A Review on Mobile Phone Acquired Blood Images to Conclude CANINE PCV for Medical Diagnosis

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Abstract—**Clinical diagnosis requiring central facilities and site visits can be burdensome for patients in resource-limited or rural areas. Therefore, development of a lowcost test that utilizes smartphone data collection and transmission would beneficially enable disease self-management and point-of-care (POC) diagnosis. Packed cell volume (PCV) measurement is a routinely performed diagnostic test in both primary care and referral settings and is often included as part of a minimum database when evaluating patients at initial presentation. Standard operating procedures, including World Health Organization guidelines for packed cell volume, are established for in-clinic laboratory tests. This paper review of a smartphone-obtained image can be used to extrapolate the PCV of canine blood samples. This paper introduces and review of canine PVC test obtained images with enhancement techniques.**

Index Terms—Image enhancement, Diagnostics, PVC, Smartphone Camera, Canine Blood Samples

I. INTRODUCTION

PCV or Packed Cell Volume Test is a test done to diagnose polycythaemia, dehydration or anaemia in certain patients. It is generally a part of the full blood count test that is used to estimate the need for certain blood transfusions and monitor the response to treatment.Packed cell volume (PCV) and total protein by refractometry (TPr) are commonly measured as part of the initial evaluationof dogs in emergency situations. In such circumstances, bloodvolume may be a limiting factor, only one type of sample may beavailable for initial analyses, and rapid diagnostic and therapeutic decisions may have to be based on the results obtained.

Packed cell volume measurements has multiple benefits in addition to its relative rapid availability, with a result available within 5–10 minutes. Only a small volume of blood is needed, which can be taken from EDTA or lithium heparin treated blood [3]. In the emergency setting or in small patients, with neonates being the classic example, blood can also be obtained directly from an IV cannula in micro hematocrit tubes pretreated with anticoagulant. The small volume facilitates repeat sampling, allowing trends to be tracked and comparisons to be made. An additional benefit is that, after the initial outlay on equipment, it is inexpensive to perform each test, making it an affordable test that can yield a large amount of information with relatively limited input.

Smartphone use is becoming increasingly widespread in the medical field and has been used to augment or improve upon current diagnostic techniques. There are previous reports of smartphone-obtained images being utilized in forensic medicine to age blood spatter. [6] Additionally, there are also reports of the use of add-on equipment, which allows hemoglobin concentration to be calculated. [7-8]

The aim of this review was to assess whether images obtained with a smartphone and reduce its noise and then widely available equipment could be used to extrapolate the PCV. The hypothesis was that images introduces obtained in a controlled environment with a standardized volume of blood spot would correlate best with them anual PCV.

II. LITERATURE REVIEW

Microhaematocrit readers are generally considered to provide a relatively accurate method for directly assessing PCV. Their use also allows for visual evaluation of plasma for haemolysis, lipaemia and hyper bilirubinaemia that may be advantageous clinically (Dubin*et al*. 1976, Thomas 2004, Rizzi*et al*. 2010). While PCV is a direct measurement, haematocrit (Hct) is calculated based on RBCcount and the mean corpuscular volume (MCV) of the erythrocyte population (Dubin*et al*. 1976, Rizzi*et al*. 2010). The potential forerror has traditionally been considered greater for Hct than for the measurement of PCV. This may be related to artefactual errors arisingfrom agglutination or changes in osmolality such as hyponatraemiaor hypernatraemia (Porter & Weiser 1990, Boisvert*et al*.1999).

However, it may also be related to the differences in erythrocytevolume between species and possible overlap with plateletsize. In particular, difficulties in counting and measuring small redblood cells can be encountered with automated impedance counters(Dubin*et al*. 1976, Brockus 2011). However, more advanced haematology analysers that use flow cytometry are now available that minimise this error (Brockus 2011).

