which can be caused by physical or functional obstruction of the biliary tract within or outside of the liver. When severe enough, cholestasis will result in elevations of bilirubin in blood. The main value of these enzymes is their greater sensitivity for this abnormality as compared to serum bilirubin levels alone.

  Alkaline phosphatase, however, is less than entirely specific for this purpose, in that its activity in serum can be influenced by a variety of other factors.

  Gamma GT is more specific in general, and more sensitive in some instances.

  Alkaline phosphatase (AP, ALP, SAP)

  Alkaline phosphatase is a non-specific metalloenzyme which hydrolyzes many types of phosphate esters at an alkaline pH in the presence of zinc and magnesium ions. There are different isoenzymes (gene products) and isoforms (posttranslationally modified gene products). The main use of ALP is as a sensitive indicator of cholestasis in the dog (it will increase before bilirubin), however it is non-specific because corticosteroids (exogenous or endogenous "stress") induce increases in this enzyme. In the cat, ALP is a very specific indicator of liver disease, whereas in large animals, the enzyme is not very useful as it is insensitive, cholestatic disorders are infrequent, and reference intervals are quite broad.

  Organ specificity

- Liver - hepatocytes, epithelium of biliary tract. These cells are the source of the L-ALP isoform (all species) and C-ALP isoform in the dog.
- Bone - this isoform is produced by osteoblasts and increases in serum in association with osteoblastic activity (young animals, certain bone disorders).
- Intestinal, renal, mammary, placental tissues - Leukocytes - ALP is found within myeloid cells, including neutrophils, eosinophils and monocytes. Cell lineage expression is species-dependent, i.e. monoblasts (immature monocytes) in dogs are particularly rich in ALP.
-

- Leukocytes - ALP is found within myeloid cells, including neutrophils, eosinophils and monocytes. Cell lineage expression is species-dependent, i.e. monoblasts (immature monocytes) in dogs are particularly rich in ALP.
-
- Drug effects
- Glucocorticoids: In dogs, increased total ALP is due mainly to synthesis of the C-ALP isoform. Marked increases are possible (50-100 fold). Total ALP may remain high for three to six weeks, depending on the drug preparation administered (ie, short-acting vs. depot forms). Interestingly, it takes approximately 10 days for C-ALP to be induced by corticosteroids; therefore the initial increases in total ALP with corticosteroid administration is due to increases in the L-ALP, and not the C-ALP, isoform.
- Anticonvulsants: phenobarbital, primidone, phenytoin - mild to marked increases in total activity occur, due mainly to raised L-ALP isoform. This is probably secondary to cholestasis because studies in dogs with phenobarbitone show that liver synthesis of ALP is not induced.
- Age effects
  ALP activity in young, growing animals of all species may be 2 - 10 times higher than in adults, due to increased B-ALP isoform. Values decrease within 3 months of age and are within adult ranges by 15 months of age.

- Disease effects
- Hepatobiliary disease: Increases in ALP (primarily the L-ALP isoform) is used as an indicator of cholestasis (intra- or extrahepatic) in animals. In cats, ALP is a specific but insensitive marker of hepatobiliary disease. Increases in ALP do occur in hepatobiliary disease, but the increase is less reliable and of lower magnitude compared to the situation in dogs (feline hepatic tissue contains much less ALP and serum half life is only six hours). Therefore, any increases in ALP in the cat are considered clinically relevant. The wide range of ALP activities and insensitivity of this test to cholestasis in large animals limits utility of ALP in these species.
  a) Extrahepatic cholestasis (bile duct obstruction): This causes very dramatic increases in ALP. Increases in ALP may occur before development of icterus, especially in the dog. Pancreatitis (acute or chronic) may result in increased ALP levels from swelling and/or fibrosis around the bile duct (especially in cats which have a common bile and pancreatic duct).
-
- b) Intrahepatic cholestasis: Localized or generalized cholestasis from hepatocyte swelling will induce ALP. Lesions that are primarily centrilobular generally cause only mild increases in ALP while lesions affecting the periphery (periportal areas) of the lobule usually result in more dramatic elevations as a result of impaired bile flow. Causes of intrahepatic cholestasis include neoplasia (primary or metastatic), hepatic lipidosis (marked increases are possible with idiopathic hepatic lipidosis in cats - lipidosis is the cause of the most dramatic increases in ALP in this species, often without concurrent elevations in GGT, which is a useful diagnostic feature), acute hepatocellular injury (intrahepatic cholestasis occurs due to hepatocellular swelling; elevated ALT levels are expected concurrently), bile sludging (occurs with anorexia, especially in cats) and periportal fibrosis and inflammation (marked increases are possible).

