CREATING CLARITY

Breezing through the biochemistry: Interpreting electrolyte profiles

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Clinical Pathologist

Friday 15th November, 2024

IDEXX

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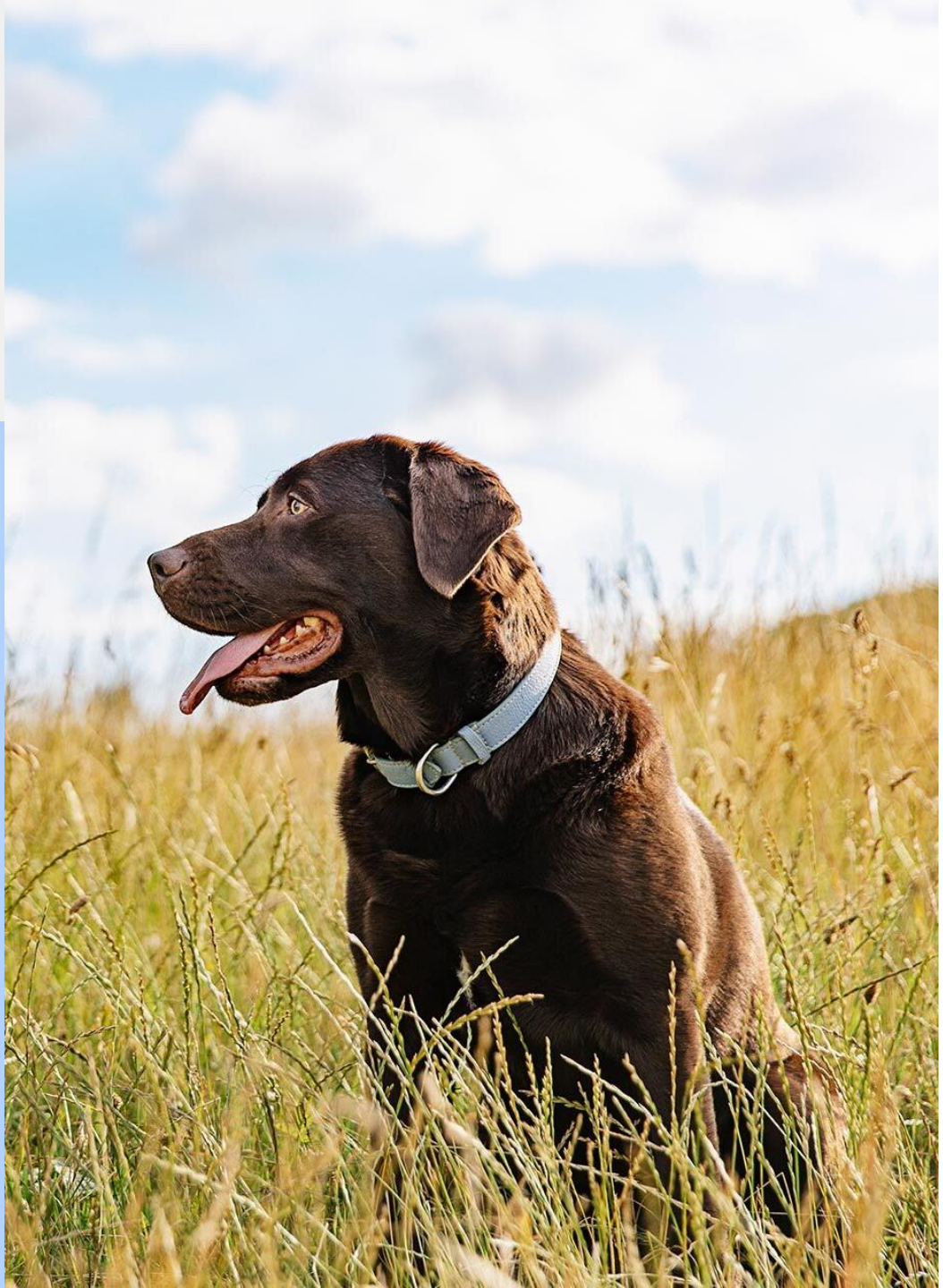

Disclosure:

I am an employee of IDEXX Laboratories Ltd.

Disclaimer:

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Learning Objectives

1. Brief introduction into the most common methodologies utilized by biochemical analysers to measure electrolyte concentrations.
2. Understanding the most common pre-analytical errors to affect electrolyte abnormalities in dogs and cats.
3. Reviewing the basic physiology of the most common electrolytes and how disturbances in the physiology will lead to electrolyte changes.
4. Use case examples to highlight the most pertinent electrolyte changes encountered in dogs and cats.

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Electrolyte Measurement Methodologies

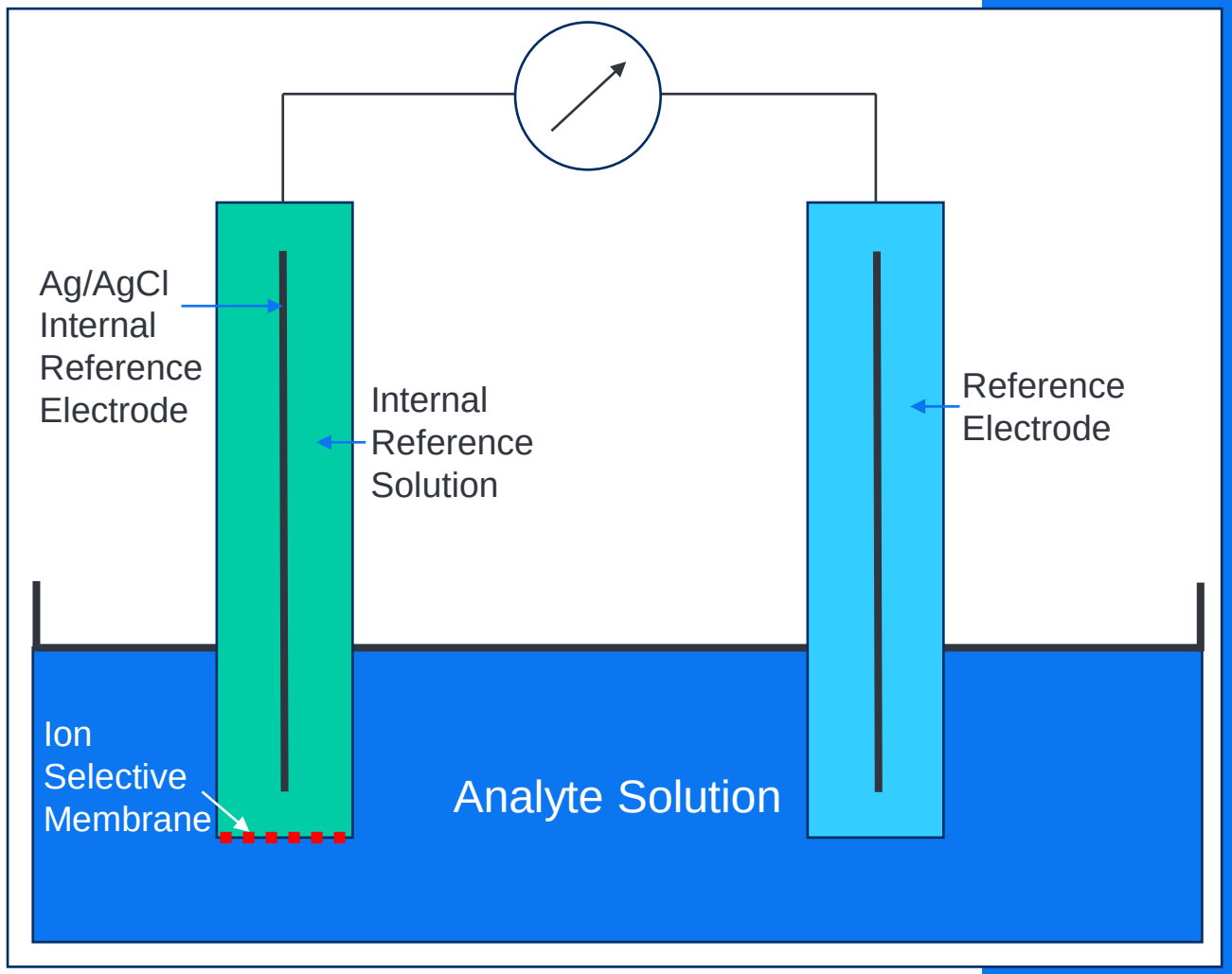
- Ion selective electrodes:
  - Determines the activity of ions in an aqueous solution by measuring the electrical potential.
  - Sodium, potassium and chloride (direct).

1. Direct potentiometry (e.g. in-house analysers): direct measurement of the electrolyte concentration – unaffected by protein/lipid changes.

[Electrolyte] ∝ Electrial Potential

2. Indirect potentiometry (e.g. reference laboratory analysers): measurement of the electrolyte in a diluted sample – affected by protein/lipid changes.

