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Erythrocyte Parameters as a Diagnostic Tool in Canine Medicine: A Review

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ABSTRACT

The haematological analysis is one of the essential diagnostic and prognostic tools for the health practitioner. Routine hematology consists of erythrocyte, leucocyte and platelet parameters estimation. Erythrocyte parameters (RBC, RDW, haemoglobin, haematocrit, MCV, MCH, MCHC) estimation plays a crucial role in identifying anemia and several other acute and chronic conditions. Accurate and precise haematology results depend on correct blood collection procedures, suitable anticoagulants, proper storage and effective blood transport. The individual reference value variance can be due to age, sex, stress, diet, body condition, hydration status and reproductive status. Automatic haeamtology analyzer can yield quick and accurate results provided the sample is free from any artifacts. In conclusion, the accuracy of the result of automatic haematology analyzer in canine medicine is impeded by the lack of precise and rapid comparison procedure, instability and complexity of blood cells. Therefore the findings of the automatic haemotolyzer should always be corroborated with the clinical findings and another laboratory test.

Key words: Anaemia, Automatic haematology analyzer, Canine, Erythrocytes parameter, Haematology.

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The haematological analysis has helped diagnose several diseases and assess the therapy's responses in canine. It also helps in formulating the prognosis of a disease. A correct interpretation of hematological analysis can also assess the haematopoietic, urinary, digestive systems condition (Gonul *et al.,* 2020). The complete routine haematology consists of erythrocyte parameters, leucocyte parameters and platelets. Detailed erythrocyte parameters description is it's self an essential and vast topic, so this study's essences have been limited to erythrocyte parameters. The present communication objective is to provide canine clinicians with an overview of the blood sample collection process, canine erythrocyte parameters reference ranges and interpret the canine's erythrocyte parameters profile. In recent times, automated haematolyzer has become more prevalent in canine practice due to its easy to operate and quick result; the present review's focus is on an alteration detected on an automatic machine. However, as per the author's recommendation, if any alteration is detected on analysis, it must be corroborated with clinical findings and other macro or microscopic tests for confirmation.

Blood sampling

Improper blood collection techniques, unsuitable anticoagulants, improper storage and faulty blood transport will lead to measurement error and flawed clinical conclusion (Bailey and Pablo, 1998).

Equipment required for the collection of the blood sample

a) Needle and syringe or b) vacutainers (evacuated blood tubes) or c) butterfly catheter and syringe.

The needle and syringe are usually used to extract the blood sample. The needle size, gauge, syringe size, volume of the vessel, animal size, hydration status and blood quantity should be appropriate (Clark *et al.,* 2017). Evacuated blood tubes may be useful in large mammals, but for small mammals, they are undesirable because the vacuum tension damages the venous and prevents withdrawal of blood (Clark *et al.,* 2017). Under some conditions, venepuncture may be more suitable for a butterfly catheter than a needle, particularly in the absence of anesthesia and may change during the procedure (Clark *et al.,* 2017).

Blood collection sites

The most common and easily accessible blood collection vessels in canine are the cephalic and the lateral saphenous vein; Jugular vein can also be used (www.researchservices.umm.edu). Blood collection vessel selection does not have alteration in the dog. As per Jensen *et al.* (1994) no decisive difference in haematological value could be detected from the blood sample collected from the cephalic and external jugular vein.

Volume

A maximum of 1 percent of a patient's body weight can be removed every 14 days without needing additional replacement fluids as a single blood draw. It is strongly advised that we remove the minimum amount of blood required (www.researchservices.umm.edu). The volume of collected blood also depends on the health and hydration status of the animal. For haematology with most commercial automated haematolyzer, 0.5 ml of whole blood is sufficient. (Poitout Belissent *et al.,* 2016).

Restraint

Animals should be restraint properly and allowed to calm before the collection of blood as stress may alter the haematological results (Roland *et al*., 2014). Control is also necessary to prevent vessel laceration and other health issues. Restraint is usually physical or chemical. In canine, the veterinarians usually prefer physical restraints (www.researchservices.umm.edu).

