

BIOCHEMISTRY DATA SHEET



The Anion Gap

Introduction

Medical clinical chemists were the first to report on the clinical use of the calculated parameter "Anion Gap" some 30 to 40 years ago. It rapidly became a valuable index of the degree of the acid base imbalance in conditions such as severe diabetic keto-acidosis, meths drinkers and ethylene glycol toxicities. At that time, of the major plasma cations, sodium, potassium, calcium, magnesium and ammonium ions in decreasing order of concentration, only sodium, potassium and calcium were being routinely measured in hospital laboratories. At this date there was no interest in the measurement of magnesium.

The plasma anions are a much more heterogeneous assemblage and include chloride, bicarbonate, sulphate, phosphate, lactate, hydroxy-butyrate, acetoacetate, propionate, FFAs, NEFAs, nitrates, formates, oxalates etc. Of these, only chloride, phosphate and bicarbonate were being assayed routinely.

By the Law of Electrical Neutrality the sum of the total plasma cations should equal the sum of the total plasma anions however not all the cations and anions could be measured on each sample. Sodium and potassium gave by far the largest and most variable contribution to the cation pool, as relatively speaking the concentration of both calcium and magnesium was small and not very variable and levels of ammonium ions were so low as to make little difference. In the anion camp the two major players were seen as chloride and bicarbonate. The other anions likely to be present in a plasma sample either could not be measured or occurred at a relatively insignificant concentration in normal samples.

The medics found that in practice the sum of the two principal anions, chloride and bicarbonate, when subtracted from the sum of the two principal cations, sodium and potassium, gives a mathematically derived index called the "Anion Gap". This anion gap is fairly constant in the healthy mammal and gives a quantifiable estimate of the balance between the anions and cations present. The normal gap is produced by the higher concentration and number of different anions present in plasma that are not measured, compared to the lower number and concentration of the remaining cations.

$$\text{Anion Gap} = (\text{Sodium} + \text{Potassium}) - (\text{Chloride} + \text{Bicarbonate})$$

Mean cation and anion concentrations for the Bovine(mmol/l)

Cations		Anions	
Sodium	143	Chloride	105
Potassium	4.5	Bicarbonate	25
Calcium	2.5	Phosphate	1.5
Magnesium	1.0	Sulphate	1.0
Ammonium	0.1	Lactate	3.0
		b-OH-Butyrate	0.5

For the figures used above (143 + 4.5) - (105 + 25) the Anion Gap = 17.5 mmol/l

Assay

Four analyses must be performed to allow the calculation of the anion gap which gives the only numeric index of the degree of metabolic or respiratory acidosis or alkalosis.

Sodium and potassium may be measured either by flame emission spectrophotometry or by the use of automated ion selective electrodes mounted on a random access analyser. Both methods are calibrated and controlled by the use of serum-based products as per the manufacturers instructions.

Chloride and bicarbonate are measured by the use of commercial kits using a random access analyser and are calibrated and controlled by the use of serum-based products.

Sample Requirements

Samples for the estimation of the anion gap may be heparinised plasma or serum.

IMPORTANT NOTES:

- o If heparin tubes are being collected the more usual types using ammonium or lithium heparin salts should be used. On no account take samples into potassium or sodium heparin tubes. Plasma taken from EDTA, oxalate, oxalate-fluoride or citrated blood samples cannot be tested.
- o Plasma bicarbonate is at a higher concentration than the carbon dioxide in the air. To avoid loss of bicarbonate when the samples are centrifuged always leave the stoppers on the tubes. Quickly remove the plasma sample into a small 2ml CFT tube and stopper immediately.
- o Erythrocytes, leucocytes and platelets contain substantially higher concentrations of potassium (x25) than plasma and any haemolysis of the sample must be avoided.

- o On hot days in summer it is advisable to centrifuge and separate the sample before forwarding it for analysis as many samples are “cooked” each year in transit.
- o Haemolysed samples and samples taken from recently dead animals are unsatisfactory and will not be tested.

Volume required 200 µl. Samples are stable if separated from the cells and well stoppered to avoid loss of CO₂ for **24 hours at room temperature**, 4 days if refrigerated and 3 months when frozen.

Reference Ranges for Plasma Anion Gap

Units are mmol/l

Species	Range
Cattle	10-20
Sheep	10-20
Goats	10-20
Pigs	10-20
Horses	10-20
Dogs	10-25
Cats	10-25

Clinical Interpretation

The Anion Gap varies with normal physiological functions within approximately 10mmol/l limits, however it may shrink or widen dramatically in certain disease situations or with some toxicological insults.

Increased Anion Gap: most changes occur as a result of a shift in concentration of one or more constituents of the un-measured anions and can occur with: _

- **Lactate** accumulation from a x25 rise in **lactic acidosis** and during **shock** can increase the Anion gap up to 35 mmol/l
- **Ketones** in **Acetonaemia/Pregnancy Toxaemia** and **severe keto-acidotic diabetes mellitus** can rise by up to 10 mmol/l and this can produce a rise in the Anion Gap to around 30 mmol/l
- **Phosphate and sulphate** accumulation in **severe uraemia** can increase the Anion Gap by 8 to 10 mmol/l
- **Oxalic, glycolic, glyoxilic and hippuric acids** resulting from the accidental ingestion of **oxalates** or **ethylene glycol** may increase the Anion Gap by 5 to 15 mmol/l
- **Formate** resulting from the metabolism of **methyl alcohol**

- **Hypochloridaemia** often due to **displacement of the abomasum** or in the single-stomached animals as vomiting after eating produces a loss of hydrochloric acid.
- **Hypernatraemia**: this can originate from increased salt intake from saline waters or food, as loss of water by evaporation, vomiting and diarrhoea or by reduced water intake as a result of injury, coma, illness or un-availability however the effect on the Anion Gap is marginal as chloride is similarly affected.

Decreased Anion Gap; is found if one or more of the measured or un-measured cations increase(s) or when one or more of the measured or un-measured anions decrease(s) in concentration. It is not as commonly seen but **Hypoalbuminaemia** will lower the protein-bound calcium. The effect, however on the Anion gap is only small even when there is a severe hypoalbuminaemia.

The un-measured cations, calcium, magnesium and ammonium ions have a total concentration of approximately 3.6 mmol/l. Even in the most marked clinical conditions their fall or rise is so relatively small compared to the concentration of sodium and potassium as to have hardly any influence on the Anion Gap. For example, the reference range for calcium in most species is between 2.0 and 3.0 mmol/l. During hypocalcaemia the calcium will fall to no lower than 1.0 mmol/l and in hypercalcaemia the rise is never greater than to 4.0 mmol/l. The maximum expected difference is no more than 1.5 mmol/l from the mean value and thus the Anion gap can only change by +/- 1.5 mmol/l in conditions relating to changes in plasma calcium and then only if the anion content remains unchanged. Applying the same interpretation to magnesium the Anion gap can only change by a maximum of +/- 1.0 mmol/l and for ammonium ions the maximum effect is only of the order of +/- 0.8 mmol/l.

Further Reading

Gabow P.A. and Kaehny W.D. 1980. Diagnostic importance of an increased serum Anion gap. N.Engl.J.Med. 303: p854

Shull R.M. 1978. The gaps game. Vet.Clin.Pathol. 7: (2), p19

Shull R.M. 1978. The value of the Anion gap and Osmolal gap in veterinary medicine. Vet.Clin.Pathol. 7: (3), p12