~~[Electrolyte] ∝ Electrial Potential~~



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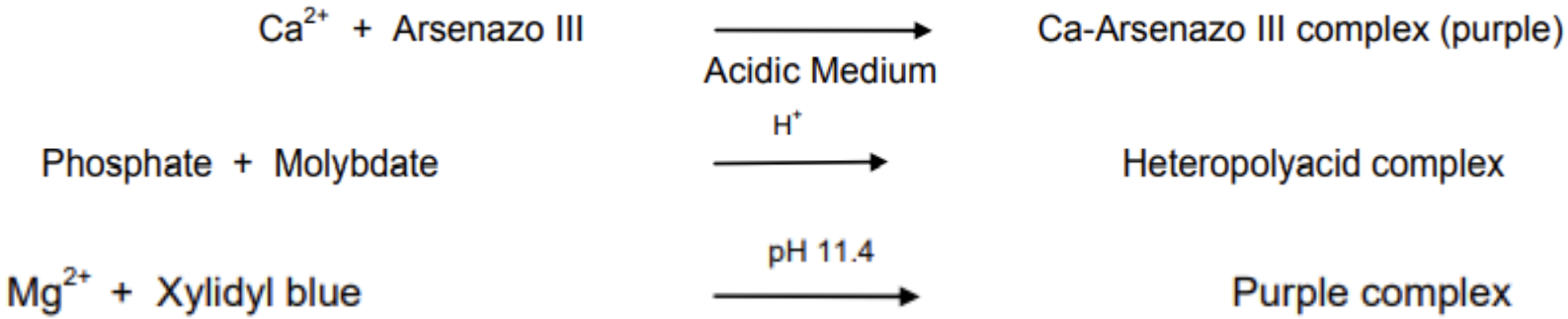
Electrolyte Measurement Methodologies



- Photometric assays:
  - Colour change is elicited by a chemical reaction and the wavelength (for example) of the colour change is detected.
  - Calcium, phosphate, and magnesium are measured in this way.
  - Also starting to be used for point-of-care analysers.

- Out of date assays:
  - Chloride: colourmetric and amperometric titration.
  - Flame photometric assays.

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Error Types

+ Pre-analytical:

These are variables associated with the patient, sample collection and sample handling.

  + Biological/Patient or Non-Biological/Sample

+ Analytical:

These are factors which influence the analytical procedure, such as precision and accuracy, and sample quality.

  + Assay performance

+ Post-analytical:

This involves the different ways data from the laboratory is presented, stored and transferred to the clinician.

  + Results delivery system

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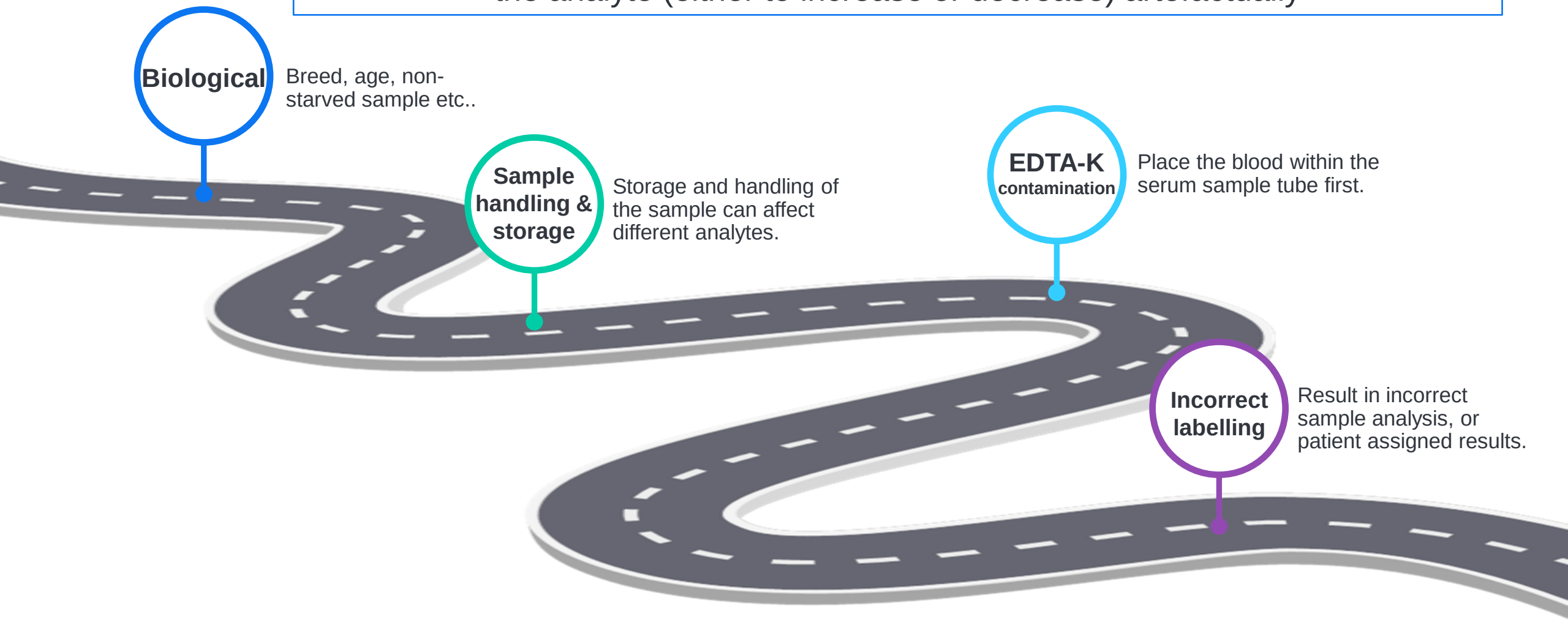
Pre-Analytical Errors

Changes that occur after sampling, prior to analysing that alter the magnitude of the analyte (either to increase or decrease) artefactually



Biological Breed, age, non-starved sample etc..

Sample handling & storage Storage and handling of the sample can affect different analytes.

EDTA-K contamination Place the blood within the serum sample tube first.

Incorrect labelling Result in incorrect sample analysis, or patient assigned results.

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Interferences; Haemolysis, Lipaemia and Icterus

+ Can be assessed visually, and automatically by the analyser.
+ Levels of interference will vary with each different analyser/brand.
+ Drugs (Br^-) and paraproteins can also cause interferences.
+ Sodium, potassium and chloride can be falsely decreased.

Lipaemia Index	Gross Appearance
<30	Clear
30-60	Hazy/Slight
60-120	Milky/Moderate
>120	Creamy/Marked

Haemolysis Index	Gross Appearance
<20	Clear
20-100	Pink tinged/Slight
100-300	Red/Moderate
>300	Dark red/Marked

+ Potassium can be falsely increased in certain dog breeds.
+ Marked changes can affect each individual analyser in different ways.

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Effects of Dilution; Heparin

- Electrolyte concentrations measured on POC will be affected by pre-loading of syringes with heparin
    1. Partly due to direct dilution effects
    2. Direct addition of NaCl

- Falsely high Na and Cl, and falsely decreased K.
- iCa and Mg will be falsely decreased due to chelation, and iCa possibly by pH changes also.

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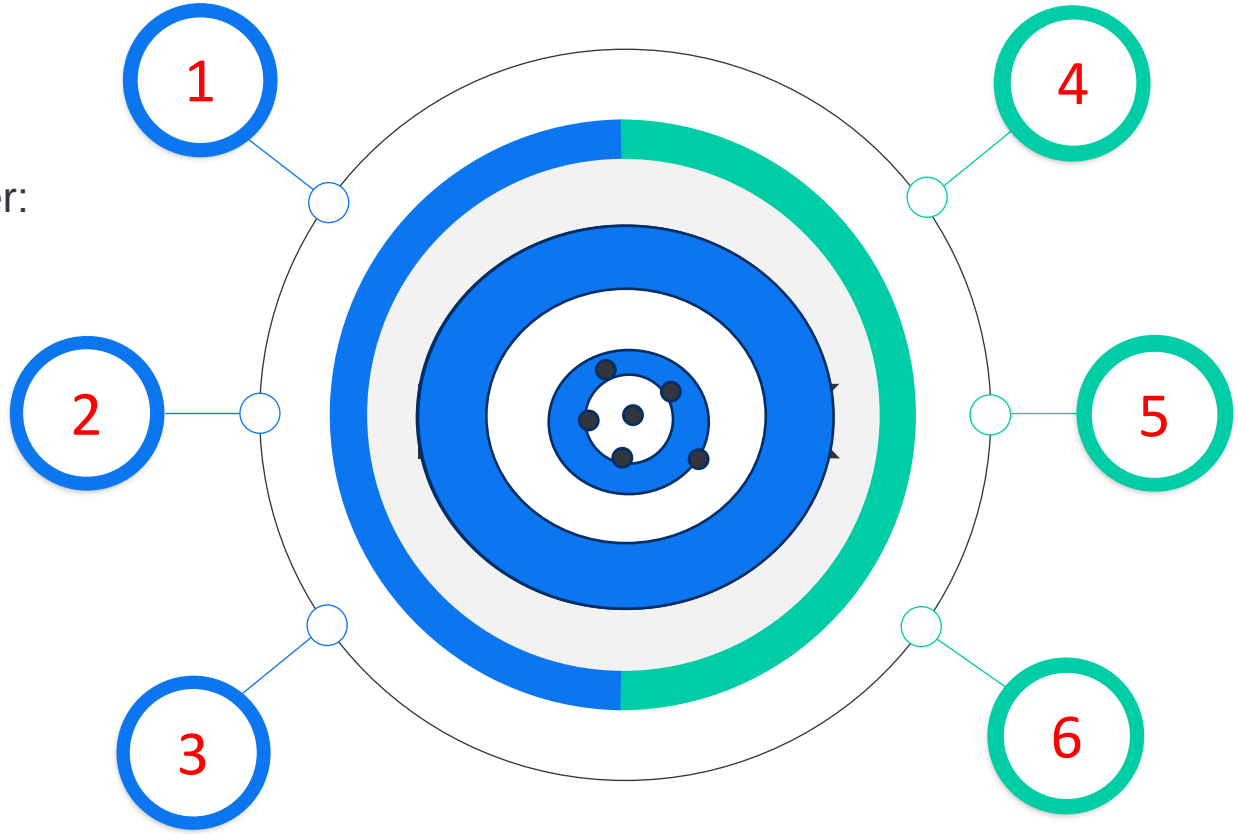
Hopper K *et al.* 2005

Analytical Error

Requirements from the analyser:

1. Trueness **No**
2. Precision **No**



For each analyte the chemistry analyser will be calibrated to trueness and precisely determine the [electrolyte] over a defined concentration range. QC material is used to determine the analyser's accuracy and precision.

