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Blood in Urine (Multistix 10 SG) - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

A. This test is based on the peroxidase-like activity of heme, which is part of the hemoglobin molecule. The heme moiety is also present in Myoglobin and in various cytochromes. Heme catalyzes the reaction of tetramethylbenzidine (TMB) and diisopropylbenzene dihydroperoxide. The resulting color ranges from orange through green. Very high levels of blood may cause the color development to continue to blue.

$$H_2O_2 + TMB \xrightarrow[\text{(peroxidase activity)}]{\text{heme}} \text{oxidized TMB} + H_2O \quad \text{(green)}$$

B. Hematuria (the presence of intact red blood cells [RBCs] in urine) may occur with disease or trauma anywhere in the kidneys or urinary tract, with bleeding diatheses and anticoagulant therapy, and with the use of some drugs, e.g. cyclophosphamide. Hematuria is also seen in healthy persons under-taking vigorous exercise (e.g. marathon runners), where the RBCs emanate from the bladder mucosa.

C. Hemoglobinuria (the presence of free hemoglobin in urine) indicates either intra- or extra vascular hemolysis of RBCs. In the absence of hematuria, hemoglobinuria implies a severe intravascular hemolysis where plasma haptoglobin has been saturated. Both hemoglobin and RBC's will usually be present in urine where extravascular hemolysis has occurred.

D. Myoglobinuria will also be detected by these strips. Myoglobinuria follows rhabdomyolysis, skeletal muscle injury, strenuous exercise, cardiac muscle injury, seizures, infections, phenylcyclidine intake, acute alcohol overdose, ethylene glycol ingestion, and rare hereditary causes.

E. This document describes the steps for this procedure to assist technologists.

II. SPECIMEN COLLECTION AND HANDLING:

Fresh, well-mixed uncentrifuged urine. It is recommended that testing be done within one hour after voiding. Otherwise, immediately refrigerate the specimen and return to room temperature before testing.

III. REAGENTS/SUPPLIES:

A. Siemens Multistix 10 SG (#2161)
B. 6.8 % weight for weight (w/w) diisopropylbenzene dihydroperoxide
C. 4.0 % w/w 3,3',5,5'-tetramethylbenzidine
D. 48.0 % w/w buffer
E. 41.2 % w/w nonreactive ingredients

IV. QUALITY CONTROL (QC):

A. Both Normal and Abnormal Kova-Trols are run and results are recorded:
    1. at the beginning of each shift
    2. whenever a new lot number of reagent strips is opened
    3. whenever a new shipment of reagent strips is received
    4. whenever troubleshooting warrants it

V. PROCEDURE:

A. Briefly dip the test area of the strip in fresh, **well-mixed** uncentrifuged urine.
B. While removing the strip, run the edge against the rim of the urine container to remove excess urine. Hold the strip in a horizontal position to prevent mixing of chemicals from adjacent reagent areas and/or contaminating the hands with urine.
    1. If reading visually, compare the **BLOOD** reagent area to the corresponding Color Chart on the bottle label at **60 seconds**. Hold strip close to color blocks and match carefully.
    2. If reading instrumentally, follow directions given in the Clinitek Advantus procedure.

VI. REPORTABLE RANGE:

Multistix 10 SG has a color comparison chart with **SEVEN** color blocks ranging from orange through green. These represent heme as negative or present in increasing amounts. The appearance of green spots on the reacted test pad indicates the presence of intact RBC's. Reactions ranging from trace to large with proportionately more numerous spots may be observed. The color chart includes examples of trace and moderate non-hemolyzed color blocks. Results are reported as follows:

Report	Observed
Negative	---

Trace	trace (hemolyzed or non-)
1+	small (hemolyzed or non-)
2+	moderate (hemolyzed or non-)
3+	large

VII. REFERENCE RANGE:

Negative

VIII. SENSITIVITY:

0.015 - 0.062 mg/dL hemoglobin concentration - this is approximately equivalent to 5-20 intact RBCs/ microliter. This test is equally sensitive to Myoglobin as to hemoglobin. Test pad sensitivity may be reduced in urines with high specific gravity. Because of the optical system of the Atlas, the sensitivity to intact RBCs is better than that perceived visually. This test complements the microscopic examination for intact RBCs.

IX. INTERPRETATION:

Blood is often, but not always, found in the urine of menstruating females. The significance of the "Trace" reaction may vary among patients, and clinical judgment is required for assessment of individual cases. Development of green spots (intact RBCs) or green color (free hemoglobin/Myoglobin) on the reagent area within 60 seconds warrants further investigation.

X. LIMITATIONS/INTERFERING SUBSTANCES:

A. URINE MUST BE WELL MIXED! FALSE NEGATIVES MAY OCCUR WHEN RBC'S ARE ALLOWED TO SETTLE.

B. Refer to following table from AMES, **Factors Affecting Urine Chemistry Tests,** Miles Inc., 1992.

Test for Occult Blood		
Urine Constituents Affecting Blood Results	**Multistix 10 SG**	**Microscopic Examination of Urine Sediment**
High specific gravity urine	May reduce reactivity	No effect
Oxidizing contaminants, e.g., hypochlorite	May produce false positive	No effect
Microbial peroxidase from urinary tract infection	May produce false positive	No effect
Ascorbic acid levels normally found in urine	No effect	No effect
Myoglobin	Reacts positively	Will not be detected

Erythrocytes settled out in container (not a well-mixed urine)	May cause false negative	May not be detected
Lysed erythrocytes	Reacts positively	May not be detected
Captopril (Capoten)	Decreased reactivity	No effect

XI. REFERENCES:

1. Multistix 10 SG Miles, Inc. Diagnostic Division, Elkhart, IN 46515, rev. 4/99.
2. Henry, J.B., Clinical diagnosis and Management by Laboratory Methods, 20th edition, Philadelphia, W.B. Saunders Co., 2001, pp 380-382.
3. Hundley, J.M. and Fleming, J.K., Urine Analysis American Society of Clinical Pathologists Workshop, Dearborn, MI 1991.
4. Ames, Factors Affecting Urine Chemistry Tests, Miles Inc., 1992.

Approval Signatures

Step Description	Approver	Date
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