Many practitioners relyon laboratory-derived results for clinical decisions on a routinebasis, but in critical situations they may base decisions on a rapidlyobtained PCV measurement. Few studies have evaluated the correlation between PCV and Hct measured using flow cytometry (Boisvert*et al*. 1999, Vatn*et al*. 2000). Such information is necessary toensure confidence in interpreting the results obtained, particularlyin situations where both measurements are used interchangeably. Refractometry is often used for rapid assessment of total proteinconcentration at the same time PCV is measured. Althougha reasonable

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reflection of total protein concentration, it assumesthat non-protein solutes such as electrolytes, urea, glucose andlipids contribute in a predictable manner (George 2001, Evans2011). Reportedly affected by glucose, cholesterol, lipaemia and haemolysis, there appear to be no reports of interference by triglyceride concentration (Dubin*et al*. 1978, Briend-Marchal*et al*. 2005, Hayes *et al*. 2011).
By contrast, laboratory-derived total protein (TP) concentrations based on biuret techniques are unaffectedby other solutes. However, there can be significant variationin the difference between TPr and TP depending on the type of refractometer used and laboratory variables such as compositionof biuret reagents, differing standards and changes in reactionconditions (George 2001). For the practitioner, clinical decisionsare often made on TPr values and knowledge of the actual variationfrom TP and potential interferences is therefore important.

III. Canine Blood Samples

All canine blood is not created equal—just like people, pets have different blood types and these differences are inherited. Giving incompatible blood can have life-threatening consequences.

- *Blood Types*

Blood groups and types vary and the differences are inherited. Antigens on the surface of the blood cells define a blood type. Antigens are proteins, carbohydrates, toxins or other substances to which the body responds by producing antibodies.

When a dog has those specific antigens on its red cells, it's said to be positive for that particular group. If the red cells do not have a given antigen, then the pet is negative for that blood group. This is important, because when a puppy is injured or ill, a transfusion with whole blood or blood components may be necessary to save the pet's life. Giving the wrong type of blood can have dire consequences.

- *Transfusion Reactions*

People (and cats) have very strong antibodies against the wrong type of blood. Our immune system recognizes non-compatible blood as foreign, and attacks and destroys the blood as if it were a virus or bacteria. When a person receives a blood transfusion and the wrong blood is given, this transfusion reaction can quickly kill the individual. The signs, though, are non-specific so it can be difficult to know what's gone wrong. Signs include a change in heartbeat, difficulty breathing, collapse, drooling, tremors, convulsions, weakness, vomiting, and fever.

- *First Transfusions*

Dogs rarely have naturally occurring antibodies the way people and cats do. The dog's immune system doesn't seem to immediately recognize incompatible blood, but must be first exposed to incompatible blood before building antibodies against it. For that reason, most dogs can receive a transfusion from any other blood group the first time. After that, though, the immune system is "primed" to recognize the foreign blood and if it's given again, a life-threatening transfusion reaction can happen.

Many times, a dog's first transfusion takes place under emergency circumstances to save the dog's life. If he's never before been transfused, it's likely he'll have no adverse reaction to the blood, even if it is incompatible. It's advisable whenever possible -- and always after your puppy has been previously transfused -- to identify the dog's blood type so that sensitization of your dog's blood and/or a possible life-threatening reaction can be avoided.

- *Canine Blood Types and Breeds*

You'll find different numbers of dog blood types listed; as many as 13 group systems have been identified but six are most commonly recognized. Dogs can be classified as positive or negative for each DEA (dog erythrocyte antigen). An erythrocyte is a red blood cell. The canine blood groups most commonly recognized are DEA-1.1, DEA-1.2, DEA-3, DEA-4, DEA-5, and DEA-7. Some blood types cause more dangerous reactions than others, and the DEA-1.1 group is the worst offender. Dogs that are negative for DEA 1.1 and other blood types are considered "universal donors" able to give to any other blood typed dog. DEA 1.1 negative is in the minority of dogs.

The majorities of dogs are DEA 1.1 positive and may only give blood safely to other DEA 1.1 positive dogs. An incompatible transfusion can result in both clumping and destruction of the red cells. Usually, the reaction is immediate, but it may be delayed up to four days. Some breeds have a predisposition to being DEA 1.1 positive or negative. On the negative column, breeds likely to be DEA 1.1 negative include Greyhounds, Boxers, Irish Wolfhounds, German Shepherds, Dobermans, and Pit Bulls. Breeds more commonly DEA 1.1 positive are Golden Retrievers and Labradors. If your puppy is one of these breeds, it would be a good idea to have your furry wonder's blood typed.