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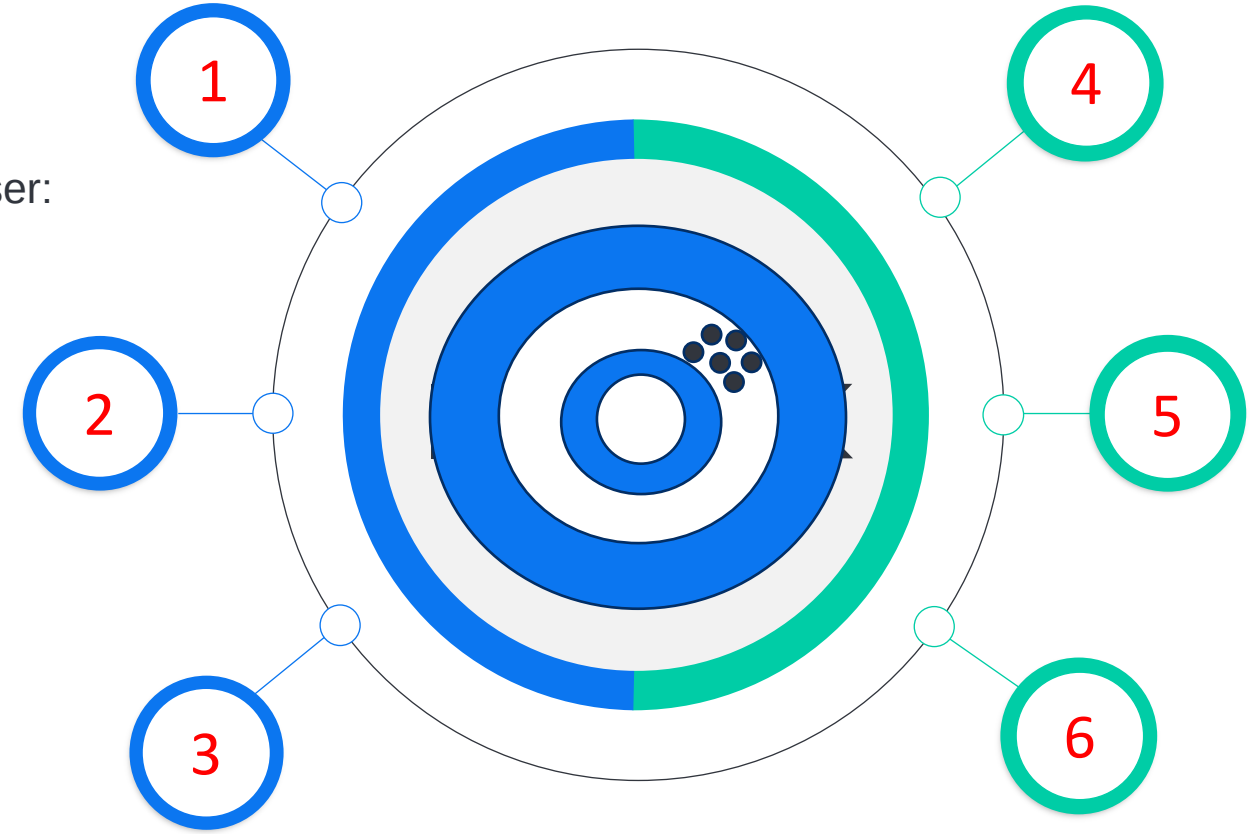
Analytical Error

Requirements from the analyser:

1. Trueness **No**
2. Precision **Yes**



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Analytical Error

Requirements from the analyser:

1. Trueness **Yes**
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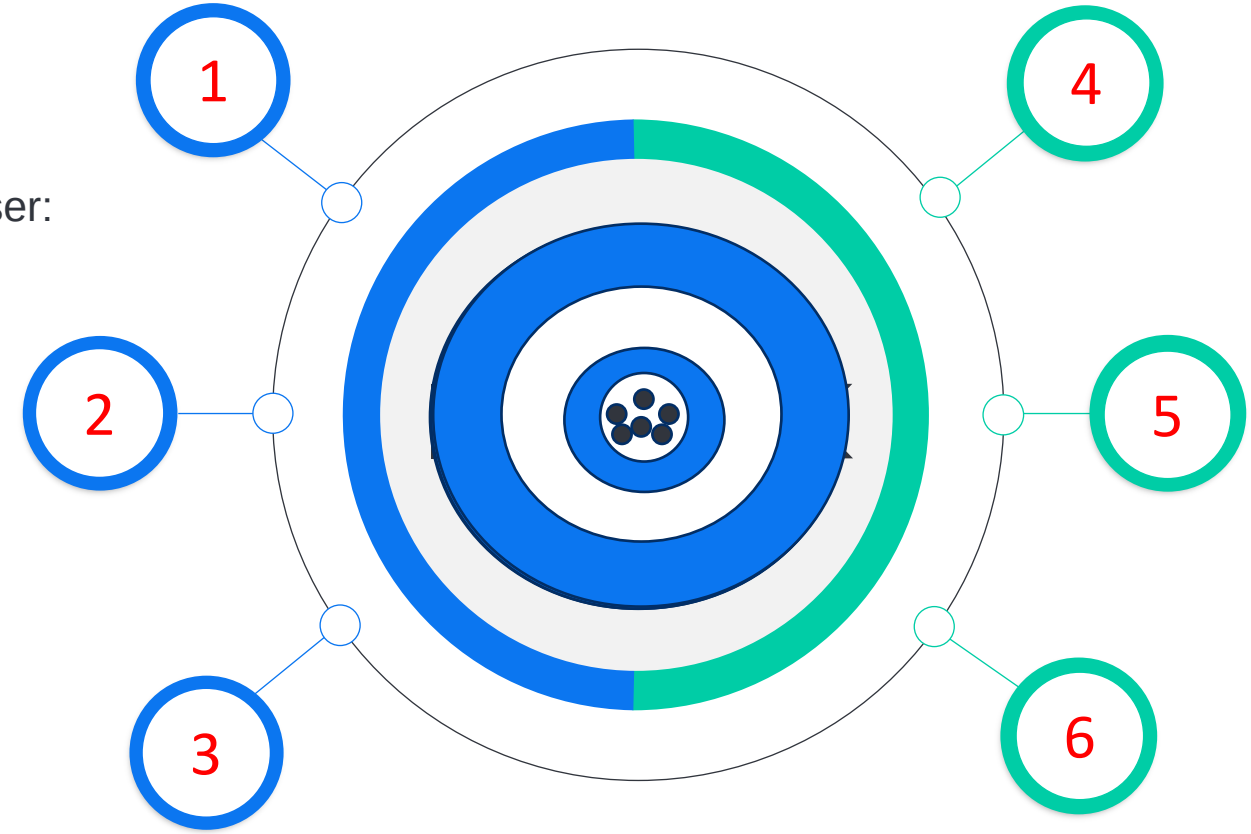

Trueness and precision are maintained through regular QC analyses and following QA protocols.

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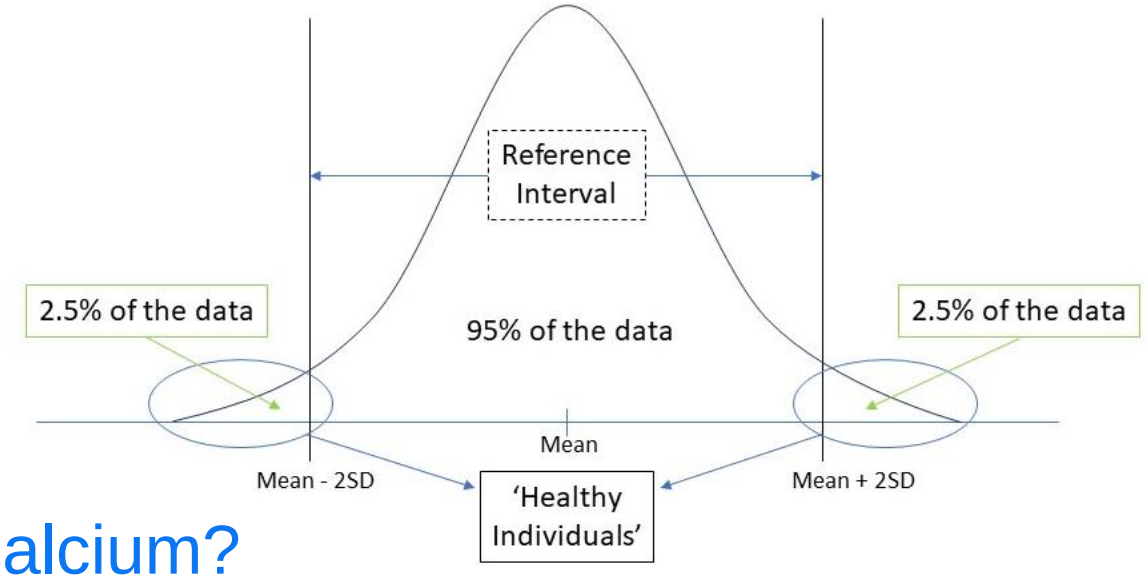
The use of reference intervals

Population based reference intervals comprise 95% of the healthy reference population.

What about biologic variation?

Pregnancy/Lactating – calcium?

Age/skeletal immaturity – phosphate/calcium?