-
- c) Functional cholestasis: This is defined as decreased bile flow due to downregulation or inhibition of transporters responsible for excreting bile salts or conjugated bilirubin into bile. It occurs without any physical obstruction or

impairment to bile flow. It is frequently mediated by inflammatory cytokines and has been reported in dogs with E coli infections. It likely occurs in other species as well. Cytokine-mediated cholestasis is usually characterized by high total bilirubin (due to direct and indirect bilirubin) with mild increases in hepatocellular leakage enzymes (ALT, SDH, GLDH). ALP levels may be normal in this condition.
d) Neoplasia: In primary liver cancer (hepatocellular/biliary), marked increases in ALP are possible (due to L-ALP or C-ALP in dogs). Metastatic neoplasia often increases ALP due to localized cholestasis. In many cases of hepatic neoplasia, ALP may be the only enzyme that is increased on a panel.

- e) Acute hepatocellular injury: Mild to moderate elevations in ALP are attributed to intrahepatic cholestasis associated with hepatocellular swelling rather than hepatocellular injury per se. Concomitantly high values of ALT, SDH and GLDH would be expected.
- Hyperadrenocorticism - Levels vary from moderate to marked (up to 100- fold) and are frequently due to induction of the C-ALP isoform (although L-ALP increases are also seen) in dogs. Up to 83-100% of dogs with Cushings disease have high C-ALP levels, but chronic endogenous stress (due to any underlying disease) may increase C-ALP and total serum ALP (up to 2-3 x normal). Therefore, C-ALP levels are a sensitive, but not specific, test for hyperadrenocorticism in dogs.
- Increased osteoblastic activity - osteoblastic activity in response to hormones (PTH, thyroxine) or neoplasia (osteosarcoma) may increase total serum ALP due to the B-ALP isoform.
  1) Primary and secondary hyperparathyroidism (2-3x increase).
  2) Osteosarcoma
  3) Fracture healing in dogs.
  4) Hyperthyroidism in cats: Affected animals may have mild increases in total serum ALP, which is mostly due to high B-ALP with a lesser increase in the L-ALP isoform.

- Gamma-glutamyl transferase - GGT
  Gamma-glutamyl transpeptidase, GGTP

Organ specificity

GGT is found primarily in the membrane and in microsomes (from SER) as aggregates. A small portion (< 5%) is found in the cytoplasm. Disaggregation (solubilization) and increased synthesis result in increased activity in serum.

- Biliary tract - source of serum activity in health and disease.
- Pancreas, gastrointestinal tract - GGT does not increase in serum in disorders involving these tissues (it is usually shed into the lumen of these organs).
- Kidney - proximal renal tubules. GGT is shed into urine, rather than blood.
- Mammary glands - GGT is excreted into milk.
- Reproductive tract - Epididymis (GGT is secreted into semen).

- Causes of increased GGT
- Drug effects
  Increases in GGT occur secondary to therapeutic drugs causing cholestasis. Increases may also be seen with anticonvulsant or corticosteroid therapy, presumably secondary to liver injury causing localized cholestasis or biliary

hyperplasia. Induction of GGT synthesis may also contribute to high GGT levels with corticosteroids. Increases in GGT occur as soon as 5 days after corticosteroid administration.

- Physiologic

  a) Neonates: Colostrum in all species, except for horses. contains high GGT concentrations. Increases in GGT occur within 24 hours of suckling and are a sensitive indicator of passive transfer.
- b) Breed: Donkeys and burros have 2-3 x GGT levels of horses.
-
-
- Disease effects

  Increases in GGT occur secondary to hyperplasia or induction of synthesis.