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➢ *Blood Banks and Dogs*

Transfusion medicine has made great strides in the past decade since dogs and cats often require a transfusion as a part of their treatment. In 1989, one of the first blood banks for pets was launched by Angell Memorial Animal Hospital in Boston. A standard unit of whole blood is 500cc, or almost 17 ounces, while packed red blood cells and plasma units are smaller. A pet's size and degree of illness determine how much he'll need. A number of programs run by veterinary teaching hospitals, as well as private commercial entities, are currently available. Some blood donor programs enlist pet dogs, based on several criteria including health, weight, and age. Others at teaching facilities may already have colonies of dogs (Greyhounds are common because most are DEA1.1 negative--but they're positive for DEA 3) that get lots of attention and treats for their participation and later may be adopted. Veterinarians now have easy-to-use canine and feline typing cards to screen for the most problematic blood types in their office. Cross-matching can also be easily done, and although it won't determine the type, it will tell whether a transfusion reaction will occur or not. A drop of serum or plasma from the recipient animal's blood mixed with a drop of blood from the prospective donor will clump when the blood is incompatible.

IV. IMAGE ENHANCEMENT

Image enhancement includes sharpening, contrast manipulation, filtering, interpolation and magnification, pseudo coloring, and so on. The greatest difficulty in image enhancement is quantifying the criterion for enhancement. Therefore, a large number of image enhancement techniques are empirical and require interactive procedures to obtain satisfactory results. However, image enhancement remains very important because of its usefulness in virtually all image processing applications. Color image enhancement may require improvement of color balance or color contrast in a color image. Enhancement of color images becomes a more difficult task not only because of the added dimension of the data but also due to the added complexity of color perception [11].

Image enhancement techniques are used to improve the appearance of the image or to extract the finer details in the degraded images. The principal objective of image enhancement is to process an image so that the result is more suitable than the original image for a specific application. A method that is quite useful for enhancing one category of images may not be necessarily be the best approach for enhancing other category of images. Color image enhancement using RGB color space is found to be inappropriate as it destroys the color composition in the original image. Due to this reason, most of the image enhancement techniques, especially contrast enhancement techniques, use HSV color space [12].

Image enhancement methods may be categorized into two broad classes: transform domain methods and spatial domain methods. The techniques in the first category are based on modifying the frequency transform of an image, whereas techniques in the second category directly operate on the pixels. However, computing a two dimensional (2-D) transform for a large array (image) is a very time consuming task even with fast transformation techniques and is not suitable for real time processing.

Image enhancement is basically improving the interpretability or perception of information in images for human viewers and providing `better' input for other automated image processing techniques. The principal objective of image enhancement is to modify attributes of an image to make it more suitable for a given task and a specific observer. During this process, one or more attributes of the image are modified. The choice of attributes and the way they are modified are specific to a given task.

1) Local Enhancement of the Image

The local enhancement is employed to get the minute details of an image. It enhances the local details in terms of the gradient of the image which gives useful information to the analyzer of the image. It addresses those pixels which would be ignored by the global method. The local enhancement method employed here is un-sharp masking [8]. In this method the image is sharpened by subtracting an un-sharp image that is a blurred or smoothed from the original image, so the name Un-sharp masking is derived. In this method the following steps are involved:

- Blurring of the image.
- Subtracting the blurred image from the original image to make the mask.
- Adding the mask to the original image.

If the blurred image is denoted as b (I,j) and the image as p (I,j) then the mask m (I,j) is given according to equation (1).

$$M(I, j) = p(I, j) - b(I, j) \quad (1)$$

The weighted portion of the mask is added to the original image to get the sharpened images (I,j) given by equation (2).

$$S(I, j) = p(I, j) + w * m(I, j) \quad (2)$$

Where 'w' is the weight, generally greater than zero. When the weight is equal to 1, it is the un-sharp masking and when greater than 1 then it is called high boost filtering. This sharpened image is given as input to the global contrast enhancement process for further improvement in the image quality or to improve the visual quality of the image.

