Point of Care Animal Side Simple Phosphorous Detection Test Kit in Cattle

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1. Introduction

Phosphorus is the second most abundant element in cattle's body after calcium [1]. Decreased blood phosphorous level in cattle due to heavy calving, intense milk production and improper diet cause Hypophosphatemia which may lead to failure in growth, loss of appetite, decreased milk production, impaired reproduction & skeletal abnormalities. If the phosphorus level is not within the required range it also affects the calcium level, as the amount of calcium absorbed depends on the amount of phosphorus. Hence it is crucial to timely diagnose animals for phosphorous deficiency [2]. The treatment cost for phosphorous deficiency is expensive (Rs.800-1000/-per day per animal) and recovery is slow. It thus causes chronic cost burden on farmer, with severe economic losses to dairy industry. Currently phosphorus deficiency is detected with the help of clinical signs and symptoms which is very ambiguous. Laboratory testing (Rs.200 per animal per test) is also available but it increases the cost, requires transport facilities and are time consuming. Neither phosphorus deficiency nor excess of phosphorus last throughout the life cycle of the cattle, as soon as one of them is detected, one can take preventive measures by making changes in their feed. Blood processing and far off laboratory testing is not a practical or economic approach for farm cattle and rural settings which has limited outreach. Due to the lack of animal-side phosphorous level monitoring kits, currently phosphorous supplementation (Cost of Phosphorous rich feed per animal per day is Rs.500-1000) is provided without measurement of blood phosphorous levels which create a chain of other problems. Therefore, we have designed Phosphorous Detection Kit which gives rapid visual results immediately on animal side.

2. Aim of the Project

To develop simple point of care, animal side, portable, affordable, visual colour based blood serum phosphorous detection test kit.

3. Our Solution

We proposed a simple colour based test which would help detect low, normal & high Phosphorous content in the serum obtained from blood of cattle. More importantly this colour change would usually indicate level of phosphorous high, normal, low is based on phosphorous level reported in cattle.

Cattle Serum Phosphorous Range: High > Normal (5.6-8.3 mg/dL) > Low [3]

4. Procedural Details

4.1 Materials: Phosphorous specific reagent (P-reagent), dil. H_2SO_4 (Sulphuric acid), Ascorbic acid

4.2 Reagent Preparation: P-reagent was prepared by adding phosphorus specific dye (P dye), ascorbic acid as reducing agent, and sulphuric acid as catalysing agent and final volume was made up using water (10mL). The P reagent was colourless. [4,5]

4.3 Preparation of Phosphorous standard solutions

Low P Concentration: 20 mg of dipotassium hydrogen phosphate (K_2HPO_4) was taken in 100 mL of water which is equivalent to 3.3 mg of phosphorus/dL.

Normal P concentration: 40 mg of K_2HPO_4 was taken in 100 mL of water which is equivalent to 6.6 mg of phosphorus/dL.

High P concentration: 60 mg of K_2HPO_4 was taken in 100mL which is equivalent to 9.9 mg of phosphorus/dL.

4.4 Procedure for detection

In Eppendorf tube 500µL of P-reagent was taken. To this 50µL of standard phosphorous solution was added (total 550µL). Mixed and observed the developed colour. The colour for phosphorous standards is as shown below.

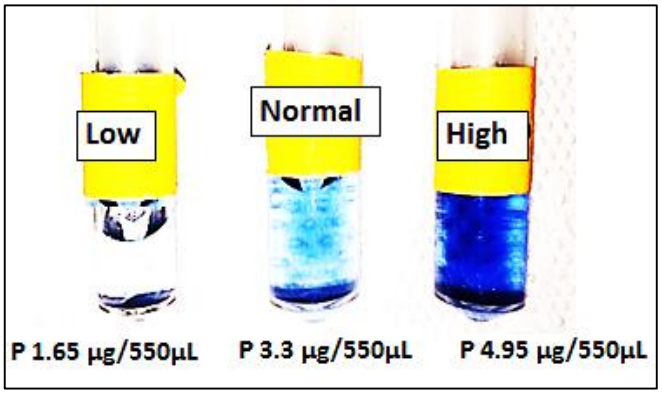


Figure 1: Standard aqueous Phosphorous solutions and their respective colour differences