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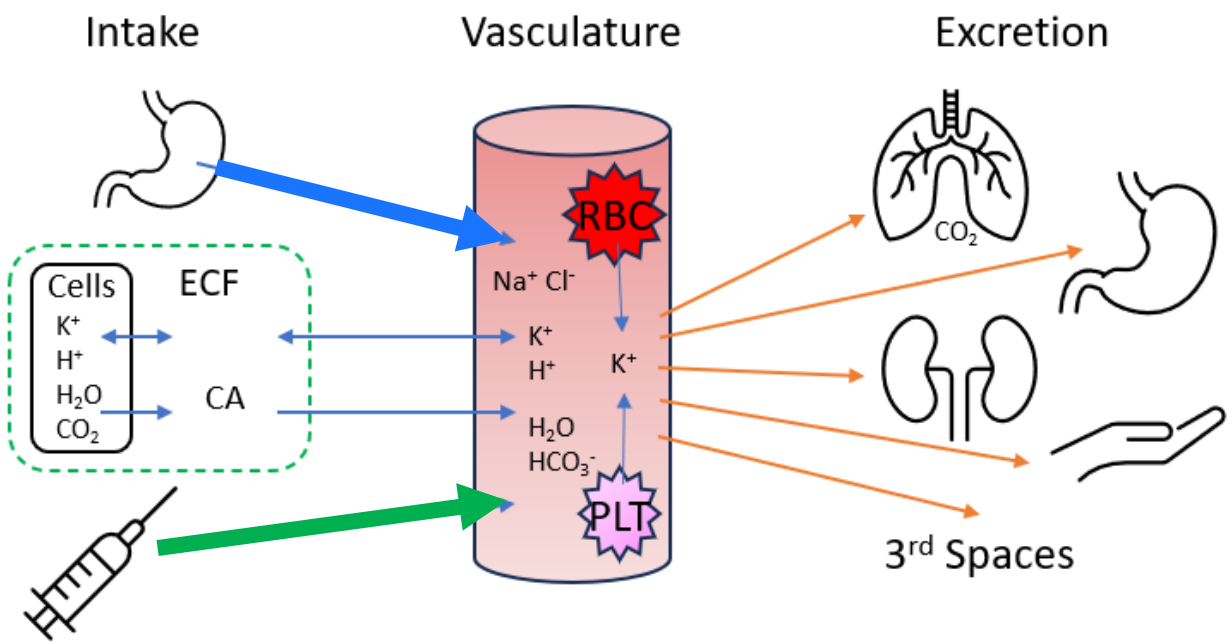
Electrolyte Physiology

+ Sodium, chloride and potassium enter via food/fluids.
+ HCO_3^- generated in lungs etc.
+ ECF: rich in NaCl.
+ ICF: rich in K.



Adapted from: Stockham and Scott Fundamentals of Veterinary Clinical Pathology, 2nd Edition.

+ Changes in electrolytes will result from:

1. Intake changes
2. ECF to/from ICF flux
3. Increased renal retention
4. Increased losses via the excretion routes

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Sodium and Chloride

- Assess in terms of hydration and ECF content
- NaCl control is performed through:
  - 1) Blood volume and RAAS
  - 2) Osmolality change – thirst change
- Hypernatraemia/chloraemia:

  H_2O loss > NaCl loss
  - Inadequate H_2O intake
  - Insensible loss, or DI (central or nephrogenic)
  - Osmotic losses; GI or renal

  NaCl intake > H_2O loss
  - Na poisoning, hypertonic saline administration
  - Hyperaldosteronism
  - Severe exercise in Greyhounds (Na mainly)

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Sodium and Chloride

- Assess in terms of hydration and ECF content
- NaCl control is performed through:
  - 1) Blood volume and RAAS
  - 2) Osmolality change – thirst change

- Hyponatraemia/chloraemia:

  NaCl loss > H_2O loss
  - GI or renal losses (Addison's etc)
  - Third space losses

  H_2O excess
  - Oedematous disorders; CHF, cirrhosis, nephrotic syndrome
  - Na-poor fluids, excessive ADH

  Na Shifts: ECF to ICF
  - Acute severe rhabdomyolysis

  H_2O Shifts: ICF to ECF
  - Hyperglycaemia (dilution effect)

  (K^+ depletion causing Na shifts intracellularly)

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Chloride Only Changes

Hyperchloraemia (only)
Metabolic acidosis (normal AG)
+ HCO_3- losses (GI)
+ HCO_3- losses – renal; Proximal/Distal RTA
Respiratory alkalosis (chronic)

Hypochloraemia (only)
Metabolic alkalosis
+ Cl losses (GI – vomiting/pyloric obstruction)
+ Diuretics
Metabolic acidosis w/ increased AG gap
+ Ketoacidosis, lactic acidosis, ethylene glycol toxicity

3 yo Crossbreed FN dog

+ No history provided

Sodium	140.0		mmol/L	135.0 - 155.0
Potassium	5.01		mmol/L	3.60 - 5.60
Sodium/Potassium ratio	**27.94**	***Low***		**28.80 - 40.00**
Chloride	**157.0**	***High***	**mmol/L**	**100.0 - 116.0**

+ Marked hyperchloremia
+ Normal Na and K

+ DDx: KBr therapy

Chloride	**157.0**	***High***	**mmol/L**	**100.0 - 116.0**
*Phenobarbital	28.0		mg/L	

15yo WHWT ME dog

+ Poor dental health, skin diseases and alopecia, clinical suspicions of calcinosis cutis, panting for many months

Neutrophils	**12.73**	2.94 - 12.67 x10^9/L
Bands	0.17	0.00 - 0.17 x10^9/L
Lymphocytes	1.72	1.06 - 4.95 x10^9/L
Monocytes	**2.58**	0.13 - 1.15 x10^9/L
Eosinophils	**0.00**	0.07 - 1.49 x10^9/L
ALP	**494.0**	<= 130.0 U/L

+ Stress or inflammatory leukogram
+ Increased ALP, inadequately concentrated USG
+ Hyperchloraemia

Suspicious for hyperadrenocorticism

Sodium	150.0	135.0 - 155.0 mmol/L
Potassium	5.30	3.60 - 5.60 mmol/L
Na: K Ratio	**28.30**	28.80 - 40.00
Chloride	**117.5**	100.0 - 116.0 mmol/L

+ DDx:
  + Chronic panting; respiratory alkalosis
  + Chronic panting; free water loss

2yo SBT MN dog

+ History of recent pyrexia, diarrhoea, and inappetence.
+ Long standing skin issues, and current injection site reaction on dorsum.

***Sodium**	**179.0**	**High**	**mmol/L**	**135.0 - 155.0**
*Potassium	3.71		mmol/L	3.60 - 5.60
***Sodium/Potassium ratio**	**48.25**	**High**		**28.80 - 40.00**
***Chloride**	**151.0**	**High**	**mmol/L**	**100.0 - 116.0**

Persistent

+ No azotaemia, mild hyperlipidaemia, Ca and PO_4 are WNL.

+ Causes:

H_2O-deficit	NaCl Excess
Inadequate water intake (e.g. congenital adipsia)	NaCl poisoning/hypertonic fluid
Insensible losses (fever is present)	Hyperaldosteronism
DI – central/nephrogenic	
H_2O > NaCl losses: Renal or GI osmotic losses	

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2yo SBT MN dog

***Sodium**	**179.0**	**High**	**mmol/L**	**135.0 - 155.0**
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***Sodium/Potassium ratio**	**48.25**	**High**		**28.80 - 40.00**
***Chloride**	**151.0**	**High**	**mmol/L**	**100.0 - 116.0**

+ Need to assess the USG/UA; 1.037 + no glucosuria.
+ Investigate the dog's water intake, full history.
+ Neurological examination, and imaging (hypothalamic lesion), osmolarity concentration.

H_2O-deficit	NaCl Excess
Inadequate water intake (e.g. congenital adipsia)	NaCl poisoning/hypertonic fluid
Insensible losses (fever is present) ≠	~~Hyperaldosteronism~~
~~DI – central/nephrogenic~~	
~~H_2O > NaCl losses: Renal or GI osmotic losses~~	

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Electrolyte disturbances: Potassium

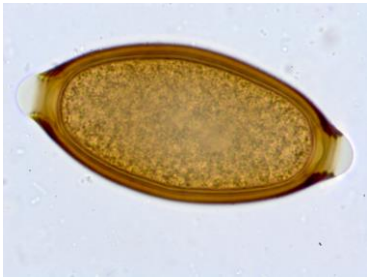
Serum [K^+] may not reflect total body potassium

Hypokalemia

- ↓ intake; Anorexia,
- ↑ loss;
  - Renal (e.g osmotic diuresis, diuretics, ketonuria)
  - GI (e.g vomiting, excessive salivation)
  - Endocrine; hyperaldosteronism
- Intracellular shift
  - Rapid increase in insulin activity
  - Endotoxaemia

Hyperkalemia

- Artefactual (EDTA contamination, haemolysis, thrombocytosis)
- ↑ total body K+; reduced loss, and increased intake
  - Renal insufficiency/failure or Post-renal obstruction
  - Endocrine; Hypoadrenocorticism/Hypoaldosteronism
- Extracellular shift
  - Metabolic acidosis from inorganic ions
  - Severe rhabdomyolysis
- Massive tissue necrosis
- Exercise in hypothyroid dogs



Trichuris vulpis egg
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Low Na/K Ratio; What does it mean?

01

Hypoadrenocorticism – poster child; <27

↓ Na and ↑ K due to hypoaldosteronism, ↓ Na due to ↑ H_2O retention due to ↑ ADH secondary to ↑ cortisol.