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2) Global Enhancement of the Image

The global enhancement of the image is used to increase the contrast of the image. In this process each pixel of the image is adjusted so that it gives a better visualization of the image. In spatial contrast enhancement, the operation is performed directly on the pixel. The pixels are arranged in such a way that it is distributed throughout the range of desired intensity level. Global contrast stretching method is used as global method of enhancing the image. There are many global techniques like histogram equalization (HE), contrast limited adaptive histogram equalization and many other transformation methods like discrete cosine transform (DCT), discrete shearlet transform (DST), adaptive inverse hyperbolic tangent function transformation, etc. Among these, HE is the one used widely as global method [8].

Any of the method can be used to enhance the image globally. In all the global methods they did not consider the local details of the image and look for the global information of the image. So we first apply the local enhancement in order to verify the algorithm, the simple HE is used. It is not mandatory to use only this method; different methods can be used to improve the image quality. For the discrete image, the probabilities of the pixel value are taken in HE. To take the probabilities, first the corresponding number of pixels should have particular pixel intensity value; it is calculated and divided by the total number of the pixels present in the image. The probability of occurrence of pixel intensity level 'k' in the digital image is stated by equation (2.10).

$$p(r_k) = \frac{n_k}{N*M} \tag{3}$$

Where N*M is the total number of pixels in the image and n_k is the total number of pixels having intensity level "k". The pixels are transformed according to the following transformation equation in discrete form [8].

$$t_k = L(r_k) = (G-1)\sum_{i=1}^{k} p(r_i) = \frac{G-1}{N*M}\sum_{i=0}^{k} n_i \tag{4}$$

Where 'G' is the highest intensity level or value, L (r_k) is the transform function and k = 0, 1, 2, 3, ..., G-1. So the output image pixel is obtained by mapping each input pixel r_i to the new transformed value tk. The processed output value may have fractional value so a rounding function to the nearest integer value is needed. While doing so some of the image pixels may go to the new value and some of the intensity pixel values may not be present in the transformed image.

- **Image Acquisition**

Volume of blood and the environment in which images were acquired were the 2 variables that needed to be standardized. The potentially confounding variables ameliorated by the controlled environment include the distance from the camera to the blood spot, the angle of the camera in relation to the filter paper, degree of movement while image was acquired, and ambient lighting.

A drop of mixed blood was applied on to the center of the filter paper. To standardize volume, a pipette and tip were used to aliquot exactly 20 μL of blood. For non-standardized volume, disposable 2mL plastic droppers, which have a drop volume stated to be 22 μL according to the manufacturer, was used to deliver 1 drop. For each sample, the sample conditions were analyzed in the same order that is, starting with pipette in uncontrolled environment and finishing with dropper in controlled environment. After 30 seconds, the filter paper was either left in place or transferred to the box if the sample was to be assessed in a controlled environment. Five images were acquired in rapid succession with the smartphone, taking approximately 1 second to obtain, in order to provide a selection of samples to evaluate for unaccounted variation between images.

This was to provide an adequate amount of time for the blood spot to wick into the paper, while allowing the technique to still be performed more rapidly than the standard PCV measurement method. In uncontrolled conditions, the photograph was obtained at a height so that the filter paper filled the screen. This distance was not measured. There were no adjustments made to the lighting of the room, although images were taken in the absence of daylight and with only ambient overhead fluorescent strip bulbs. The images were evaluated grossly for any evidence of movement artifact and would have been reacquired had this been evident.

For a standardized environment, the filter paper with the blood spot was placed within the box. Thus, images were obtained from 4 different experimental conditions: dropper used out, with a standardized environment; pipette used out, with a standardized environment; dropper used inside a standardized environment; pipette used inside a standardized environment.For the alternative technique, the coverslips were placed at either end of one of the microscope slides, with the other microscope slide then overlaid. A dropper was then used to fill the created aperture between the slides via capillary action. Enough blood was instilled to fill the area between the 2 coverslips. This was then transferred into the box and images acquired.

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V. PCV TEST

Despite the fact that a packed cell volume is measured dozens of times a day at the Animal Medical Center, most pet owners have never heard of packed cell volume, sometimes referred to as a hematocrit. If one of your pets has experienced a serious issue with anemia, then you might have heard your veterinarian talk about this test. Also known as PCV, packed cell volume is one measure of the number of red blood cells in the blood. There are other methods to assess the number of red blood cells, but these take more time and much more sophisticated laboratory equipment. The laboratory can count the number of red blood cells; there are millions in a drop of blood. The oxygen-carrying protein hemoglobin contained inside of red blood cells can also be measured; like red blood cells, hemoglobin decreases when a patient is anemic.