Collection procedure

The collection site should be clipped with scissors or clippers to make the vein visible. An alcohol solution (*e.g.,* 70% ethanol or 100% methanol) or a detergent with an alcohol solution should be used to clean the site. The alcohol should be required to 'air-dry' before venepuncture as contamination of the sample by alcohol can lead to haemolysis (Wu, 2006). The same collecting equipment should not be used for other individuals to prevent infection.

In the direction of the vein, position the needle with the bevel going upward and the syringe about 15° in the direction horizontal to vein. Advance the needle to perforate muscle, subcutaneous tissue and vessel wall. Avoid lateral motion of the needle's tip as laceration of the vein can result in the subsequent formation of haemorrhage and haematoma (Clark *et al.,* 2017). When we put the needle in the vein, sufficient blood should be withdrawn by applying moderate and even pressure over the length of the syringe A collector should avoid intense suction as it can induce haemolysis (Clark *et al.,* 2017). Following blood collection, the needle should be withdrawn and digital pressure applied to the vein 30 seconds to 1 minute in order to prevent blood leaking from the perforated vein (Clark *et al.,* 2017).

Collecting tubes

For haematological analysis, blood should be collected in a tube containing anticoagulant. A universal anticoagulant cannot be documented capable of providing maximum preservation of all the cellular elements in blood samples, *e.g.,* heparin has been reported to reveal better haematocrit value in canine (Penny *et al*., 1967) but unsatisfactory for total and differential leukocyte counts (T.L.C. and DLC). Canine blood samples collected in oxalates give inferior results for TLC. Ethylene diamine tetra-acetic acid (EDTA) is recommended for TLC and DLC, but acceptable values recorded for limited times after sample collection. We suggest if it is not practical for the concerned to collect samples into different anticoagulants for the various haematological tests, then the anticoagulant with the least alteration on the accuracy of haematological value over the longest duration should be selected, *e.g.,* potassium ethylene diamine tetra-acetic acid [EDTA (K3)] @ 1.02 mg/ml of blood and di-sodium ethylene diamine tetra-acetic acid [EDTA (Na2)] @ 1.5 mg/ml of blood. Other coagulants used for the specific test are citrate (coagulation studies), heparin (plasma separation), diatomaceous earth (activated clotting time). Consequently, tube manufacturers and laboratory recommendations should be followed to minimize errors and repeat testing.

Special consideration

When blood is to be obtained from animals during winter conditions, vasoconstriction of peripheral vessels is triggered that can hamper venepuncture. Directing a local heat source over the site typically provides sufficient warmth to facilitate local vasodilatation and efficient blood collection (Clark *et al.,* 2007).

Storage

It is always suggested to perform the haemato-biochemical analysis as soon as possible after the collection of blood. Rim *et al.* (2018) have previously reported effect on complete blood counts (CBC's) typical values due to cold agglutinin. However, if it is not possible, collected blood in vacutainer can be stored for up to 24 hr at 2 to 4°C, which is associated with the least artifactual alteration (Roland *et al*., 2014; Athanasiou *et al*., 2016; Udegbunam *et al.,* 2020).

The canine haematological reference value

International Federation for clinical chemistry recommended "reference value" as the preferred term instead of the reference range, normal value, or normal range (Klaassen, 1999). The most appropriate reference value to be selected from a group of healthy animals having a similar environment and physiologic status as a patient is as much as possible. The blood vascular system consists of two compartments: the central pool and the marginal pool. (Wood, 2014) The marginal pool is the capillary-tissue interface microcirculation. The central pool is composed of larger vessels. Venipuncture blood samples are inherently the most indicative of the central pool. Flow rate, fluid motion and selective leucocyte adhesion to endothelium are variables that can lead to marked cell concentration differences in the two pools (Wood, 2014). Variation in animals' reference value may be attributed to age, sex, stress (activity, temperature, altitude), diet, body condition, hydration status and reproductive status (Wood and Quiroz-Rocha, 2010; Krimer, 2011; Roland *et al*., 2014). A 2016 study in dog reported age-related variation in the haematological value (Braten *et al*., 2016). Sex and age-related changes in beagle dogs were also reported in another study (Jeong *et al*., 2007). In another survey of 2011, transportation and vaccination stress were attributed to an

alteration in haematological value (Choi *et al*., 2011). Canine red blood cell (RBC) count, mean corpuscular haemoglobin value (MCHC) and mean corpuscular volume (MCV) differs significantly in pregnancy (Khan *et al*. 2011). The breeds of dogs have no affect on the haematology values (Nadia, 2019). For interpretation, harmony reference value from four different sources is placed in Table 1. We recommend laboratories to set up their reference value considering all the variants.