The flow sheet of phosphorous detection is depicted below

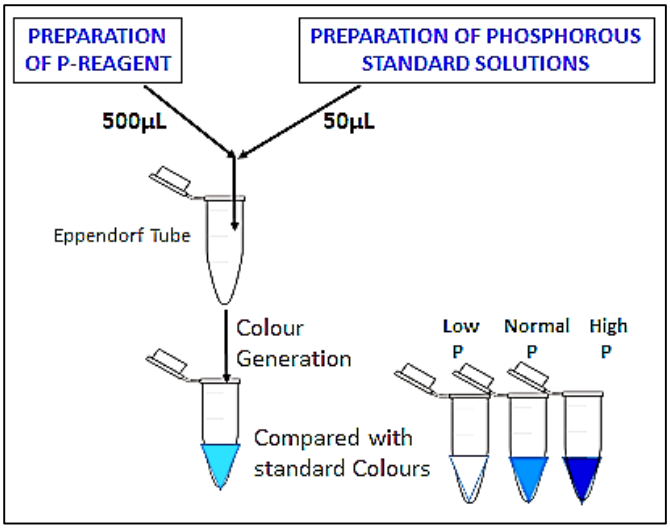
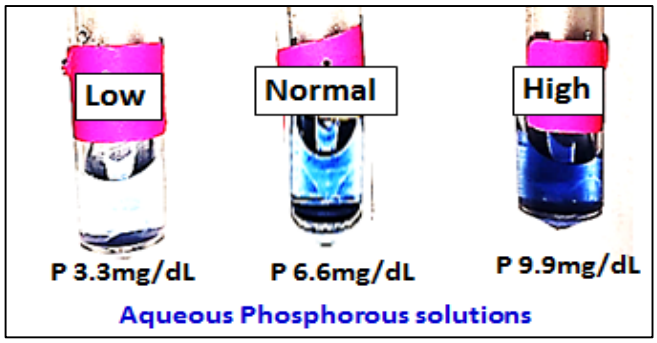


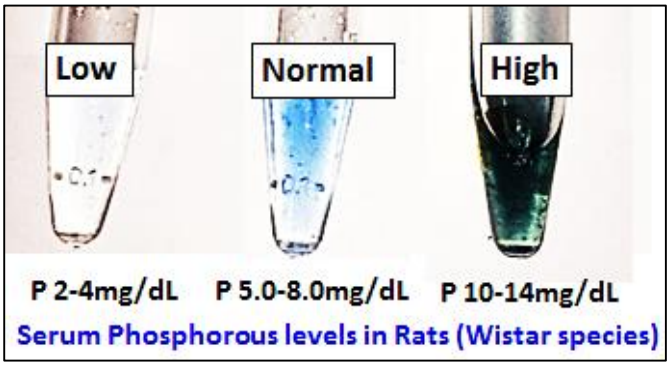
Figure 2: Flow sheet for Phosphorus Detection

5. Results & Inference

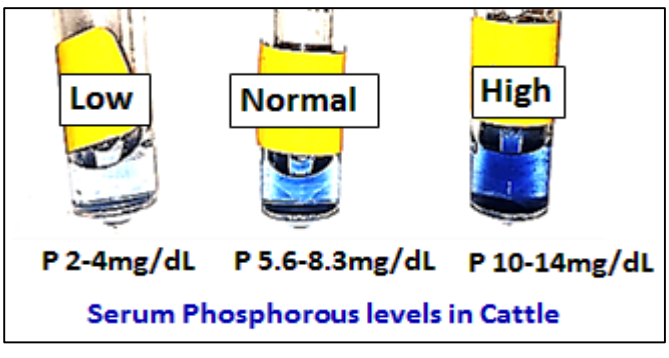
5.1 Phosphorous detected in Aqueous solution



