I. PRINCIPLE:

The enzyme, Pyruvate Kinase, catalyzes the following reaction:

$$\text{ADP} + \text{Phosphoenolpyruvic Acid} \xrightarrow{\text{Pyruvate Kinase}} \text{ATP} + \text{Pyruvic Acid}$$

The pyruvic acid formed then takes part in the following reaction:

$$\underset{\text{(High Fluorescence)}}{\text{Pyruvic Acid} + \text{NADH}} \xrightarrow{\text{Lactic Dehydrogenase}} \underset{\text{(No Fluorescence)}}{\text{Lactic Acid} + \text{NAD}}$$

In this procedure, the red cell sample is incubated with a reagent which contains ADP, phosphoenolpyruvic acid and NADH. Lactic dehydrogenase, which is also required for the reaction to proceed, is provided by the red cells. When Pyruvate Kinase is present, the NADH is oxidized to NAD resulting in a loss of fluorescence (spots made on filter paper are viewed under a long wave ultraviolet light). When Pyruvate Kinase is deficient in the sample, the NADH remains intact and no loss in fluorescence is observed.

II. SPECIMEN:

Blood collected in EDTA is suitable for the screening test, even after several weeks of storage at 4°C.

EDTA microtainer specimens with volumes of less than 500µl are not suitable for this screening test. Low volume specimens increase the likelihood of WBC contamination. WBC's contain PK and will possibly produce a normal result in a deficient patient.

III. REAGENTS/EQUIPMENT:

1. Long wave ultraviolet light

2. Whatman No. 1 paper (use **only** this filter paper)

3. 0.15 M Phosphoenolpyruvic Acid (PEP)
   51.3 mg Phosphoenolpyruvic Acid Trisodium Hydrate, MW 342 (stored in freezer) in 1ml H_20

4. .03 M ADP (adenosine-5'-diphosphate) (monosodium salt)
   If the molecular weight changes the formula is:
   M.W. x molarity (.03) x 5 ml = mg of ADP/ 5 ml H_2O
   M.W. 449.2, 67.38 mg in 5 ml H_2O

5. 3 M NaDH
   1 vial of 2mg NaDH, in staining cabinet at room temperature, in 1ml H_2O

6. .08 M Mg $CL_2 \cdot 6\ H_2O$ magnesium chloride
   M.W. 203, .163 gm in 10 ml H_2O, stored at **room temperature, stable for 1 year**.

7. **.25 M K Phosphate Buffer, pH = 7.4**
   a. .25 M K_2HPO_4 M.W. 174.2
      4.35 gm in100 ml H_2O

   . b .25 M KH_2PO_4 M.W. 136
      3.4 gm in 100 ml H_2O

   Add 20 ml of .25M KH_2PO_4 to 100 ml of .25M K_2HPO_4 and adjust to pH 7.4.
   Store at **room temperature, stable for 1 year**.

9. PK screening solution:

PEP	.6 ml:	prepared as above
0.3 M ADP	2.0 ml:	prepared as above
0.8 M $MgCL_2.6H_2O$	2.0 ml:	prepared as above, bottle in cabinet
.25 M K Phosphate	1.0 ml:	prepared as above, bottle in cabinet
H_2O	4.4 ml	

Aliquot .5 ml in tubes and freeze. Stable indefinitely.

IV. QUALITY CONTROL:

Normal EDTA blood

V. PROCEDURE:

1. Prepare NaDH (add 1 ml of distilled water to 2 mg vial NaDH) and mix well.

2. Prepare screening solution: remove tube from the freezer, thaw and add .5 ml of NaDH.

3. Patient and normal control samples are centrifuged at 3000 rpm for 10 minutes. Remove all the plasma and buffy coat layer. **Leukocytes contain PK and may produce normal results in a deficient patient.**

4. Measure 20 µl of red cells and resuspend with 80 µl of 0.9% NaCL

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5. 20 µl of the above cell suspension is mixed with 200 µl of screening solution, and the mixture is incubated in the 37°C waterbath.

6. This solution is spotted on **Whatman No. 1 paper** immediately after mixing and every 15 minutes thereafter for 1 hour. See example. .

7. The paper is examined under illumination with long-wave ultraviolet light after the spots are dry. (See the new safety procedure for long-wave ultraviolet light at end of procedure.)

8. To read PK: use the ultraviolet light in Molecular Biology 6th floor. Use appropriate face shield provided.

VI. RESULTS:

Must be entered as **normal or deficient**.
Do not enter as positive or negative.
Normal = Fluorescence disappears after 15 minutes of incubation.
Deficient = No Disappearance of fluorescence after 60 minutes.

VII NOTES:

It has been recognized that inherited deficiencies in certain red cell enzymes may be responsible for many forms of hemolytic disease. Drug-induced hemolytic anemia may be attributed to an innate deficiency in glutathione, or glutathione reductase. Nonspherocytic congenital hemolytic anemia may be the result of deficiency in glucose-6 phosphate dehydrogenase, pyruvate kinase, or, in much rarer instances, glutathione reductase or other enzymes.

Pyruvate Kinase deficiency has become the second most common erythrocytic enzyme deficiency associated with hemolytic anemia. This deficiency has been documented in over 150 cases. Although found primarily in subjects of northern European extraction, some cases have also been reported in other parts of the world.

This atypical form of congenital hemolytic anemia has been characterized by the presence of marked autohemolysis that is not corrected by glucose. Jaundice and the appearance of anemia are reported as common in infancy in homozygotes. Splemomegaly has been noted as invariably present. This disease does not favorably respond to splenectomy. Heterozygotes may demonstrate lower than normal enzyme activity.

Medical conditions, such as acute leukemia, preleukemia, and refractory sideroblastic anemia, as well as complications from chemotherapy, can cause an acquired pyruvate kinase deficiency (PKD). This type is more common and milder than the hereditary type.

VIII. REFERENCES:

1. Williams W.J., Beutler E., Erslev A.J., Hematology 3rd edition.
2. Sigma Technical Bulletin #205, Qualitative Screening Procedure for the detection of Pyruvate Kinase Deficiency, Sigma Diagnostics, St. Louis Missouri.
3. Cartwright, George E., Diagnostic Laboratory Hematology, 4th edition. Grune & Stratton, Inc, 1968.
4. http://emedicine.medscape.com, Pyruvate Kinase Deficiency

IX. HISTORY:

H-1 This procedure was written by P. Morris on 10-21-97.
H-2 This procedure was revised by Paula Morris on 2/17/11.
H-3 This procedure was revised by S. Richardson on 3/27/12.