R.B.C./Erythrocyte parameters

The canine erythrocytes diameter range from 6 to 8 ìm and larger compared to other species (Adili *et al*., 2016). It has a life span of 110-115 days (Adili and Melezi, 2014). A red blood corpuscular count typically includes the total number of RBCs, packed cell volume (PCV)/ haematocrit, haemoglobin, erythrocyte indices and occasionally the red cell distribution width (RDW). Erythrocyte indices include MCV, mean corpuscular haemoglobin (MCH) and MCHC.

There is a significant increase in the RBC, haemoglobin and haeamtocrit concentration with the age as there is a positive correlation with age between RBC life span and haemoglobin concentration (Brenten *et al.,* 2016). Young animals noticeably have lower haemotocrit plus lower plasma protein and a higher percentage of reticulocytes than the reference value of adults resembling the blood report as blood loss anaemia (Tvedten, 2005). Breed values also differ with, for example, haematocrit in Greyhounds is higher than non-greyhounds (Tvedten, 2005). Tvedten (2005) reported hydration status and fear could alter the haematocrit value, haemoglobin and RBC. In dog breed variation in erythrocyte volume is recorded (Kumiega *et al.,* 2020). Greyhounds have an MCV, which is physiologically higher than other breeds (Porter and Canaday, 1971). Macrocytosis (enlargement of red blood cell) is commonly seen in miniature dogs and poodles (Schalm, 1976), while microcytosis (smaller red blood cell) is recorded in Asian dog breeds (Akita or Shiba Inu) (Tanabe, 2006). Organ insufficiency is also one of the factors for alteration in haematological study (Indhu *et al.,* 2019).

Evaluation of RBC Counts, haematocrit and haemoglobin

These data are crucial to assessing the existence and to the severity of anaemia. Counting may be accomplished by hand, using haemocytometers or, faster, using automated instruments. Later one depicts more reliable results provided the sample does not include interferent due to improper pre-analytical handling (Paltrinieri, 2014). Villiers and Blackwood (2005), Roland *et al*. (2014) and Athanasiou *et al*. (2016) have well documented the pre-analytical handling artifactual alteration of blood that can interfere with automated results. Analytical artifacts impact the microhaematocrit less than other approaches. This technique's usage can also be helpful if automated instrument findings are uncertain or may not correspond to clinical presentation (Paltrinieri, 2014).

In veterinary medicine, microhaematocrit is important as haematocrit (PCV) is the primary indicator of RBC mass (Ettinger and Feldman, 2010). Calorimetric based automated cell counter calculate haematocrit based on the formula: RBC (10^{12}/L) × MCV (fL/10) (Thongsahuan *et al.,* 2020) and expressed as %. Anaemia is considered mild, moderate, severe and very severe when haematocrit is 30-37%, 20-29%, 13-19% and <10%, respectively (Tvedten, 2010). Pseudoincrease, by estimation with automated cell counter in haeamtocrit value, is detected due to high white blood cell (WBC) count (>50,000/L, hyperosmolar states, RBC agglutination, cryoglobulin, cryofibrinogen and giant platelets (Brigden and Dalal, 1999). The false decrease is observed in hyperosmolar states, *in vitro* haemolysis, microcytic RBCs and cold agglutinin (Brigden and Dalal, 1999).