02

Diarrhoea; GI losses

↓ Na due to GI losses, and resultant thirst; whipworm infection. HCO_3- losses can also ↑ K+.

03

Renal Failure/Urinary tract obstruction/Uroperitoneum

04

Third-space loss, cavitary effusions

Multifactorial, drainage of ECF Na, dilution of ECF by thirst driven by hypovolaemia, etc.

05

Diabetes with ketonuria

↓ Na due to osmotic diuresis, and ICF to ECF fluid shift due to ↑ glucose.

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1yo Cocker Spaniel MN

Peripheral LN enlargement

Phosphorus	1.28	0.81 - 2.20 mmol/L	
Calcium	1.71	1.98 - 3.00 mmol/L	
Sodium	150	144 - 160 mmol/L	
Potassium	8.1	3.5 - 5.8 mmol/L	
Na: K Ratio	19		
Chloride	112	109 - 122 mmol/L	

1. Is the [K+] compatible with life?
2. [Ca] is also low

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3yo ME Boxer dog – Lethargy, inappetence, possible ataxia.

+ Marked hyperkalaemia – exclude pre-analytical errors

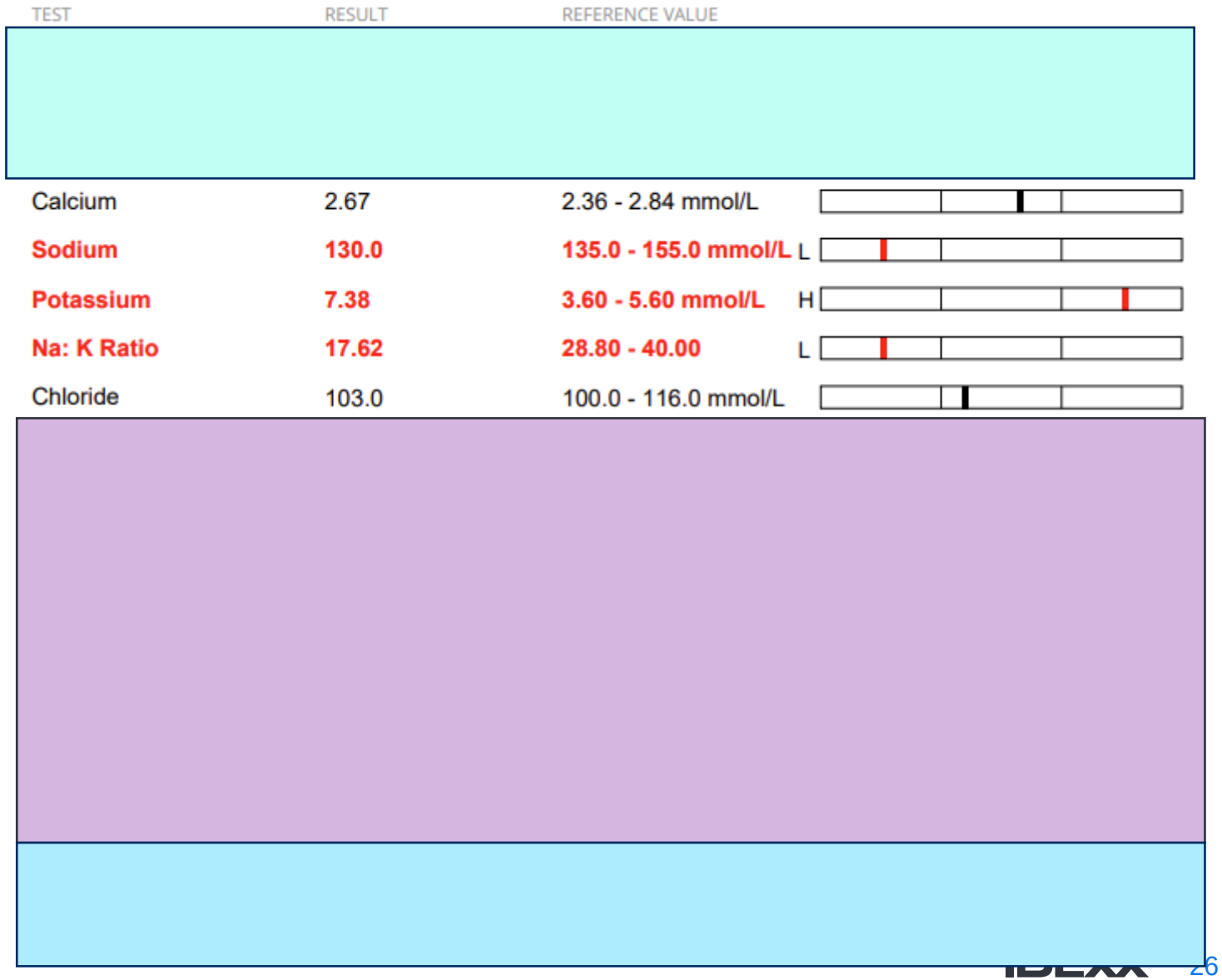
+ DDx:
  + Hypoadrenocorticism
  + ~~Oliguric/Anuric urinary failure~~
  + ~~Post-renal obstruction/uroabdomen~~
  + Gastrointestinal losses – Whipworm
  + Other gastrointestinal diseases.

+ Renal values – are they elevated?

+ Calcium is within the RI – albumin?
+ Albumin is low, so could there be a hypercalcaemia? iCa?

+ Other markers of hypoadrenocorticism

+ ACTH stimulation test confirmed lack of adrenal stimulation



TEST	RESULT	REFERENCE VALUE	
Calcium	2.67	2.36 - 2.84 mmol/L	
Sodium	130.0	135.0 - 155.0 mmol/L	L
Potassium	7.38	3.60 - 5.60 mmol/L	H
Na: K Ratio	17.62	28.80 - 40.00	L
Chloride	103.0	100.0 - 116.0 mmol/L	

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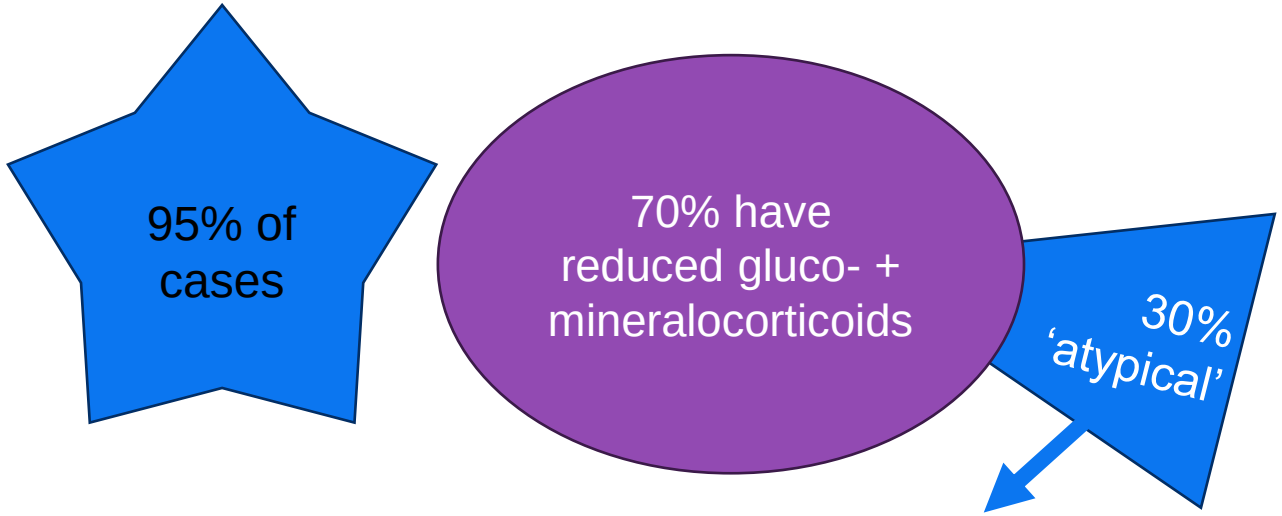
Hypoadrenocorticism

+ Primary hypoadrenocorticism

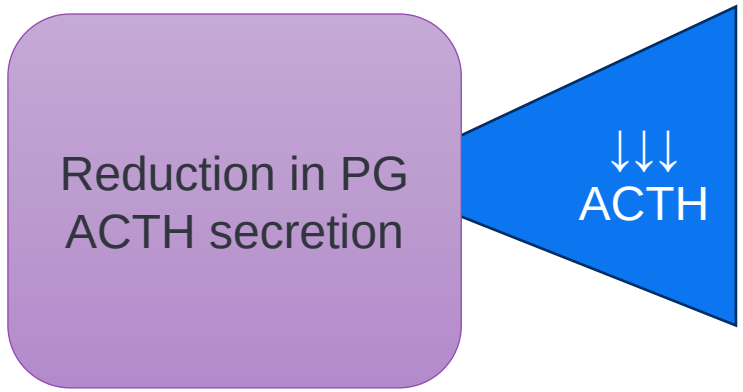


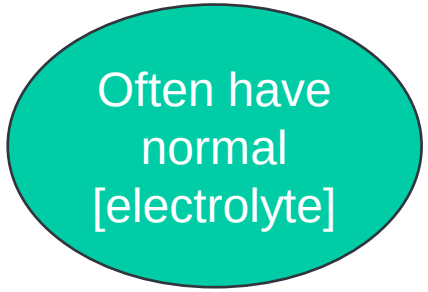
- Increased K+ excretion (↑ tubular flow rate)
- Increased aldosterone sensitivity
- Na intake compensation to ↑ K+ excretion

- May develop electrolyte abnormalities later on

Prior glucocorticoid therapy can give false positive ACTH stimulation results; basal ACTH ↑↑↑↑

+ Secondary hypoadrenocorticism





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Tardy et al. JVIM 2024

Feline Hypoadrenocorticism

- Only 48 cases within the literature (1983-2023)
- Primary disease:
  - Presumed immune-mediated cortical destruction
  - Secondary to lymphoma
  - Trauma
  - Congenital disease
- Secondary disease:
  - Iatrogenic suppression; reversible
  - Rare hypophyseal neoplasia

- Common bloodwork:
  - ↓ Na 32/48 (Median: 132 mmol/l)
  - ↓ Cl 14/48 (Median: 100 mmol/l)
  - ↑ K 27/48 (Median: 5.8 mmol/l)
  - ↑ PO_4 11/48
  - Normal Na:K Ratio 10/48
  - Na:K Ratio <27 12/48
  - Azotaemia 30/48
  - Lymphocytosis 6/48, lack of stress leukogram 4/48

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Reference: Glebocka MJ and Boag A *et al*. 2024

Are electrolytes measured by POC and Reference Laboratory analyses interchangeable?