Blood, in general, is a mix of plasma as well as cells. The PCV test measures how much of the blood consists of cells. If the PCV returns a reading of 50%, it means that 50 ml of the cells are present in exactly 100 ml of blood. If the RBC number increases, then the total reading of the PCV is also up. This number can also increase due to dehydration.

Performing the PCV tests and the total solids is a pretty routine and simple test undertaken at many hospitals. All medical members can easily perform the test but interpreting them is the tricky part. The readings can provide a lot of information regarding the patient's status and also help plan the next treatment step.

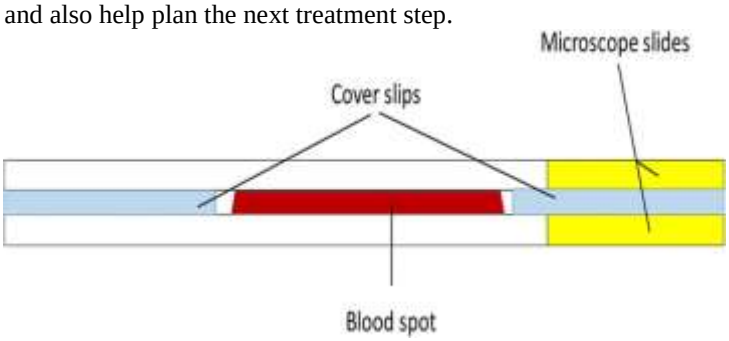

Fig 1: Packed Cell Volume

PCV [2] is the percentage of red blood cells in circulating blood. A decreased PCV generally means red blood cell loss from any variety of reasons like cell destruction, blood loss, and failure of bone marrow production. An increased PCV generally means dehydration or an abnormal increase in red blood cell production. TS are a measurement of plasma proteins. These proteins include albumin, globulins, and fibrinogens. Decreased TS generally means the animal is suffering from protein loss from any variety of reasons like blood loss, PLE, PLN, or malnutrition. Increased TS usually means dehydration but can be present in certain chronic diseases.

By looking at a hematocrit tube fresh out of the centrifuge, you can also get some idea for white blood cell content by examining the buffy coat. The buffy coat sits between the red cell layer and the plasma (and should not be counted as part of the PCV) and is normally 1 percent or less. A large buffy coat can signify a large increase in WBC count. The plasma layer should also be examined for hemolysis, lipemia, and icterus. These characteristics should be noted along with the PCV/TS results. Remember to look at both values together to get the entire clinical picture of your patient, and continue to monitor throughout their hospital stay.

- **↑PCV, ↑TS**: This patient is most likely suffering from dehydration. As the water portion of blood is decreased you will see an elevation in both the PCV and TS. Both of these values should decrease as fluid therapy rehydrates the patient.
- **↑PCV, normal TS**: This patient also may be dehydrated, but remember that the addition of fluids will bring both values down, so there is a loss of proteins occurring. Watch this patient for the development of hypoproteinemia and associated clinical signs (hypotension, peripheral edema) as fluids are administered. This patient could be suffering from polycythemia, a rare condition where the body overproduces red blood cells.
- **↑PCV, ↓TS** : This patient could be suffering from dehydration and a profound protein loss. More commonly, you may see this with a recent trauma. This patient may be suffering from acute blood loss and splenic contraction has temporarily increased the PCV, but the low TS point you towards blood loss. In a trauma patient where you expect blood loss it is important to recheck the PCV and TS after starting treatment.
- **Normal PCV, ↑TS**: This is a common scenario with CKD cats. This patient is most likely suffering from anemia and dehydration. The normal PCV may fool you into thinking this animal is okay, but as you rehydrate remember that both numbers will drop leaving you with an anemia to treat.
- **Normal PCV, normal TS**: Normal is good, right? Be sure to match the results to the patient. If this patient sustained recent trauma there may be blood loss that isn't apparent on blood work yet. If the results are different than what you expected, then recheck the PCV/TS as you begin treatment.
- **Normal PCV, ↓TS**: This patient is most likely suffering from a protein losing disease (PLN, PLE), chronic diarrhea, or certain liver and kidney diseases. Be prepared for clinical signs of hypoproteinemia (hypotension, peripheral edema) and their treatment.
- **↓PCV, ↑TS**: The elevated TS most often points to dehydration, and remembering that the addition of fluids will further drop the PCV, this patient is anemic and needs close monitoring of the PCV and most likely the addition of blood products.