The automated cell counter calculates the number of RBCs of a given blood volume by electrical impedance and is usually expressed as the number of RBCs/microlitre or liter (http://www.clinlabnavigator.com/complete-blood-count-cbc). False increase of RBC can be seen due to high WBC count or giant platelet, cryofibrinogen, cryoglobulin and pseudo decrease in case of warm agglutinin, cold agglutinin, EDTA dependent agglutination, MCV<50 (*e.g*., burn patient), *in vitro* haemolysis (Brigden and Dalal, 1999).

Table 1: Reference value of routine erythrocytes parameter[#] in the adult dog.

Haematological	Klassen *et al.* (1999)	Kahn and Line (2010)	Chakorbarty (2008)	www.ecourses.icar.gov.in	Mazotta *et al.* (2016)
Haemoglobin	12.1-20.3 g/dl	12-19 g/dl	13 g/100 ml	12-18 g/dl	*
RDW	*	*	*	*	13.3% 12.3-13.7
RBC	4.8-9.3×10^6/µL	5-7.9 ×10^6/ µL	6.20 million/C.mm	5.5-8.5×10^4/ml	*
Haematocrit	36-60%	35-57%	45.50%	37-55%	48.6% 45.6-50.5
Reticulocyte		0-0.10%			
MCV	59-79 fl	66-77 fl	67 cubic micron	60-72 fl	69.2 fl 66.7-70.7
MCH	19-28 pg	21-26.2 pg	*	*	*
MCHC	30-38%	32-36.3%	24 micro microgram	31-37 g/dl	*

[#]Unit mentioned as described by respective authors *Not available. RDW: Red Cell distribution width; RBC: Red blood cell, PCV: Packed cell volume, MCHC: Mean corpuscular haemoglobin concentration, MCH: Mean corpuscular haemoglobin, MCV: Mean corpuscular volume.

Haemoglobin concentration is measured by converting haemoglobin to haemoglobincynide and spectrophotometrically measuring its light absorption at 540 nm (http://www.clinlabnavigator.com/complete-blood-count-cbc) and usually expressed in gram per deciliter (g/dl) or %. Pseudoincrease in haemoglobin's value is observed when there is overfilling of tubes, carboxyhaemoglobin, high WBC count, hyperlipidemia, abnormal haemoglobin, hyperbilirubinemia, cryoglobulinemia, paraproteinemia and false decrease in sulfhaemoglobin (Brigden and Dalal, 1999).

A decreased value of haematocrit or PCV or RBC is detected in case of anaemia. The detailed interpretation is described under specific headings. An increase in the number of red blood cells is termed as erythrocytosis. Some authors also describe it as polycythemia, but polycythemia also denotes a rise in all blood cells (including white blood cells and platelets) (Randolph, 2010).

Randolph (2010) has classified erythrocytosis as

- Relative erythrocytosis is caused due to any etiologies of fluid loss (Dehydration, vomiting, diarrhea, *etc.*).
- Transient erythrocytosis is caused due to condition which leads to the spleen to contract such as fear excitement *etc.*
- Absolute erythrocytosis- a) Primary absolute erythrocytosis occurs in case of bone marrow disorder b) Secondary absolute erythrocytosis occurs excessive release of endocrines, which can stimulate red blood cell production.

Increase oxygen demand, chronic pulmonary obstructive diseases, obstructive airways diseases and chronic respiratory diseases are also enlisted as the cause of erythrocytosis (Anonymous, 2013).

Evaluation of erythrocyte indices

Erythrocyte indices include MCV, MCHC and RDW. These values can be generated directly with an automated analyzer. These values' calculation procedure with an automated analyzer differs from the manual approach (Stockham and Scott, 2008; Moritz and Becker, 2010; Paltrinieri, 2014). Paltrinieri (2014) described these indices briefly as below

i. **MCV:** Refers to the mean value for each RBC, which is the base of RBC classification into normocytic, microcytic and macrocytic. SI unit of MCV is femtoliters (fL).
ii. **MCHC:** Refers to the percentage of Haemoglobin per RBC, which is the base of classification of normochromic, hypochromic and hyperchromic RBC SI unit of MCHC is gram per deciliter (g/dl). Hyperchromic RBC generally reflects the overestimation of Haemoglobin during analysis.
iii. **MCH:** Refers to the content of Haemoglobin in each RBC. It can also be used for the classification of normochromic and the hypochromic RBC SI unit of MCH is pictogram (pg).
iv. **RDW:** Refers to the quantity of RBC distributed around the MCV. RDW calculation depends on the histogram of RBC width on the Y-axis and MCV on the X-axis. SI unit of RDW is %.