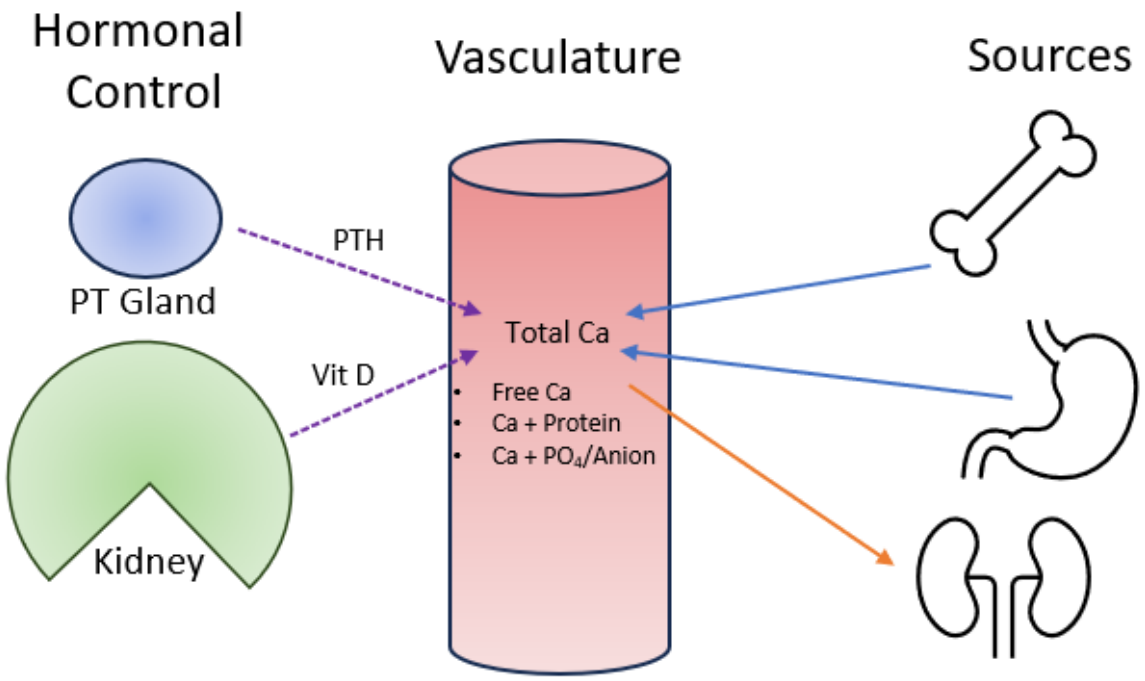
- No…..
- Comparable only between the same analyser
- Methodologies and reference intervals will differ

- In house results are going to be as good as your quality control system
- Reference labs may have stricter QC/QA protocols

- Fowlie S *et al*. 2020

Electrolyte Physiology; Calcium

+ PTH will:
  + Mobilise bone Ca
  + ↑ Vit D
+ ↓ fCa and ↓ Vit D increases [PTH]
+ Vit D will:
  + ↑ Ca absorption in GI
  + Mobilise bone Ca
  + ↓ calciuria
+ ↓ fCa and ↑ PTH increases [Vit D]



Adapted from: Stockham and Scott Fundamentals of Veterinary Clinical Pathology, 2nd Edition.

+ Changes in electrolytes will result from:

1. Intake changes
2. ECF to/from ICF flux
3. Increased renal retention
4. Increased losses via the excretion routes

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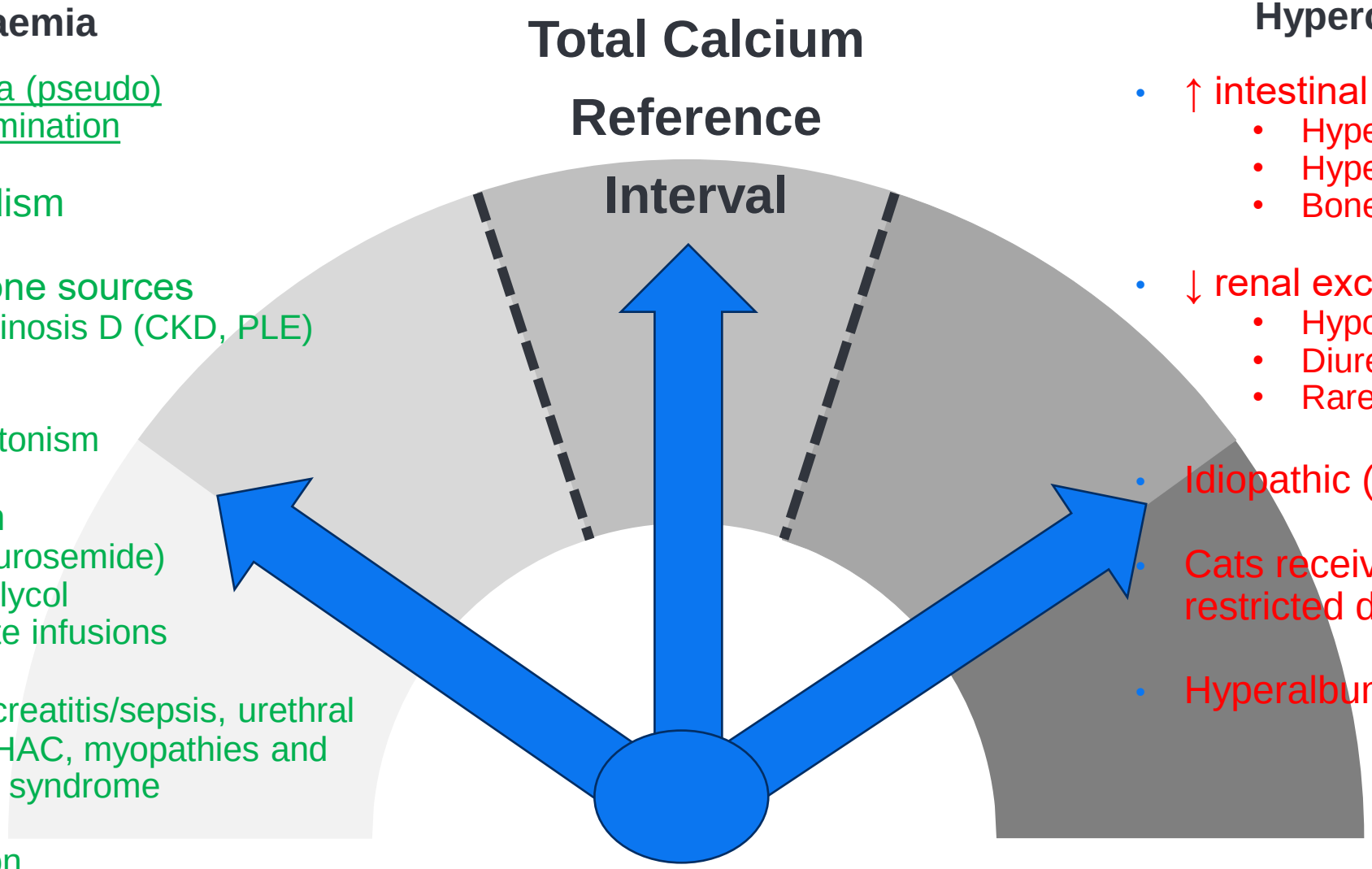
Total Calcium; all calcium is iCa; 50% bound to anions, 50% free (hormonal). major anions (90%) are albumin (80%) and globulins (20%), small proportion (10%) bound to other anions (PO_4, lactate etc).