  a) Hepatobiliary disease: In small animals, GGT is a sensitive indicator of biliary hyperplasia and cholestasis.
- b) Renal disease: GGT is expressed on the membranes of proximal renal tubular epithelial cells. Cell injury causes GGT to be shed into the urine and not into blood. Urinary GGT:creatinine ratio has been studied as an early indicator of renal tubular injury.
-
- Bilirubin
- The bilirubin panel provides information about the total bilirubin concentration in the sample, as well as the composition of that total in terms of proportion conjugated vs. unconjugated
- Total Bilirubin
- The majority of bilirubin (80%) is produced from the degradation of hemoglobin from erythrocytes undergoing normal (removal of aged or effete cells) or abnormal destruction (i.e. intravascular or extravascular hemolysis) within mononuclear phagocytes (principally splenic, hepatic and bone marrow macrophages). A small percentage (20%) is derived from the catabolism of various hepatic hemoproteins (myoglobin, cytochrome P450) as well as from the overproduction of heme from ineffective erythropoiesis in the bone marrow. Within macrophages, a free heme group (iron + porphyrin ring) is oxidized by microsomal heme oxygenase into biliverdin and the iron is released (the iron is then stored as ferritin or released into plasma, where it is bound to the transport protein, transferrin).
-
-). Biliverdin reductase then reduces the green water-soluble biliverdin into unconjugated bilirubin. Heme oxygenase is also located in renal and hepatic parenchyma, enabling these tissues to take up heme and convert it to bilirubin. Birds lack biliverdin reductase, thus they excrete heme breakdown products as biliverdin rather than bilirubin. Unconjugated or free bilirubin is then released into plasma where it binds to albumin. Uptake of unconjugated bilirubin occurs in the liver and is carrier-mediated. The carrier-mediated uptake is shared with unconjugated bile acids and dyes such as BSP. Once within the hepatocyte, unconjugated bilirubin is transported with ligand (Y protein) or other proteins (e.g. Z protein) and the majority is conjugated to glucuronic acid by UDP-glucuronyl transferase. The remainder is conjugated to a variety of neutral glycosides (glucose, xylose). In the horse, the majority of bilirubin is conjugated to glucose. Bilirubin must be conjugated before it can be excreted into bile (conjugation makes bilirubin water soluble). Excretion into biliary canaliculi is the rate-limiting step of the entire bilirubin metabolism pathway and occurs via specific transporters, which are energy (ATP) dependent
-
- Transfer into the canaliculi is facilitated by bile salt-dependent and bile salt-

independent biliary flow (the latter of which is generated by a basolateral (sinusoidal or blood-side) Na/K ATPase pump). Bilirubin is secreted, along with bile salts (and sodium) into the intestine, where the bile salts form micelles facilitating absorption of fat. In the intestine, bacterial enzymes deconjugate bilirubin and degrade it to urobilinogen. Urobilinogen is reabsorbed (about 10%) or broken down (90%) into urobilin and stercobilin (both of which are excreted in the feces). Of the resorbed urobilinogen, most is taken up by the liver (enterohepatic circulation, i.e. the urobilinogen is absorbed into the portal vein, taken up by the liver and re-excreted into bile), whilst the rest bypasses the liver and is excreted into the urine. Conjugated bilirubin is not normally found in the urine of domestic animals, although small to 1+ amounts of conjugated bilirubin may be seen in concentrated urine from dogs (particularly males), due to the low canine renal threshold for bilirubin. In all species (but dogs, in particular), bilirubinuria may precede an increase in serum bilirubin in cholestatic disorders. Remember, only conjugated bilirubin can be excreted in urine as it is water soluble.

-
- Circulating bilirubin exists in two main forms as determined by the Van den Bergh reaction, which differentiates bilirubin into conjugated (direct) and unconjugated (indirect) forms. There is a third form of bilirubin, called delta bilirubin (or biliprotein), which is conjugated bilirubin bound to proteins. Delta bilirubin increases in serum when hepatic excretion of conjugated bilirubin is impaired (cholestasis) and the liver retains intact conjugation mechanisms. Delta bilirubin may be responsible for a persistent bilirubinemia without bilirubinuria seen in some animals with cholestasis.
- Causes of increased total bilirubin
- Artifact: With some analyzers and reagents, hemolysis and lipemia (even mild) will cause artifactually high bilirubin values.
- Hemolysis: Destruction of red cells, whether through extravascular or intravascular hemolysis will increase the production of unconjugated bilirubin because of enhanced hemoglobin metabolism by mononuclear phagocytes.
- Liver disease: Hepatic disease may cause increases in both unconjugated and conjugated bilirubin. Increases in bilirubin in dogs often occur after elevation of cholestatic enzymes (GGT, SAP) due to the low renal threshold for bilirubin. In acutely developing icterus, SAP and GGT levels may be normal because they require time for induction. In large animals with liver disease, increases in bilirubin are usually due to unconjugated bilirubin.
-
- Cholestasis: This is defined as decreased bile flow and can be due to physical obstruction of bile flow or functional defects in the transporters that deliver bile salts or bilirubin into the biliary system. Physical obstructions to bile flow can be intrahepatic ((e.g. hepatocyte swelling due to hepatic lipidosis in cats) or extrahepatic (e.g. bile duct obstruction from pancreatic neoplasia, cholelithiasis, Fasciola hepatica in cattle). Functional defects in bile salt or bilirubin transporters occur secondary to inflammatory cytokines (e.g. endotoxemia) and drugs.
- Amylase and Lipase
- Serum activity of these enzymes is measured as an (imperfect) aid to the clinical diagnosis of pancreatic injury.