Anicytosis reflects the unequal size of R.B.C. Anicyotosis increases with the presence of macrocytes and microcytes. RDW detects regeneration anaemia earlier than MCV, as MCV value is not affected at the early stage of few reticulocyte generations. However, these few reticulocytes generated can influence RDW and cause an increase in its value.

Martinez *et al.* (2019) reported higher RDW in dogs with primary immune-mediated haemolytic anaemia, immune-mediated thrombocytopenia, hyperadrenocorticism, hepatic vascular anomaly, hypothyroidism, pneumonia, chronic kidney disease, multicentric lymphoma and myxomatous degeneration. Significantly higher median RDW value has also been reported in patients of pre-capillary and post-capillary arterial hypertension (Mazzotta *et al.,* 2016). Increased RDW and decreased MCV is said to be good indicators of haemorrhagic diseases (Arslan, 2017). Zhao *et al.* (2014) reported elevated RDW levels due to oxidative stress and inflammation in canine.

As per the author's suggestion, RDW is part of a set of data and should never be interpreted individually. As with the MCV, RDW is an essential tool for anaemia differential diagnosis and other systemic disorders. For example, higher MCV with an RDW within the reference value denotes aplastic anaemia and chronic liver disease, while MCV within the reference value and higher RDW with early cobalamin, iron, or folate deficiency and chronic hepatobiliary disease (Martinez *et al.,* 2019).

Most of the veterinary laboratory automatic instrument calculates the total haemoglobin by the calorimetric cynamethaemoglobin method (March *et al.,* 2005). This method does not discriminate between cellular haemoglobin from the extracellular haemoglobin, from the free haemoglobin of the solution used to treat acute anaemia in the dog (March *et al.,* 2005). Consequently, the result reflects a false increase in MCH and MCHC (March *et al.,* 2005). The remedy for this artifactual error is the laser-based automated cell counter, which along with MCH and MCHC also reveals corpuscular haemoglobin concentration mean (CHCM) and the cellular hemoglobin. Additionally, laser-based counter read the volume of Haemoglobin reticulocytes (MCVr) and Haemoglobin content of reticulocytes (CHr), which is better indices for identification of reticulocyte response and confirmation of iron deficiency anaemia (Steinberg and Olver, 2005; Paltrinieri, 2014).

Anaemia

Clinical anaemia or haemorrhage is the common condition warranting RBC analysis. Anaemia reduces red blood cell mass (PCV, haematocrit, haemoglobin concentration, or RBC count) (Stockham and Scott, 2008; Paltrinieri, 2014). Absolute anaemia is caused by an actual decrease in the RBC mass. Relative anaemia is seen in pregnancy, the introduction of vast amounts of fluids intravenously, or hyperproteinemia, which causes an increase in plasma concentration leading to dilution effect to average RBC mass, which mimics anaemia (Roland *et al.,* 2014). On the

response of bone marrow anaemia can be categorized as i) non-regenerative anaemia when there is an inadequate response from bone marrow (Roland *et al.,* 2014; Paltrinieri, 2014) ii) regenerative anaemia when the bone marrow responds adequately (Roland *et al.,* 2014; Paltrinieri, 2014).

Classification of anaemia based on the cell size, *i.e.*, normocytic anaemia, macrocytic anaemia and microcytic anaemia, suggesting normal, increased and decreased MCV, respectively (Roland *et al.* 2014) and based on the haemoglobin concentration, *i.e.*, normochromic anaemia, hypochromic anaemia and hyperchromic anaemia suggesting normal, decreased and increased haemoglobin concentration, respectively (Roland *et al.,* 2014).