Hypocalcaemia

- Hypoalbuminaemia (pseudo)
- EDTA (etc.) contamination
- Hypoparathyroidism
- ↓ intestinal or bone sources
  - Hypovitaminosis D (CKD, PLE)
  - Rickets
  - EPI
  - Hypercalcitonism
- ↑ renal excretion
  - Diuresis (furosemide)
  - Ethylene glycol
  - Bicarbonate infusions
- Other: acute pancreatitis/sepsis, urethral obstruction, ARF, HAC, myopathies and acute tumour lysis syndrome
- Pregnancy/lactation



Hypercalcaemia

- ↑ intestinal or bone sources
  - HyperPTH/PTHrP
  - Hypervitaminosis D
  - Bone neoplasias
- ↓ renal excretion
  - Hypoadrenocorticism
  - Diuretics (thiazide)
  - Rarely, renal failure
- Idiopathic (cats)
- Cats receiving PO_4-restricted diets (ref)
- Hyperalbuminaemia

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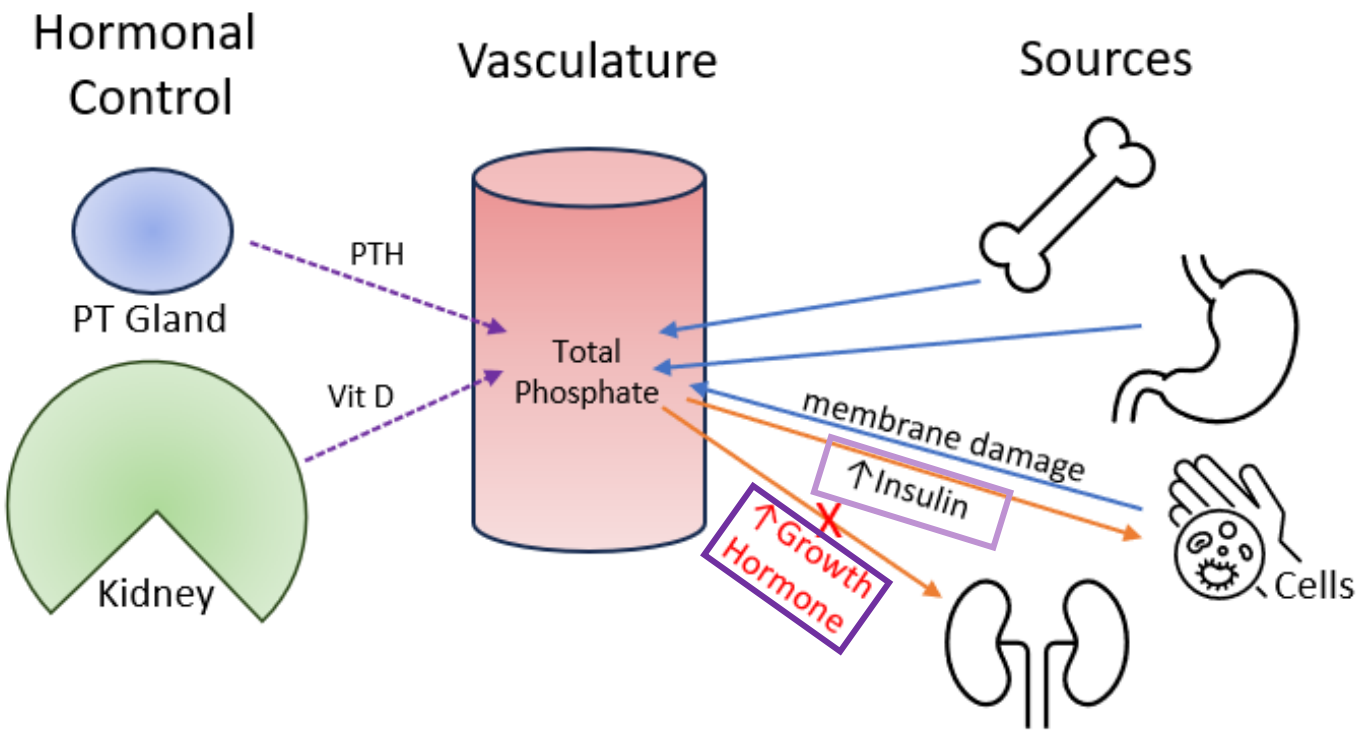
Electrolyte Physiology; Phosphate

- PTH will:
  - Mobilise bone PO_4
  - ↑ Vit D
  - ↑ phosphaturia
- ↓ fCa and ↓ Vit D increases [PTH]
- Vit D will:
  - ↑ PO_4 absorption in GI
  - Mobilise bone PO_4
- ↓ fCa and ↑ PTH & PO_4 increases [Vit D]
- Insulin
- Growth Hormone



Adapted from: Stockham and Scott Fundamentals of Veterinary Clinical Pathology, 2nd Edition.

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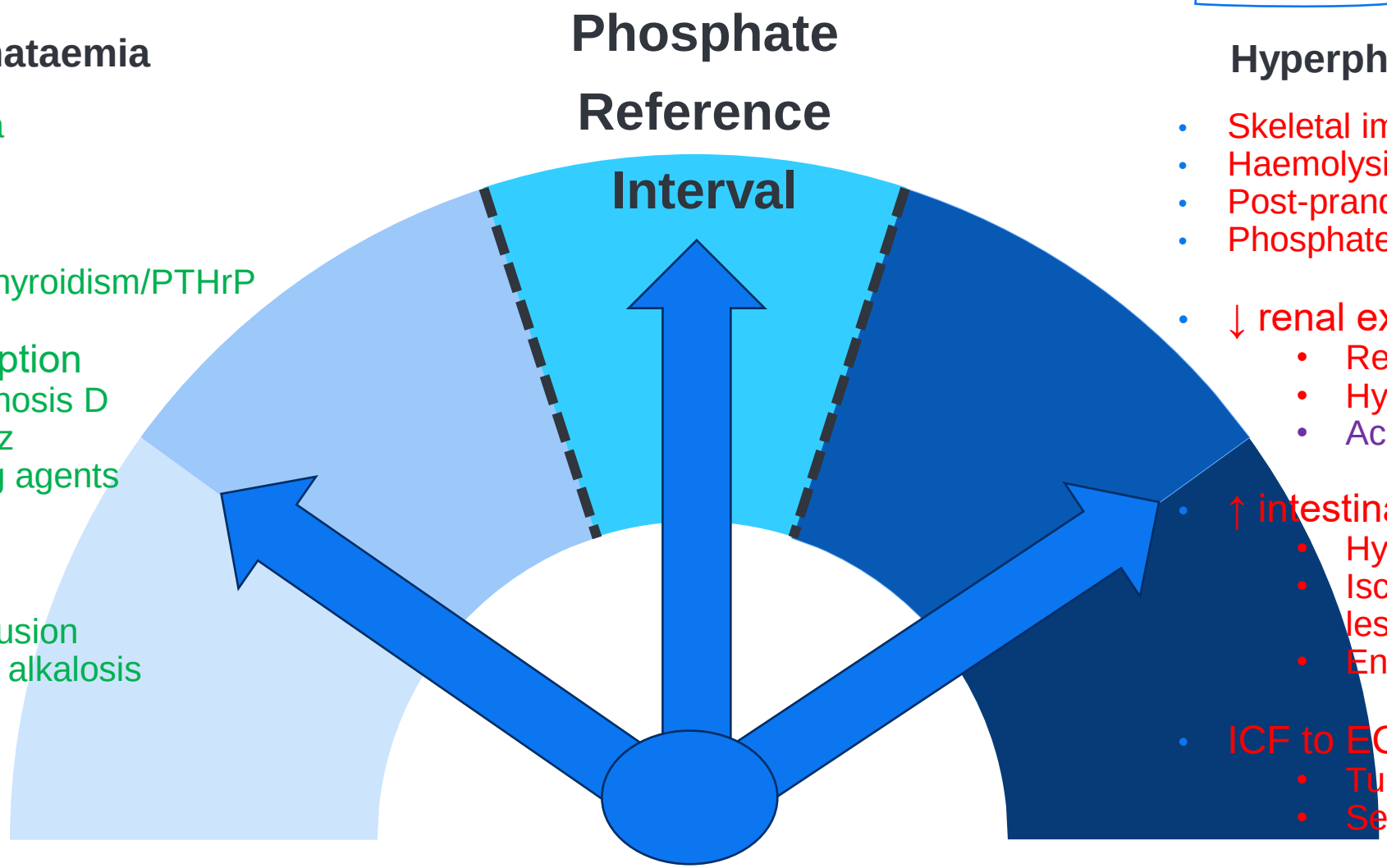
Phosphorus; 85% unbound, 10% protein bound (globulins etc.) and 5% bound to calcium or magnesium

Hyperadrenocorticism/increased steroids
Hyperthyroidism

Hypophosphataemia

- Prolonged anorexia
- ↑ renal excretion
  - Diuresis
  - Hyperparathyroidism/PTHrP
- ↓ intestinal absorption
  - Hypovitaminosis D
  - Intestinal diz
  - PO_4 binding agents
- ECF to ICF
  - ↑ Insulin
  - Glucose infusion
  - Respiratory alkalosis
- Eclampsia



Hyperphosphataemia

- Skeletal immaturity
- Haemolysis
- Post-prandial – mild
- Phosphate-rich diets/fluids
- ↓ renal excretion
  - Reduced GFR
  - Hypoparathyroidism
  - Acromegaly
- ↑ intestinal absorption
  - Hypervitaminosis D
  - Ischaemic intestinal lesion
  - Enema
- ICF to ECF
  - Tumour lysis syndrome
  - Severe rhabdomyolysis

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Ionized Calcium; biologically active ionised form (hormonal control)

+ Protein bound Ca does not act as a reservoir for Ca

+ iCa is measured by ion-selective electrodes (direct potentiometry)
+ Measured in serum (preferred), or heparinised plasma

Preanalytical Errors!!

- EDTA or citrate will chelate the Ca ions
- Heparinised plasma can falsely decrease the Ca however

- Gel separator tubes contain Ca so cannot be used

- pH; delayed sample separation (↑ iCa), air exposure (10 mins) (↓ iCa)…..