  Idiopathic inflammatory disease (acute-to-chronic pancreatitis) is the most common disease entity (mainly dogs, occasionally cats, rarely, horses). Less commonly, pancreatic tumors (adenocarcinoma) or trauma (HBC) can be the cause of clinical signs and elevated enzyme activity.

  With regard to the diagnosis of pancreatitis, results must be interpreted in light of the history and clinical signs in the patient, and correlated with physical,

radiographic, hematologic, and other clinical chemistry findings:

- vomiting, diarrhea, history of recent fatty meal, table scraps, garbage ingestion
- painful abdomen, dehydration, sometimes icterus
- hazy cranial abdomen on plain films
- neutrophilic leukocytosis
- azotemia (often), lipemia (sometimes), hypocalcemia (occasionally), cholestasis
- Newer tests, such as trypsin-like immunoreactivity (TLI) can be used to diagnose pancreatitis and are thought to be more sensitive than measurement of these enzymes in dogs.
- Amylase
- Organ specificity
- Pancreas: Found in zymogen granules. The pancreas has higher concentrations of amylase than other tissues.
- Intestine: Duodenum, ileum
- Ovary and testes
- Salivary gland: Salivary amylase is found in high concentration in pigs, resultilng in high reference intervals for amylase in this species. Dogs lack salivary amylase.
- Causes of hyperamylasemia
- Acute pancreatitis: The increase and decrease of serum amylase tends to parallel that of lipase. Amylase values peak at 12-48 hours and are normal within 8-14 days after about of pancreatitis in dogs. It is rare to observe increased amylase in cats with pancreatitis. Note that amylase concentrations are often higher in ascitic fluid than in blood in animals with pancreatitis (or intestinal disease).
- Chronic renal insufficiency: As discussed above, the increase in amylase is due to macroamylase formation (polymerization with globulins).
- Decreased GFR: This can cause increased amylase (up to 2-3 x normal) in the absence of significant pancreatic disease. If an azotemic patient has amylase values greater than 2 to 3 times the reference values, pancreatitis should receive consideration as a diagnosis.
- Intestinal disease/obstruction: Moderate elevations in amylase are possible.
- Lipase
- Lipase hydrolyzes triglycerides. There are several forms of lipase: pancreatic lipase, colipase and lipoprotein lipase.

Causes of increased lipase

- Drugs : Corticosteroids are reported to increase lipase values.
- Acute pancreatitis: Destruction of pancreatic acinar tissue results in the escape of pancreatic enzymes into the pancreas and peritoneal cavity. The enzymes enter the blood by way of lymphatics or capillaries with subsequent elevation of serum levels. Increases of at least 2 x normal are seen in pancreatitis.
  In the cat, lipase is not consistently elevated in pancreatitis. It was hoped that measurement of trypsin-like immunoreactivity (TLI) would be better for diagnosis in cats, as high values are seen in dogs with pancreatitis and are very useful for diagnosis in dogs with normal lipase values.
- Gastrointestinal disease: Peritonitis, gastritis, bowel obstruction, visceral manipulation (laparotomy), hepatic disease and neoplasia may increase lipase values by 2-3 x normal.
- Decreased GFR: Increases of up to 4 x normal may be seen in patients with decreased GFR. In azotemic patients with lipase levels greater than 3-4 times normal, the diagnosis of pancreatitis should still be considered.

- Cholesterol
- Cholesterol is the most commonly occurring steroid. It is an important precursor of cholesterol esters, bile acids and steroid hormones. It is derived from dietary sources and synthesized in vivo from acetyl-CoA in the liver (main site) and other tissues (intestines, adrenal glands and reproductive organs). The bile is the main route of excretion of cholesterol. Note that visible hyperlipemia in a blood sample is usually due to increased triglycerides not due to increased cholesterol.