Non-regenerative anaemia

Non-regenerative anaemia occurs due to decreased erythrocyte production from inflammatory diseases (infectious and non-infectious), chronic renal disease, marrow hypoplasia or aplasia (infection, drug, toxins, irradiation, myelopathies, myelofibrosis, osteoporosis) and selective erythroid or hypoplasia (pure red cell aplasia, endocrine, liver disease) and less commonly defective erythropoiesis from nutritional deficiencies (Iron, copper, Vitamin B12 (cobalmin), folate), Immune-mediated destruction of erythroid progenitors and myeloid plastic syndrome (Takahira, 2009) and Inflammatory bowel disease (Bhavani *et al*., 2021). The diagnosis of non-regenerative anaemia is more difficult in comparison to regenerative anaemia (Takahira, 2009). In dogs, reticulocyte count lower than 60.000/ µL or 1% (absolute count) is indicative of non-regenerative anaemia (Takahira, 2009). Non-regenerative anaemia usually occurs in the following variations: normochromic and normocytic, normochromic and macrocytic, or hypochromic and microcytic (Kraft and Durr, 2005; Brockus, 2011; Paltrinieri, 2014).

Normochromic and normocytic anaemia

It is seen in non-specific illness, particularly in chronic inflammation. It is also seen in erythropoietin deficiency during chronic renal failure (King *et al.,* 1992), leishmaniasis (Keenan *et al.,* 1984), Hepatozoon infection (Mundim, 2008), mild to moderate in the acute phase of *Ehrlichia canis* infection (Waner, 2008), endocrine disorder (hypothyroidism, hypoadrenocorticism) (Takahira, 2009; Megha *et al.* 2018), depression of bone marrow, certain drugs (phenylbutazone, estrogen, Phenobarbital), toxin (Takahira, 2009), neoplasia. As reported by Lobetti (2002), *Ehrlichia canis* infection can cause either regenerative or non-regenerative anaemia. It can be concluded that normocytic normochromic anaemia is a non-regenerative type, with the exception termed pre-generative type in condition occurring immediately after haemolysis or acute blood loss where sufficient reticulocytes are released by the bonemarrow only after some time and attain the peak value within a week (Paltrinieri, 2014).

Hypochromic microcytic anaemia

Characteristic of anaemia due to iron deficiency is vital for haemoglobin synthesis, which effectively mediates replication of RBC precursors. Despite iron, precursors tend to replicate, forming smaller mature RBC (Paltrinieri, 2014). As stated by Paltrinieri *et al.* (2010) microcytic may not be the diagnostic feature in Iron deficiency anaemia. Hence Paltrinieri *et al*. (2010) suggested conducting an additional test, *i.e.*, a sideremic profile to confirm iron deficiency anaemia.

Normochromic macrocytic anaemia

Outcome of deficiency of vitamin B12, Folic acid and cobalt in human medicine is not prevalent in canine. In a study conducted by Stanley *et al.* (2019), normochomic and macrocytic anaemia due to folate and cobalmin deficiency was not significant in dogs. However, in a canine haematological report, it may indicate artifactual change when storage is prolonged (Paltrinieri, 2014).

Regenerative anaemia

Decrease life span of RBC primarily of two causes a) severe acute haemorrhage b) hemolytic anaemia. In both these conditions, bone marrow responds sufficiently to release immature RBC to compensate for the loss of RBC. Such anaemia is classified as regenerative anaemia. Various etiologies are mentioned as below:

Acute haemorrhage

The patient is hypovolemic but not anaemic instantly after acute blood loss, as both RBC and plasma are drained. Eventually, water is resorbed from the extracellular fluid in a few hours to compensate hypovolemia and haeamtocrit decreases and anaemia remains as pre-regenerative (See normochromic normocytic anaemia) (Paltrinieri, 2014). Usually, regeneration starts 4-7 days after the sufficient increase in reticulocyte amount (Tvedten, 2010). Paltrinieri (2014) reported that internal RBC loss in dogs might reabsorb through vascular walls, for the restoration of haematocrit.