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- **↓PCV, normal TS**: This patient is suffering from RBC destruction lack of production. In blood loss we expect to see a decrease in the TS as well. With just the red cells decreased be on the lookout for IMHA.
- **↓PCV, ↓TS**: This patient is suffering from whole blood loss and needs to be monitored very closely. Blood products should be considered in treating this patient.
- Armed with this information, you now know a great deal about your patient just by looking at the PCV/TS. By using critical thinking skills and knowledge of the treatment plan for these patients you can prepare for their future needs.

A. Reading the PCV

A lower number of the PCV means that the RBC count loss is due to reasons such as blood loss, cell destruction and less bone marrow production. Increased PCV can generally mean that a person is dehydrated and there is a higher number of RBC productions. By looking at the tube out of the centrifuge, you can get an idea of the WBC content as well. This buffy coat normally lies between the plasma and red cell layer. (This shouldn't be counted as a part of the PCV test). The layer of plasma should also be examined for lipemia, hemolysis in addition to icterus.

B. Preparation for the PCV Test

There is not any special preparation required for the PCV test. If you are anxious about the test, it is better to talk to the doctor and let him/her know. Also, any medications that you've been taking have to be relayed to the doctor. If there are any medical problems which are underlying too, you need to fast before taking a test.

C. Uses of Packed Cell Volume Test

A low PCV implies that the patient has a low number of red blood cells and is suffering from anaemia. The doctor may ask the patient to undergo further tests to determine the underlying causes of anaemia. Treatment will be given accordingly.

D. Measuring the PCV Test

The PCV test is calculated with the help of an automated analyzer which means that it isn't directly measured. By multiplying the red cell count with the mean cell volume, doctors get the final amount. PCV is slightly less accurate than the hematocrit as they include small amounts of the plasma from the blood that is generally trapped in between two red cells. By tripling the haemoglobin concentration and dropping the units, an estimated hematocrit can be determined in percentage.

The PCV can also be determined with the help of the capillary tube and the centrifuging heparinised blood in it at around 10000 RPM for roughly five minutes. This process helped in separating the blood into different layers, and the volume of the total packed RBC divided by the blood sample's total volume gives the final amount of the PCV. Since a tube is also used, it can be used to measure the lengths lying between certain layers.

There is also another way to measure the levels of hematocrit, and this is through optical methods such as spectrophotometry. With the help of differentials, the differences between the optical densities of the sample flowing through the glass tubes at isosbestic wavelengths and the product containing the luminal diameter along with the hematocrit can create a linear relationship.

E. When Does A Low PCV Reading Occur?

There are certain conditions that contribute to the low reading in the PCV. These include:

- Nutritional deficiencies of iron or vitamin (B12 or folate) and mineral deficiencies
- Bleeding
- Inflammatory conditions, for example, rheumatoid arthritis
- Kidney diseases
- Haemolysis, which is the situation where the RBCs are destroyed prematurely by the immune system. This occurs due to certain organ damages and inherited abnormalities of the RBCs
- Liver cirrhosis
- Medicines - including that of chemotherapy
- Abnormalities of RBCs or haemoglobin containing disorders such as myelodysplastic syndrome, lymphoma, bone marrow disorders and myeloma
- One of the most common causes of heightened PCV readings is that of dehydration. With adequate fluid intake, the levels return to normal, but it can also create a condition known as polycythaemia where there are more RBCs

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VI. CONCLUSION

PCV measurement method requires several pieces of equipment, which reduces its availability for both financial and logistic reasons, such as the need for electricity. The necessary equipment also reduces its portability, limiting its use in field situations. A smartphone application would overcome these limitations. The main limitation is that a controlled environment is required to improve the correlation and predictive ability of the technique. This paper reviewed of a smartphone-obtained image can be used to extrapolate the PCV of canine blood samples. This paper introduces and review of canine PVC test obtained images with enhancement techniques.

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