- Causes of hypercholesterolemia

- High cholesterol is usually due to increased numbers of cholesterol-rich lipoproteins, i.e. HDL and LDL. Because VLDL's do contain some cholesterol (12%), high cholesterol can also be seen with very high VLDL concentrations.
- Inherited disorders of lipid metabolism: Familial hypercholesterolemia has been reported in Briards, Rottweilers, Shetland Sheepdogs, and Dobermans.
- Diabetes mellitus: Insulin stimulates lipoprotein lipase, which is responsible for hydrolysis of chylomicrons (CM) and VLDL. Insulin also antagonizes hormone sensitive lipase, the hormone responsible for lipolysis of adipose tissue. Lack of inhibition of hormone sensitive lipase causes increased lipolysis, with increased non-esterified fatty acid presentation to the liver and VLDL production. In addition, LDL receptors on hepatocytes are downregulated, resulting in increased LDL levels.
-
-
- Hypothyroidism: In dogs, hypothyroidism is associated with mild to marked elevations in cholesterol, due to increased LDL and HDLs. Thyroid hormone (T3) stimulates LDL receptors (and promotes uptake of cholesterol), therefore lack of thyroid hormone results in decreased LDL receptors and decreased LDL (cholesterol) uptake.
- Nephrotic syndrome: This is characterized by edema, hypoalbuminemia, hypercholesterolemia and albuminuria and is caused by glomerular damage, e.g. amyloidosis, immune-complex glomerulonephritis. There is an increase in HDL and LDL in this syndrome, although the exact mechanism is unknown.
- Hyperadrenocorticism: Hypercholesterolemia is due to increased LDL, thought to be due to peripheral insulin resistance and down-regulation of LDL receptors in the liver. Corticosteroids also stimulate hormone-sensitive lipase, resulting in increased lipolysis and VLDL production.

- Cholestasis: In hepatobiliary disorders, especially those causing extrahepatic cholestasis (e.g. bile duct obstruction), increased cholesterol may be seen.
- Pancreatitis: Although hypertriglyceridemias are more common in this disorder, high cholesterol may be seen concurrently due to inhibition of lipoprotein lipase.
- Excessive negative energy balance: In states of excessive negative energy balance (e.g. starvation, anorexia) particularly when energy demands are high (e.g. late pregnancy, early lactation), lipolysis of fat stores in adipocytes will increase VLDL concentrations. Although VLDLs contain more triglycerides than cholesterol, increases in both of these analytes may be seen. Hyperlipemia due to excessive negative energy balance mostly occurs in horses and camelids and is associated with secondary hepatic lipidosis.
-
- Causes of abnormally low cholesterol levels
- Low cholesterol can be due to decreased numbers of cholesterol-containing lipoproteins (LDL, HDL, VLDL) or a decreased cholesterol content of these

lipoproteins.

- Decreased absorption: Malabsorption and maldigestion problems, e.g. protein losing enteropathies, exocrine pancreatic insufficiency.
- Decreased production: Since the liver is the main site of cholesterol production, low cholesterol values can be seen in chronic liver diseases (e.g. cirrhosis), synthetic liver failure (acute or chronic), and portosystemic shunts (acquired or congenital).
- Altered metabolism: Inflammatory cytokines can reduce the cholesterol content of lipoproteins by decreasing lecithin-cholesterol acyltransferase activity (the enzyme responsible for converting free cholesterol to cholesterol ester which is then incorporated into HDL and LDL)..
- Increased uptake of lipoproteins: Upregulation of LDL-receptors on cells (peripheral tissues and liver) can potentially lower cholesterol values. This occurs in rapidly proliferating tumor cells (e.g. acute myeloid leukemia in human patients) and in response to inflammatory cytokines (some acute phase proteins in human patients, such as serum amyloid A, enhance LDL removal from the circulation in acute phase reactions).
- Creatine Kinase – CK
- CK is a "leakage" enzyme present in high concentration in the cytoplasm of myocytes and is the most widely used enzyme for evaluation of neuromuscular disease.
- Causes of increased CK
- Artifact: Hemolysis will increase CK values as analytes in red blood cells (ADP or G-6-P) or their membranes (adenylate kinase) contribute to the enzymatic reaction for CK, artifactually increasing values. Inadvertant penetration of muscle during venipuncture can cause 3- to 4-fold increases in CK activity in the sample and is a common cause of mildly increased CK values in healthy (or sick) animals.
- Physiologic: CK values in young puppies are higher than in adults. Moderate increases (2-3x) are possible in exercising horses. Post exercise increase is less in well conditioned animals although the baseline level is somewhat higher. Intramuscular injections will increase CK values (2-3x), dependent on the drug,
-
- muscle binding of drug, local blood flow. CK will especially increase if the injected compound is an irritant (e.g. tetracyclines).
- Muscle disease: Detection of increased activity in serum is useful as an indicator of muscle injury. The assay is quite sensitive in this regard, but elevations are not specific as to cause (e.g., trauma, inflammation, degeneration). High CK values are observed in inherited muscular dystrophies, exercise-induced rhabdomyolysis, polymyositis, vitamin E-selenium deficiency, snake bite poisoning, etc
-