Haemolytic anaemia

Decreased life span of RBC (Intravascular or extravascular haemolysis due to toxin or oxidants) or accelerated aging of RBC (intrinsic or extrinsic factor). Common causes enlisted below:

- Enzyme deficiency (PPP, EMP and Antioxidant) an inherited issue. For example, phospofruckto kinase enzyme deficiency in the spaniel breed and pyruvate kinase in terrier, beagle and basenji breed of dog. Phospofructokinase deficiency has also been reported after exercise due to hyperventilation (Paltrinieri, 2014).
- Haemolysis caused by chemicals (bacterial haemolysins, toxins in snake/insect venom) that lead to direct lysis of RBC (Paltrinieri, 2014), Oxidants (acetaminophen, onions, propofol, zinc, Vitamin K3 endogenous oxidants produced during diabetes, hyperthyroidism, lymphoma) causing haemolysis (Paltrinieri, 2014).

- Infectious agent bacteria (Leptospira, *Escherichia coli* (0103:H12), *etc.*), viruses and parasites (*Babesia*, *Ehrlichia etc.*) are also responsible for anaemia. In this condition anaemia either occurs due to immune-mediated or toxin (Paltrinieri, 2014: eclinpath.com/haematology/anaemia/causes-of-anaemia/).
- Immune-mediated haemolytic anaemia occurs due to the drug (Penicillin), Infectious agent (antigen from parasite or bacteria) and incompatible blood transfusion (Blood type DEA 1-1).
- Autoimmune disease (Neonatal isoerythrolysis not common, systemic lupus erythematosus and autoimmune thrombocytopenia in female large breed dog) (Paltrinieri, 2014).

Automatic haematologic analyzer

The QBC VetAutoread was one of the first automatic instruments in animal health practice (Becker *et al.,* 2008). Several limitation of automatic haemotylzer in veterinary practice has been reported by Bienzle *et al.* (2000) and Tasker *et al.* (2001). One challenge is that there's no gold standard or true standard reference method since each instrument has benefits and limitations. In present scenario, three different physicals, operational principle are used in automatic haemotlyzer (Scaffon, 2014).

1. Impedance-based

A blood sample is passed through a narrow aperture between two electrodes. At a time, only a single cell can pass through the aperture due to its limited size. The impedance alters as a cell passes through the aperture. The difference reported in impedance is proportional to cell volume, resulting in a cell count and measure of volume.

2. Flow-cytometry based

More expensive than above analysis technique, as expensive reagents are used, but detailed information about the morphology of blood cells is reported. A stream of one cell passes through a laser beam. The absorbance is determined and to assess the cell's granularity, diameter and inner complexity, the scattered light is calculated at different angles. These are the same features of cell morphology that can manually be calculated from a slide.

3. Fluorescent flow-cytometry based

The addition of fluorescent reagents improves the use of fluorescent cytometry to quantify cell populations. Fluorescent dyes display each cell's nucleus-plasma ratio. It's also beneficial for nucleated RBCs and reticulocytes.

For veterinary use these three technologies are combined by companies (IDEXX Laboratories Inc, USA; Siemens, USA; Diagnovison product corporation, India; Exigo, India) with creative applications of reagents, hydrofluidics and software for data processing to generate patented methods, each of which has accuracy, speed and breadth strengths.

Becker *et al.* (2008) in a comparative efficacy study of canine total blood count for automatic instrument CELL-DYN (IDEX Laborataries Inc,USA) and ADVIA 120 analyzer (Siemens, USA) with seven in-clinic haemotylzer, reported acceptable value for total RBC count with some bias in haeamatocrit and reticulocyte results.

Parameters to be considered for suggesting automatic haemotylyzer

i. Clinical lab or research.
ii. Range of test.
iii. Time per analysis.
iv. Accuracy, precision and linearity.

CONCLUSION

In conclusion the precision of the result of automatic haematology analyzer in canine medicine hindered by the absence of precise and rapid reference process, instability and complexity of blood cell. There is definitely scope for progress on instruments and software for current and new instruments, which can make the result of automatic haematology analyzer precise and useful in canine medicine. As per authors suggestion a canine clinician to rule out any hindrance in the successful clinical conclusion of patient should always corroborate CBC result with the clinical findings and other laboratory tests.

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