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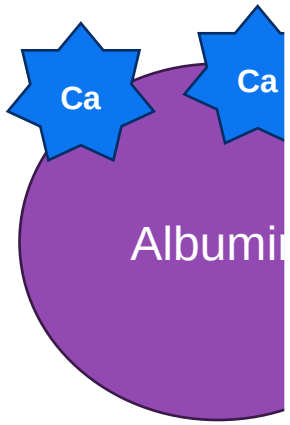
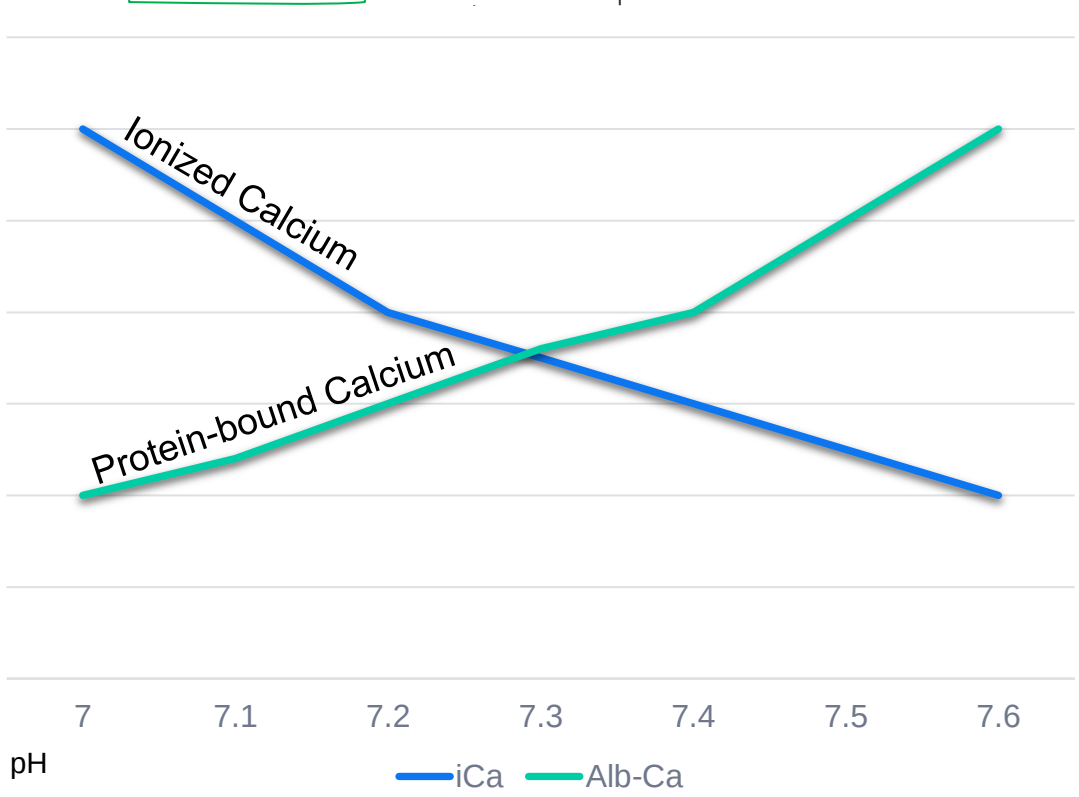
Effects of pH on iCa concentrations!

+ Alkaline – High pH

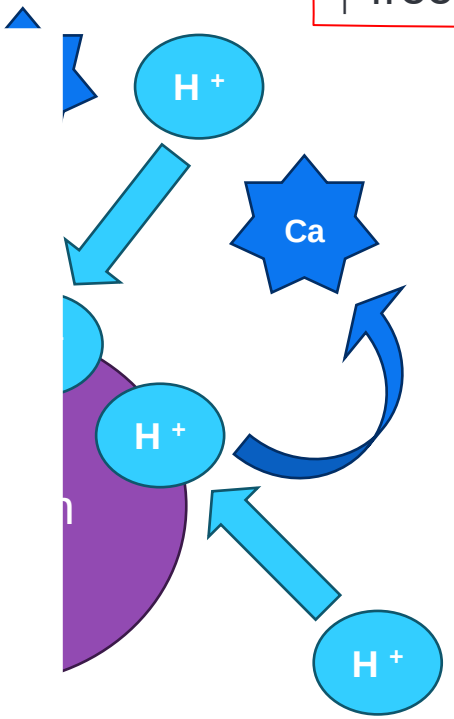
↓ free Ca





+ Acidic – Low pH

↑ free Ca



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iCa; Most common causes of feline ↑ iCa (Broughton *et al* 2023)

- True high and low ionised Ca typically reflects hormonal or hormone-related protein changes
- AKI (ureterolithiasis) (13%)
- Malignancy and idiopathic (10% each)
- CKD/renal-diet associated (8.4%)
- Iatrogenic (5.5%)
- <5%: primary PTH, Vit D toxicity or granulomatous disease

- No cause identified in 47%

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iCa; Most common causes of canine ↑ iCa (Coady *et al* 2019)

- True high and low ionised Ca typically reflects hormonal or hormone-related protein changes
- Malignancy (13%)
- Primary PTH (4.4%)
- Hypoadrenocorticism (1.7%)
- Kidney injury (1.2%: CKD > AKI)
- <1%: Vit D toxicity, granulomatous disease, non-malignant skeletal lesions

- Transient/non-pathological or inconsequential in 71%

8yo ESS MN
History of Diabetes Mellitus, suspected DKA

+ Collapsed at presentation, ketotic, hyperglycaemic, has received insulin and fluids, but still flat

Sodium	140.0		mmol/L	135.0 - 155.0
Potassium	**2.71**	***Low***	**mmol/L**	**3.60 - 5.60**
Sodium/Potassium ratio	**51.66**	***High***		**28.80 - 40.00**
Chloride	**118.0**	***High***	**mmol/L**	**100.0 - 116.0**
Inorganic phosphorus	**0.41**	***Low***	**mmol/L**	**0.80 - 1.60**
Calcium	**1.64**	***Low***	**mmol/L**	**2.36 - 2.84**
Glucose	5.1		mmol/L	3.6 - 7.0

+ Marked ↓ ↓ potassium and phosphate (normoglycaemia)
+ Cause: most likely ECF → ICF movement secondary to insulin therapy
+ (Prophylactic supplementation would be recommended)
+ (Risk of haemolysis with the hypophosphataemia)
+ Other causes: increased renal excretion (osmotic diuresis etc.) or GI loss

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8yo ESS MN dog
History of Diabetes Mellitus, suspected DKA

+ Collapsed at presentation, ketotic, hyperglycaemic, has received insulin and fluids, but still flat

Sodium	140.0		mmol/L	135.0 - 155.0
Potassium	**2.71**	***Low***	**mmol/L**	**3.60 - 5.60**
Sodium/Potassium ratio	**51.66**	***High***		**28.80 - 40.00**
Chloride	**118.0**	***High***	**mmol/L**	**100.0 - 116.0**
Inorganic phosphorus	**0.41**	***Low***	**mmol/L**	**0.80 - 1.60**
Calcium	**1.64**	***Low***	**mmol/L**	**2.36 - 2.84**
Glucose	5.1		mmol/L	3.6 - 7.0

+ Marked hypocalcaemia

Total Protein	**40.0**	***Low***	**g/L**	**54.9 - 75.3**
Albumin	**14.3**	***Low***	**g/L**	**26.3 - 38.2**
Globulin	25.7		g/L	23.4 - 42.2

+ Cause: marked hypoalbuminaemia (loss, dilution effects, liver dysfunction)
+ Other causes: pancreatitis (lipase, imaging)

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8yo ESS MN dog
History of Diabetes Mellitus, suspected DKA

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Calcium	**1.64**	***Low***	**mmol/L**	**2.36 - 2.84**
Glucose	5.1		mmol/L	3.6 - 7.0

+ Normonatruiaemic and hyperchloraemic…..check for persistence
+ Osmotic diuresis can lead to hyperchloraemia (H_20 loss > NaCl)

+ Cause: unclear; hyperchloraemic metabolic acidosis (low bicarbonate)
+ Assess pH and HCO_3^- concentration,

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12yo FN Dog

- One month history of weight loss, PU/PD and lethargy, loud heart murmur, pot bellied appearance and low BCS

Glucose	28.7	3.6 - 7.0 mmol/L

- Diabetes mellitus, likely
- Hypochloraemia, low Na/K ratio
  - Increased losses e.g. vomiting, urine,
  - Metabolic acidosis secondary to a ketoacidosis
- Hyperphosphataemia - ?azotaemia
- Hypocalcaemia - ?albumin
  - Pancreatitis
  - Hyperadrenocorticism, exogenous steroid therapy
  - iCa, monitoring for persistence

Sodium	142.0	135.0 - 155.0 mmol/L
Potassium	5.57	3.60 - 5.60 mmol/L
Na: K Ratio	25.49	28.80 - 40.00
Chloride	92.8	100.0 - 116.0 mmol/L

Creatinine	65.0	44.0 - 133.0 µmol/L
Urea	12.3	3.1 - 10.1 mmol/L
Phosphorus	1.63	0.80 - 1.60 mmol/L
Calcium	2.23	2.36 - 2.84 mmol/L

Normal albumin

Lipase	1,039.8	<= 200.0 U/L

ALP	2,056.0	<= 130.0 U/L
GGT	35.2	2.0 - 5.7 U/L

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Electrolyte Changes After Packed Red Blood Cell Transfusions in Dogs

1. Prospective study, anaemic dogs given pRBC n=26.
2. Blood was taken 0-1h prior to transfusion, and 1h post-transfusion.
3. Only minor changes noted in the electrolytes.
4. Na, K and Cl all mildly increased post-transfusion.
5. Cl was significantly higher post-transfusion

(Mean: Pre – 116.35 mmol/l, Post – 119.15 mmol/l)

- Cl^- elevation is attributed to NaCl 0.9% fluid therapy, and in IMHA patients, distal RTA may be present.

Bou P *et al*. 2024

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Summary:

1. Keep in mind pre-analytical errors, especially where results are extreme
2. Document persistence
3. Evaluate the electrolytes together, with the renal parameters and proteins, then the wider clinical pathology etc.
4. Not all cases are classical!
5. Regular QC/QA

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