5.2 Phosphorous detected in Rat Serum

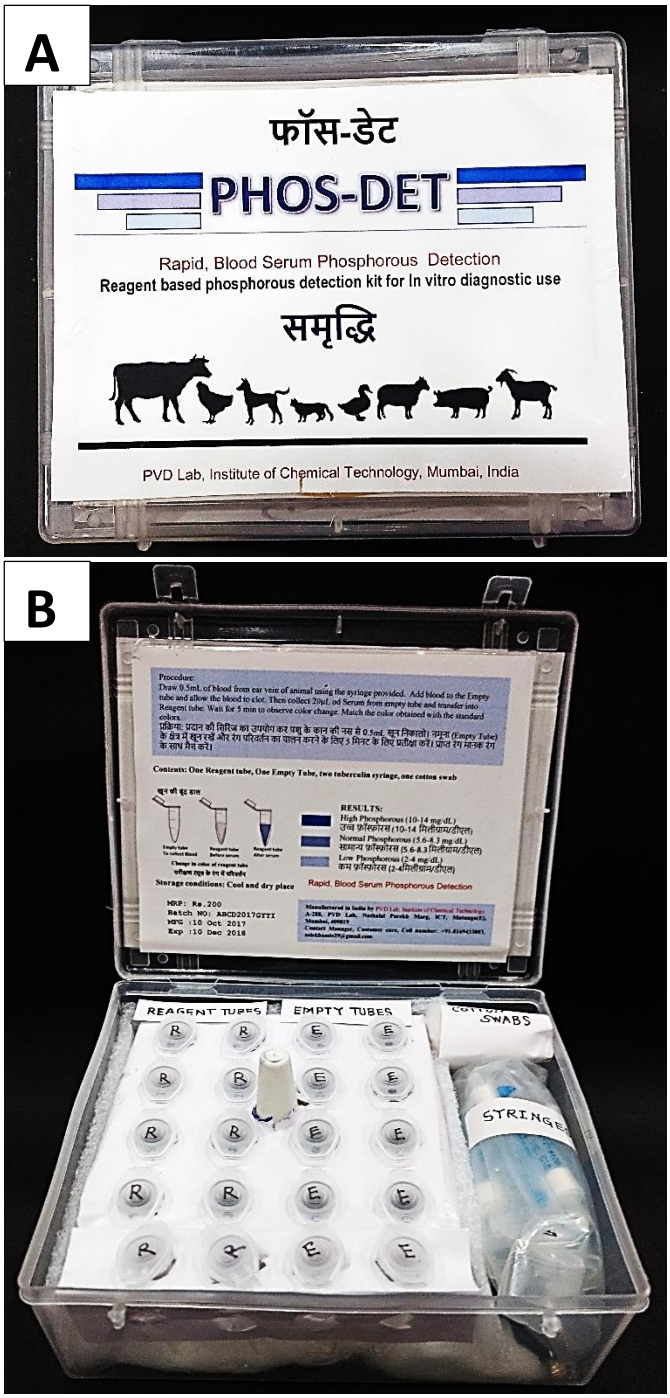


5.3 Phosphorous detected in Cattle Serum



5.4 Final Phosphorous Detection Kit Assembly

Phosphorous Detection Kit Assembly for 10 tests



Phosphorous Detection Kit assembly (A) Front View, (B) Inside View

The components of the Phosphorous Detection Kit Assembly for 1 of 10 tests

- REAGENT TUBE (R): This tube contains phosphorous specific reagent (0.5mL)
- EMPTY TUBE (E): In this tube the blood is collected and allowed to clot for 15 min to get sufficient serum (50µL)
- SYRINGES (2): first Syringe is required to withdraw 0.5mL of blood from animal's ear vein.

- Another syringe is required to collect serum from EMPTY TUBE and transfer it to REAGENT TUBE
- COTTON SWABS: to clean the pricked area
- STANDARD COLOR BANDS: This will provide colour identification of high to low phosphorous level

5.5 Procedure to use Phosphorous Detection Kit

1. Withdraw 0.5mL of blood from animal's ear vein using first syringe
2. Transfer the whole blood in EMPTY TUBE
3. Allow the blood to clot for 15min in REAGENT TUBE
4. Use another syringe to collect obtained serum (50µL) from EMPTY TUBE after blood clotting
5. Add this collected serum in REAGENT TUBE containing P-reagent
6. After 5 min visually observe the developed colour
7. Visually match the developed colour with standard colour bands to confirm the phosphorous level

Dark Blue color: High P, Faint blue color: Normal P, Very less blue color: Low P [Demonstrated in Figure 3]

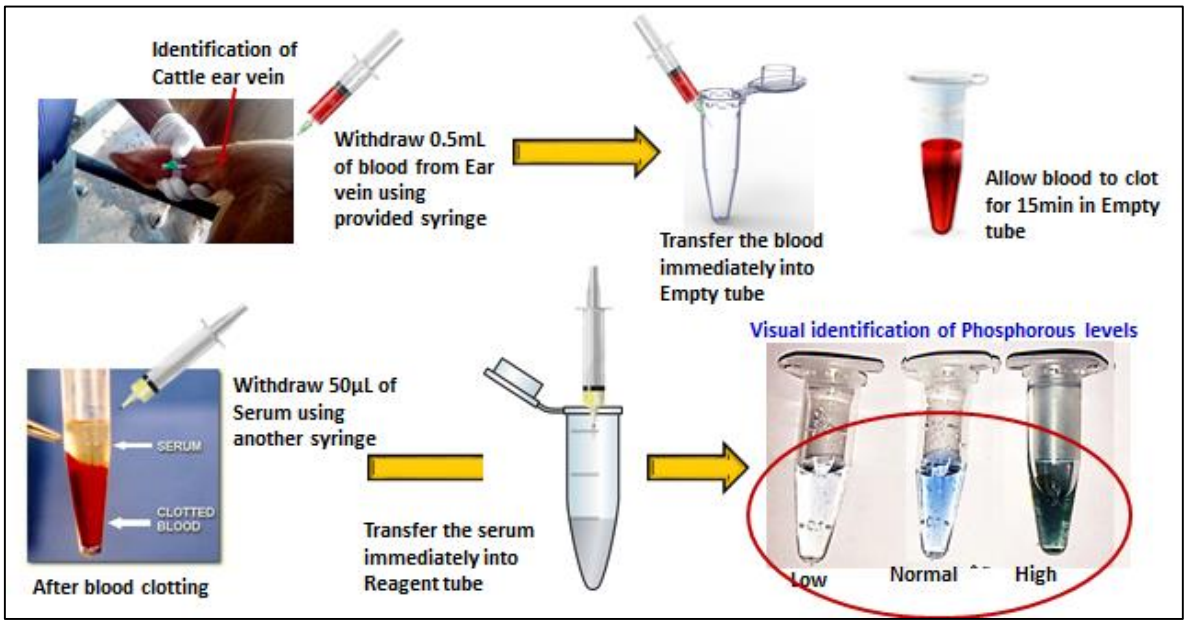


Figure 3: Schematic for the Use of Phosphorous Detection Kit

6. Advantages of Phosphorous Detection Kit

•Simple point of care, •Rapid and visual test, •Easy to use in rural area, •Do not require sophisticated instruments •No need of trained personnel, •Farmer friendly

7. Applications

•Rural/semi-rural veterinary healthcare centres, •Dairy farmers, •Dairy industries

We believe that the cost of Phosphorous Detection per kit for 10 tests is approximately Rs.200/-

8. Conclusion

Thus our Phosphorous Detection Kit is a simple yet innovative animal side, point of care, affordable blood serum phosphorous detection kit which has great outreach.

9. References

1. Brooks H. V., Cook T. G., Mansell G. P., Walker G. A. (1984), Phosphorous deficiency in dairy herd, New Zealand Vet. J.32: 174-76.
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4. Taussky H. H. & Shorr E. (1953), A microcolorimetric method for the determination of inorganic phosphorous. J Biol Chem. Jun;202(2):675-85.
5. Zinzadze Ch. (1935), Colorimetric Methods for the Determination of Phosphorus, Industrial & Engineering Chemistry Analytical Edition 1935 7 (4), 227-230.